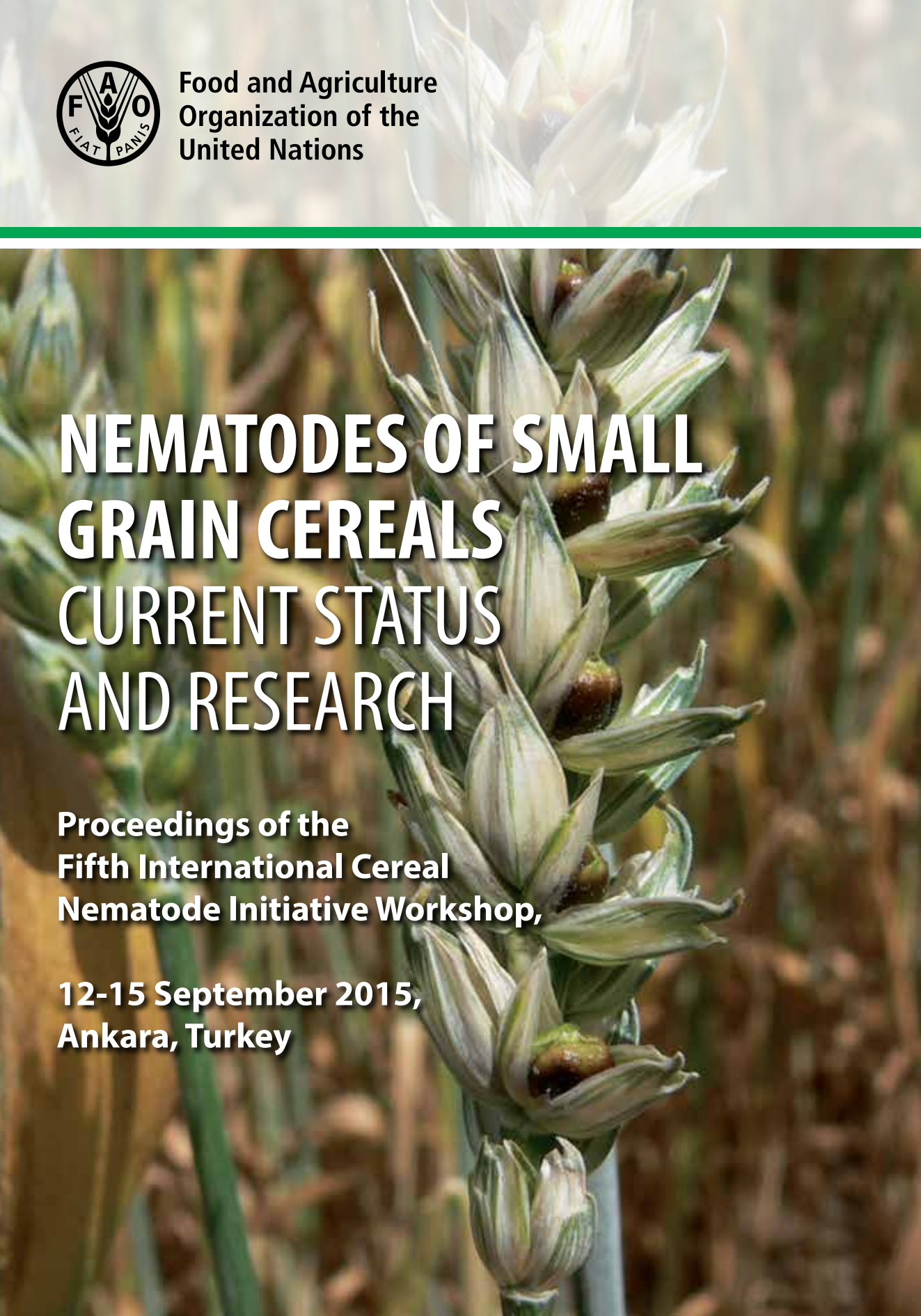


Food and Agriculture Organization of the United Nations

NEMATODES OF SMALL GRAIN CEREALS CURRENT STATUS AND RESEARCH

Proceedings of the Fifth International Cereal Nematode Initiative Workshop,

12-15 September 2015, Ankara, Turkey

NEMATODES OF SMALL GRAIN CEREALS
CURRENT STATUS AND RESEARCH

Proceedings of the

Fifth International
Cereal Nematode Initiative Workshop

12-15 September 2015,
Ankara, Turkey

ABDELFATTAH A. DABABAT
HAFIZ MUMINJANOV
RICHARD W. SMILEY
Editors

FOOD AND AGRICULTURE ORGANIZATION
OF THE UNITED NATIONS
Ankara, 2015

ISBN: 978-92-5-108878-4

Cover illustration: The seed gall nematode '*Anguina tritici*' on wheat in Turkey showing seed galls in wheat heads. Photograph: Adnan Tulek.

Printed in Turkey

THESE PROCEEDINGS ARE DEDICATED TO

MINISTRY OF FOOD, AGRICULTURE AND LIVESTOCK OF THE REPUBLIC OF TURKEY

THE MINISTRY HAS BEEN INSTRUMENTAL IN IMPROVING WHEAT IN CLOSE COLLABORATION WITH THE CIMMYT-ICARDA WHEAT IMPROVEMENT PROGRAM

CONTENTS

PART 1 - GLOBAL STATUS

PART 2 – ECONOMIC IMPORTANCE AND POPULATION DYNAMICS

PART 5 – MOLECULAR TECHNOLOGIES

FOREWORD

Small grain cereals include wheat, barley, oats, rye, triticale, rice and other species that constitute the world's most important source of food. They are critical components of local economies in developed and developing countries. These crops supply 20% of calories and account for more than half of all harvested crop areas in the world. About 70% of land devoted to producing food crops is planted to cereals.

Production of small grain crops on a per unit area basis increased linearly from 1960 until about 2005 and then began to decelerate in rate of annual gain[1]. The rate of increase is projected to continue to decline through 2050. This alone is not particularly ominous because rates of population growth are also predicted to begin a similar trajectory during the next decade. However, predicted consumption of wheat and other small grains is anticipated is continue a slow increase. Land planted to wheat, the primary small grain crop, is not anticipated to increase appreciably and any increase will likely occur on land that is of only marginal to good productive capacity since most available prime land is already being used for crop production. Additionally, land capable of being irrigated, and therefore capable of producing higher-yielding crops, is not anticipated to increase appreciably through 2050.

Additional gains in productivity of small grain cereals will continue to depend upon developments of new technologies and cereal cultivars, and identifying and addressing the production constraints associated with shifting climate patterns, degradation of cropland soils, declining availability of certain fertilizer nutrients, salinization of some currently irrigated land, reduced availability of water in some regions, competition of agriculture with other potential land uses particularly near cities, and yield reductions caused by crop pests.

Plant-parasitic nematodes are a well-recognized constraint to production of small grain cereals in fields that are highly infested by these microscopic para-

[1] Alexandratos N, Bruinsma, J (2012) In 'World Agriculture Towards 2030/2050: The 2012 Revision.' Food and Agriculture Organization of the United Nations, Agricultural Development Economics Division, *ESA Working Paper No. 12-03*.

sites. Three decades ago, it was estimated[2] that nematodes reduced global productivity of small grain cereals by nearly 750,000,000 metric tons annually, with yield reductions of 7.0%, 6.3% and 4.2% for wheat, barley and oats, respectively. A more recent estimate[3] indicates yield losses equivalent to about 10% of global production.

Nematodes that invade roots of small grain cereals typically become most numerous in direct proportion to the frequency of host crops produced on an infested field. Small grain production is often restricted by economic, agronomic and/or climatic factors that cause them to be repeatedly planted on the same tracts of land.

The most important genera of plant-parasitic nematodes include species of *Heterodera* (cyst nematodes) and *Pratylenchus* (root-lesion nematodes).[4] Many other genera and species also cause more-localized yield constraints but *Heterodera* and *Pratylenchus* species account for most of the global crop damage, especially in temperate regions where small grains well adapted and are mostly produced without supplemental irrigation. It is also important to recognize that root-invading nematodes are not only capable of directly reducing a plant's ability to withdraw water and nutrients from soil but to also enter into disease complexes with plant-pathogenic fungi that cause root diseases. Some complexes reduce yields more than is caused by the sum of the capabilities of individual pathogens within the complex.

Sources of genetic resistance to some nematodes have been identified but the rate of incorporating effective genes into commercial cultivars has been slow due to difficulties in transferring the resistance factor(s) into cultivars that have agronomic traits and productive capabilities of importance to agriculturalists. Disease management strategies other than genetic resistance are effective for some nematode species but those strategies are often neither environmentally nor economically acceptable. Such approaches include combinations of seed treatment chemicals or biological agents, planting non-host crops, or placing land into prolonged periods of fallow between plantings of susceptible crops.

2 Sasser JN, Freckman DW (1987) A world perspective of nematology: the role of the Society. In. 'Vistas on Nematology' (Eds. JA Veech and DW Dickson) pp. 7-14. Society of Nematology. Hyattsville, Maryland.

3 Dixon J, Braun HJ, Kosina P, Crouch J (Eds.). 'Wheat Facts and Futures 2009.' (CIMMYT, Mexico)

4 Smiley RW, Nicol, JN (2009) Nematodes which challenge global wheat production. In. 'Wheat: Science and Trade.' (Ed. BF Carver) pp. 171-187. (Wiley-Blackwell: Ames, Iowa).

Additional advances toward genetic resistance is especially important because it is the control strategy that is environmentally and socially most acceptable for minimizing yield losses caused by plant-parasitic nematodes.

The goal of this workshop is to build upon progress that has been made in the understanding and control of cereal nematodes since this series of workshops became launched with leadership by CIMMYT in 2009. The first workshop was in Antalya, Turkey and included more than 50 presentations from scientists in 22 countries. The third and fourth workshops were equally notable for the quality and quantity of presentations. The fifth workshop in Ankara, during 2015, will include 44 presentations from more than 70 participants from 20 countries. We express our appreciation to this global community of scientists who are dedicated to improving cereal productivity through greater understandings of nematode biology, interactions between nematodes and crops, and practices that can be used to reduce the economic impact of nematodes on productivity of small grain cereals.

Dr Abdelfattah A Dababat
ICARDA-CIMMYT Wheat Improvement Program, Ankara, Turkey

Dr Hafiz Muminjanov
Plant Production and Protection Officer, FAO Sub-regional Office for Central Asia, Ankara, Turkey

Dr Richard W Smiley
Professor Emeritus, Oregon State University, Pendleton, Oregon

ACKNOWLEDGEMENTS

The Fifth International Cereal Nematodes Initiative Workshop was coordinated by Dr Abdelfattah Dababat and could not have been successful without the assistance and support of many, in particular Prof Dr Richard Smiley for editing papers, providing images, and compiling this book.

The Turkish Ministry of Food, Agriculture and Livestock, General Directorate of Agricultural Research and Policies (GDAR), especially Dr Masum Burak, Director General, Dr Ali Osman Sari, Deputy Director General, and Dr Isa Ozkan, Head of Field Crops Research Department, for hosting the workshop in Ankara – Turkey and for providing their valuable support to this workshop.

The editors sincerely thank Dr Hans J-Braun, CIMMYT Global Wheat Director, and Dr Alexei Morgounov, Head, the International Maize and Wheat Improvement Center (CIMMYT) for being the main organizer of this workshop.

Special thanks go to Dr Hafiz Muminjanov, FAO, for his valuable contribution and support for this workshop. Thanks also go to Mr Timour Madibaev for the huge efforts he made for the layout and designing.

Contributions by donors were critical to the convening and success of this workshop. Donors included Syngenta, The Republic of Turkey's Ministry of Food, Agriculture and Livestock (MFAL), International Maize and Wheat Improvement Centre (CIMMYT), The Australian Grains Research and Development Corporation (GRDC), DuPont, The Plant Breeders Sub-Union of Turkey (BISAB), The Food and Agriculture Organization (FAO), and Dikmen Company. All are gratefully acknowledged for their support.

The local organizing committee of this workshop, Prof Halil Elekçioğlu (Cukurova University, Adana), Dr Gul Erginbas-Orakci (CIMMYT), Dr Mustafa İmren (Bolu University), and Halil Toktay (Nigde University), are acknowledged for their contributions.

Mrs Jennifer M. Nelson, Program Manager, Global Wheat Program, CIMMYT, and Mrs Emilia Arredodo Fernandez, Finance Department, CIMMYT, Mexico, are highly acknowledged for their contributions to this workshop.

Mr Suleyman Erharat, CIMMYT, Turkey, is thanked for his valuable administration and logistical support.

Mr Michael Listman, Carelia Juarez Hidalgo, and Clyde Raymond Beaver from CIMMYT- Mexico for their great support.

Dr Abdelfattah A Dababat
ICARDA-CIMMYT Wheat Improvement Program, Ankara, Turkey

Dr Hafiz Muminjanov
Plant Production and Protection Officer, FAO Sub-regional Office
for Central Asia, Ankara, Turkey

Dr Richard W Smiley
Professor Emeritus, Oregon State University, Pendleton, Oregon

ACRONYMS

BISAB	The Plant Breeders Sub-Union of Turkey
CIMMYT	International Maize and Wheat Improvement Centre
FAO	Food and Agriculture Organization of the United Nations
GDAR	General Directorate of Agricultural Research and Policies
GRDC	Grains Research & Development Corporation
ICARDA	International Centre for Agricultural Research in Dryland Agriculture
IWWIP	International Winter Wheat Improvement Program
MFAL	The Republic of Turkey's Ministry of Food, Agriculture and livestock
SBP	Soil Borne Pathogens

ABSTRACT

The Fifth International Cereal Nematodes Initiative Workshop, held in Ankara, Turkey during 12-15 September 2015, is an update to the 1st International Cereal Cyst Nematodes Initiative Workshop held in 2009 in Antalya, Turkey.

The 5th International Cereal Nematodes Initiative Workshop involved more than 70 scientists from wheat and barley producing regions in 20 countries throughout Asia, Australia, Europe, North Africa and North America. Cereal nematodes are microscopic parasites that invade roots of wheat, barley, oats and other small grain cereals. The most important of these plant-parasitic nematodes occur in the genera Heterodera (cyst nematodes) and Pratylenchus (root-lesion nematodes).

Forty four papers in this volume cover: the history and status of cereal nematodes globally and regionally; research on morphological, genetic and ecological diversity; development and deployment of host resistance including development and applications of molecular technologies; and investigations into other strategies for reducing the magnitude of economic damage caused by cereal nematodes. Special emphasis is given to opportunities to develop and deploy integrations of sustainable management practices.

The papers provide valuable insights into the impacts of cereal nematodes and endeavors to provide sustainable management options for farmers. The impact of cereal nematodes in reducing crop yields and the efficiency of cropping systems ranges from severe in resource-limited cropping systems to minor in cropping systems where it is possible to integrate a broader range of rotation crops and resource-intensive inputs.

Unacceptable levels of economic loss continue to occur in many countries. International collaboration such as occurred in this workshop is required to ensure that appropriate genetic resources and technologies are developed, communicated and deployed.

DISCLAIMER

This volume contains papers voluntarily contributed by participants of the Fifth International Cereal Nematodes Initiative Workshop, 12-15 September 2015, Ankara, Turkey.

Information and views expressed in these papers are entirely those of the authors, whose papers have not been formally peer-reviewed to independently confirm the veracity of the content.

Although the editors have taken care to guide contributors, and have assisted in technical editing, nothing in the papers presented in this volume should be taken as representative of the views or recommendations of the publisher, the editors and/or the sponsors who provided support for this workshop and/or publication.

Mention of any proprietary product or service by the authors in this volume cannot be taken as constituting an endorsement and/or recommendation of that product or service.

First International Cereal Cyst Nematode Initiative Workshop
21-23 October 2009, Antalya, Turkey

Second International Cereal Cyst Nematode workshop
24 September 2010, Austria, Vienna

Third International Cereal Cyst Nematode Initiative Workshop
22-23 September 2012, Adana, Turkey

Fourth International Cereal Nematodes Initiative Workshop
22-24 August 2013, Beijing, China

COLOUR PLATES





Plate 1. Symptoms of wheat injury caused by the cereal cyst nematodes *Heterodera avenae* or *H. filipjevi*: A. winter wheat with an uneven stand height caused by *H. avenae*, B. patchy stand in winter wheat caused by a root-disease complex including *H. filipjevi* and *Rhizoctonia solani*, C,D. distinct patches caused by *H. avenae* in a single field of winter wheat, showing more intense yellowing on an eroded hilltop with shallow soil and lower soil fertility (C) than on the flatland with deeper, more fertile soil (D), E. shallow roots of spring wheat caused by adventitious wheat growth (knotting or witches' brooming) at points where *H. avenae* females established feeding sites, F. spring wheat roots with shallow, knotted roots caused by *H. avenae*. Photographs: A-E - Richard Smiley, USA; F - Honglian Li, China.

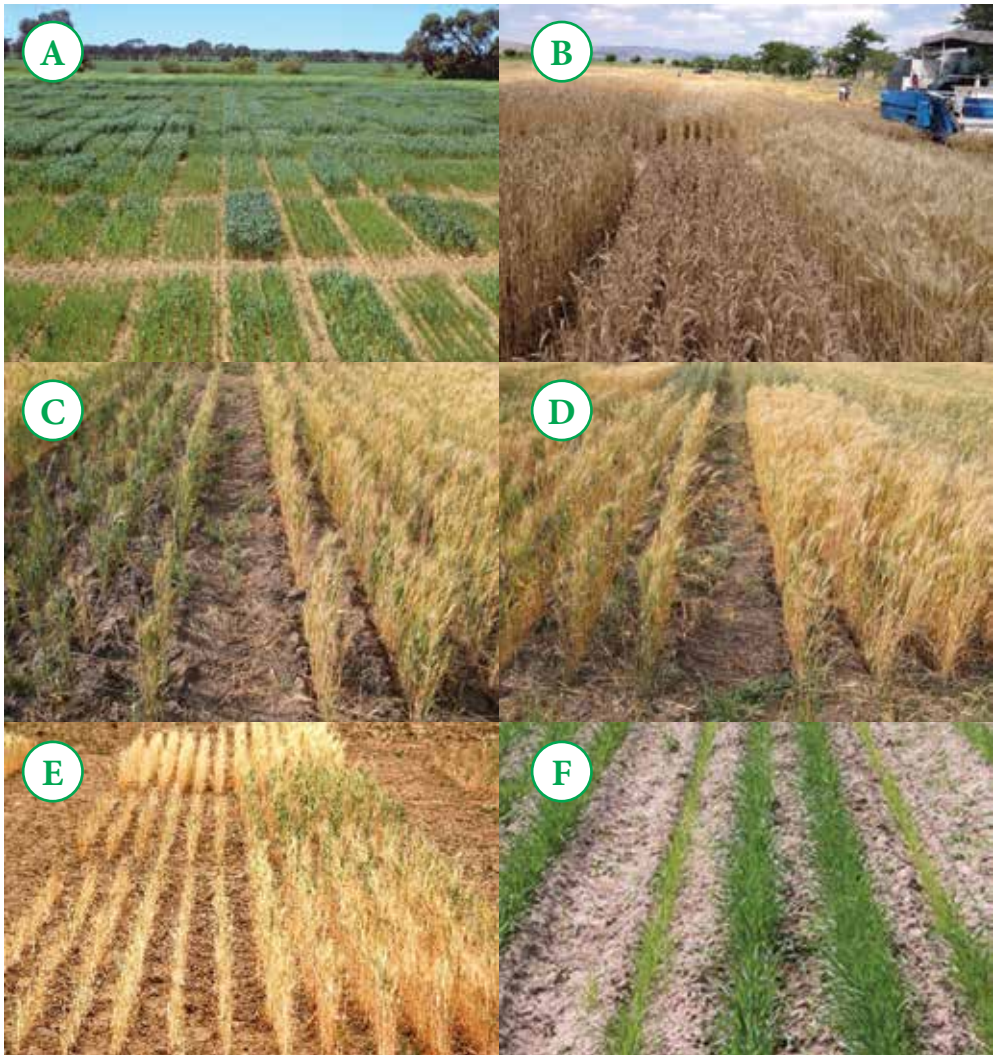


Plate 2. Screening wheat for resistance and tolerance to cereal cyst nematodes in fields naturally-infested by *Heterodera avenae* or *H. filipjevi*: A,B. differential effects of *H. avenae* (A) *or H. filipjevi* (B) on growth of wheat varieties in naturally-infested fields in South Australia and Turkey; C,D,E. identical varieties of wheat showing improved growth when aldicarb was applied while planting (right) compared to no application of nematicide (left) in genetic tolerance trials in fields that were naturally-infested with *H. avenae* in the USA (C,D) or in Tunisia (E); F. combined stresses from *H. avenae* and nutrient deficiency in spring wheat where an equipment malfunction caused starter fertilizer to be banded below the center two rows, as was desired, but not below the two outer rows after fertilizer tubes plugged while planting; Photographs: A - Sharyn Taylor, Australia; B - Abdelfattah Dababat, Turkey; C,D,F - Richard Smiley, USA; E - Ian Riley, Australia.

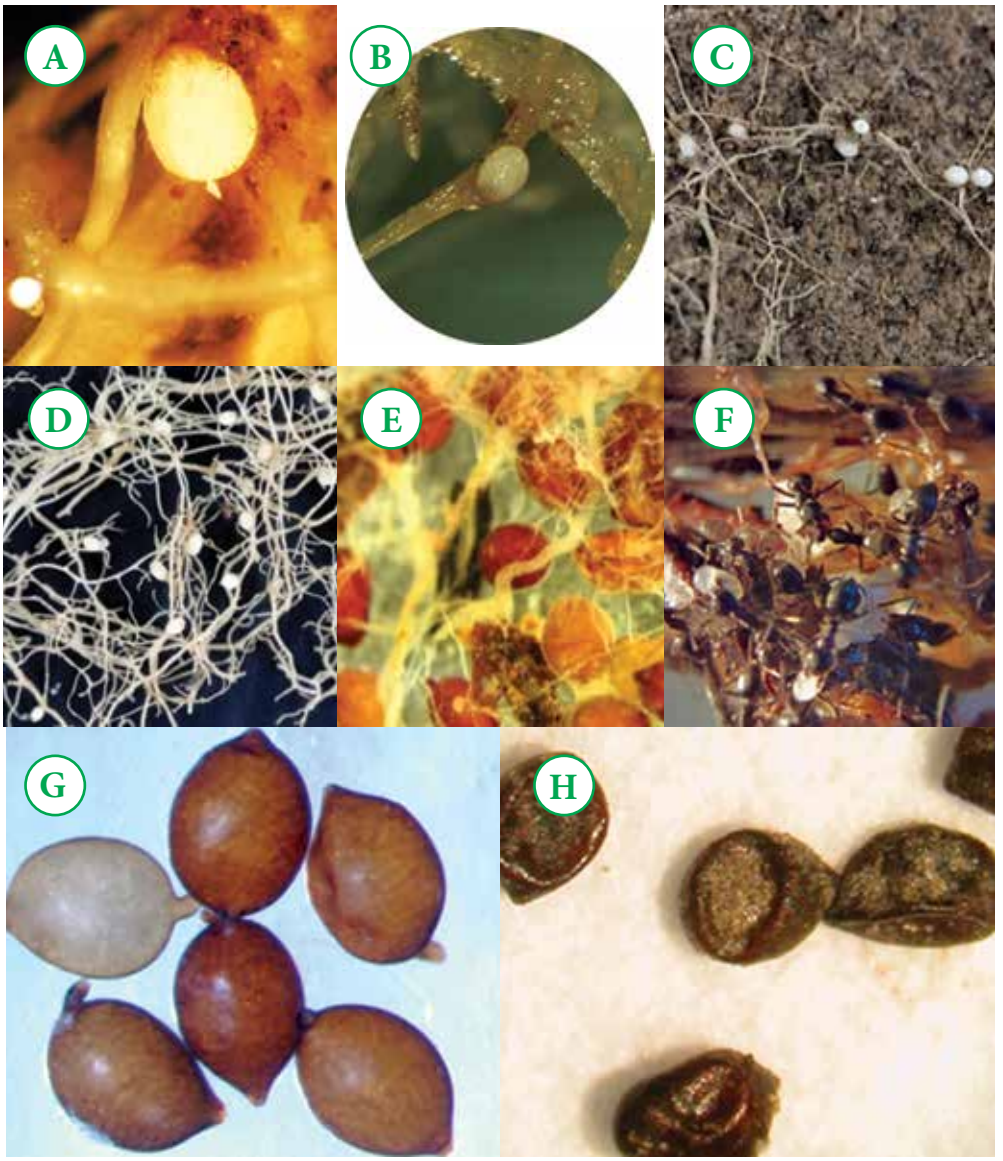
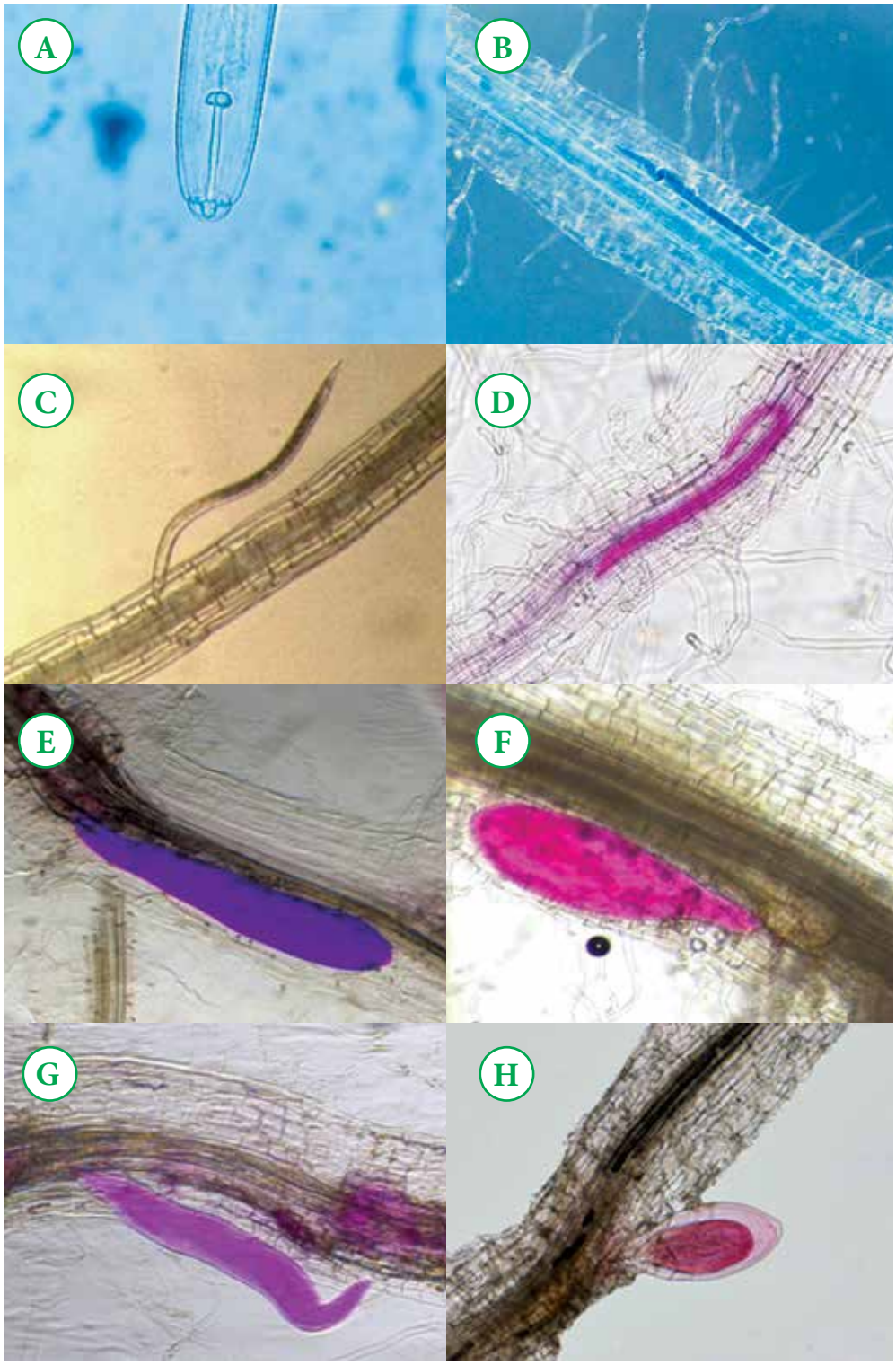


Plate 3. Cereal cyst nematodes *Heterodera avenae* (A-D,H) and *H. filipjevi* (E-G): A-D. swollen white females on washed roots (A,B) or behind a glass plate (C,D) in a controlled growth chamber experiment, E,F. young cysts amongst washed roots (E) and swollen white females being fed upon by ants (F), G. newly-produced cysts extracted from soil, H. old cysts extracted from a naturally-infested soil. Photographs: A - Hugh Wallwork, Australia; B,H - Guiping Yan, USA; C,D - Mohamed Baklawa, Egypt; E-F Samad Ashrafi and Abdelfattah Dababat, Turkey; G –Shree Pariyar and Abdelfattah Dababat, Turkey.


A
B
C
D
E
F
G
H

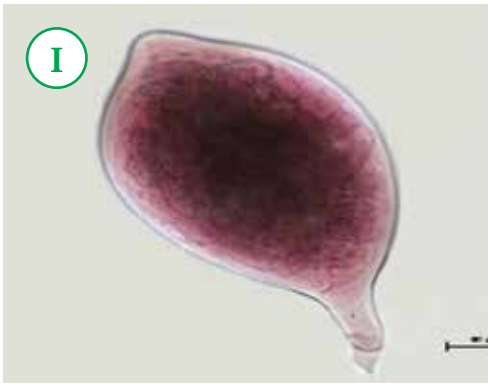


Plate 4. Cereal cyst nematodes *H. avenae* (A,B) and *Heterodera filipjevi* (C-G): A. head and stylet of a 2nd-stage juvenile, B. stained 2nd-stage juvenile migrating in the cortex towards the root tip, C. 2nd-stage juvenile probing root surface at two days post-inoculation (dpi), D. 2nd-stage juvenile inside the root at 3 dpi, E. 3rd-stage female at 10 dpi, F. 4th-stage female at 15 dpi, G. male in root at 15 dpi, H. developing female, I. fully-developed female. Photographs: A,B – Hugh Wallwork, Australia; C-G – Shree Pariyar and Abdelfattah Dababat, Turkey; H,I - Hai Yan Wu, China.

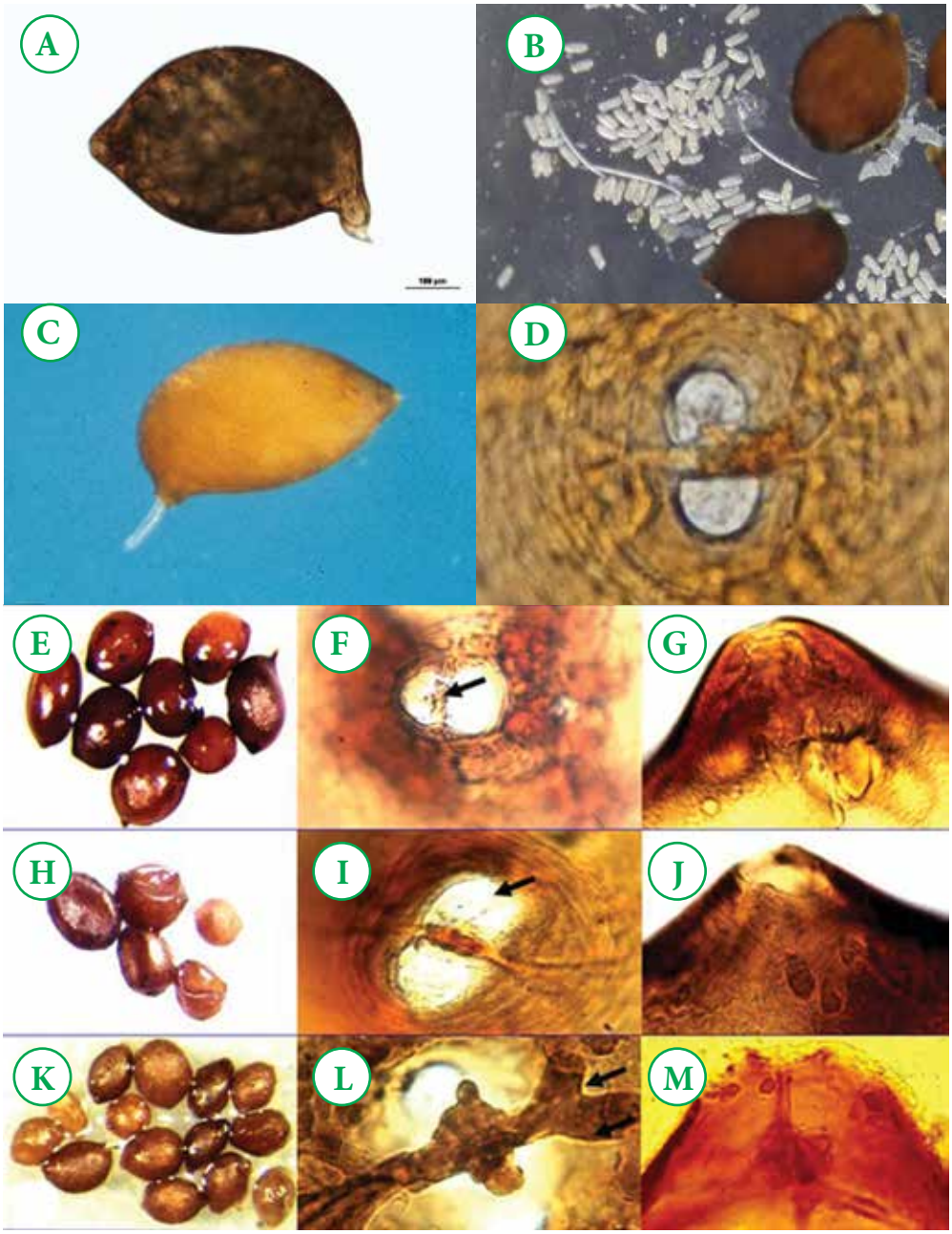


Plate 5. *Heterodera avenae* (B-G), *H. filipjevi* (H-J) and *H. latipons* (K-M); showing an encysted female with eggs visible through the body wall (A), disrupted cysts showing eggs and 2nd-stage juveniles (B), a 2nd-stage juvenile emerging (C) through the fenestra (D) of the vulval cone, and cysts, underbridge and vulval cone of each species. Photographs: A - Hai Yan Wu, China; B – Najoua Namouchi-Kachouri, Tunisia; C – John Lewis, Australia; D – Guiping Yan, USA; E-M – Hussam Abiedo, Syria.

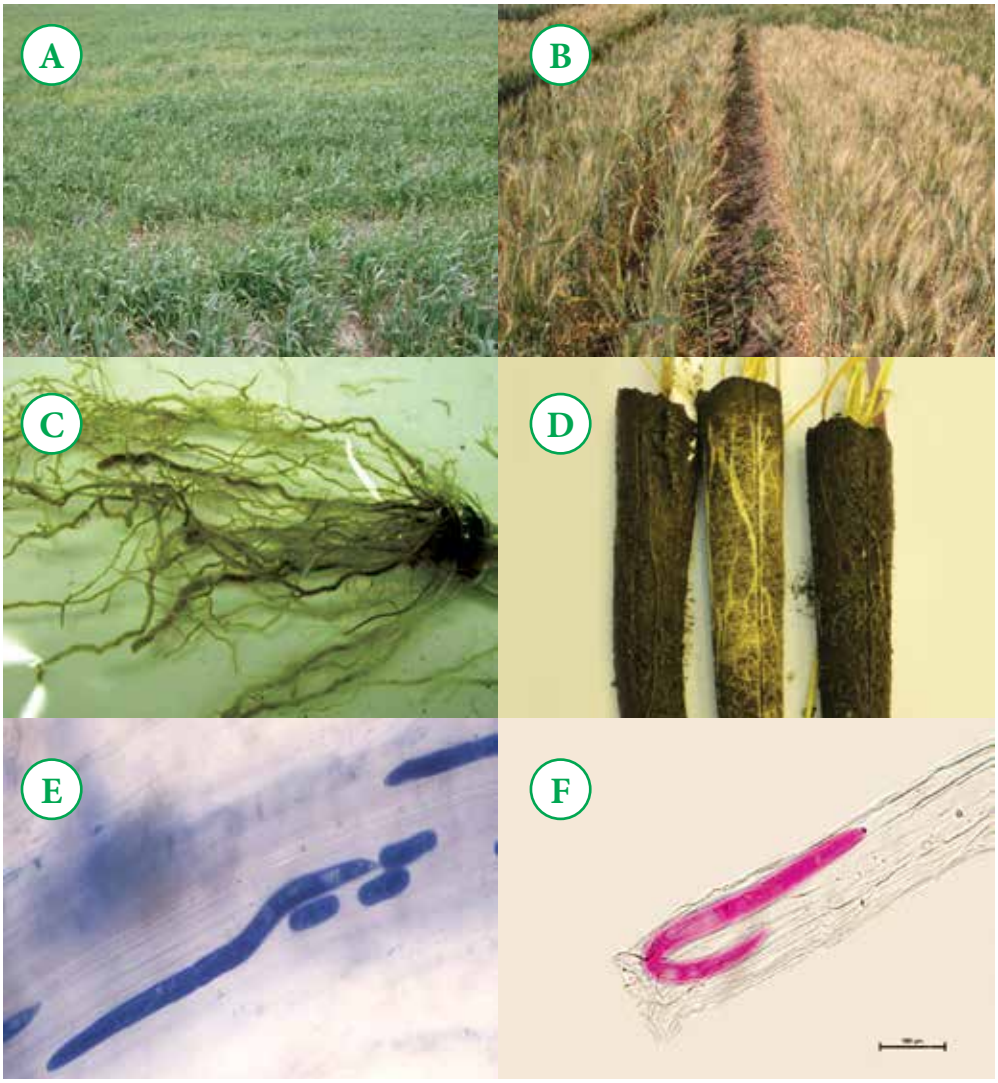


Plate 6. The root-lesion nematodes *Pratylenchus neglectus* and *P. thornei* on wheat: A. winter wheat in a field infested with high densities of *P. thornei* + *P. neglectus* (2:1 ratio), *Pythium* spp., and *Pasteuria* spp.; B. spring wheat tolerance trial in a field naturally-infested by *P. neglectus*, with drill strips either treated with aldicarb (right) or not treated at the time of planting (left); C. wheat roots darkened by lesions caused by *P. neglectus*; D. spring wheat growing in cones of soil infested with *P. neglectus*, showing two susceptible commercial varieties (left and right) and a resistant Iranian landrace wheat ('AUS28451') being used in wheat breeding programs (each variety produced substantial foliar growth and had white roots emerging from the bottoms of the cones); E. adult female and eggs of *P. neglectus* stained blue in the root cortex; F. an adult *Pratylenchus* spp. in a root. Photographs: A,B - Richard Smiley, USA; C,E - Vivien Vanstone, Australia; D - Alison Thompson, USA; F - Hai Yan Wu, China.

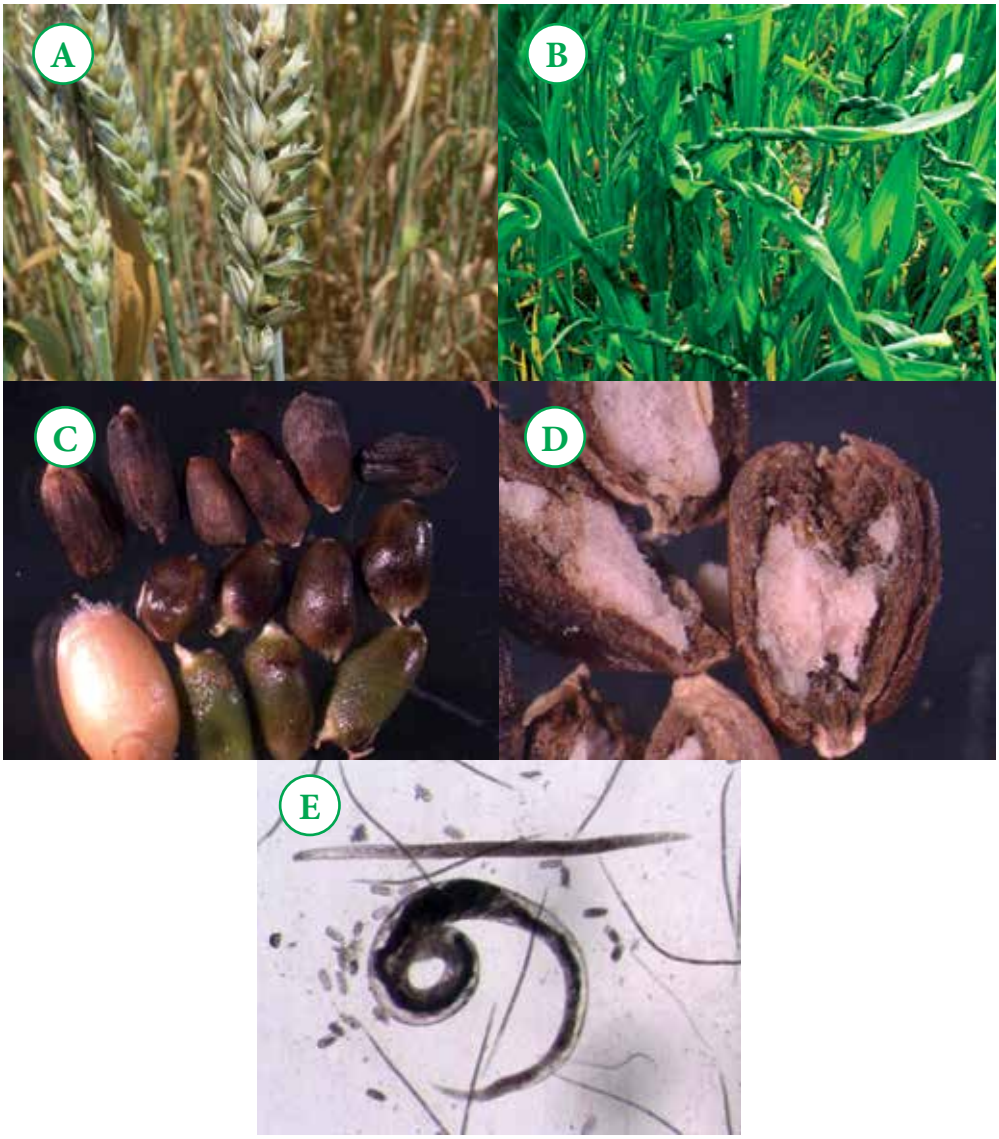


Plate 7. The seed gall nematode *Anguina tritici* on wheat in Turkey: A. seed galls in wheat heads; B. rolling, curling or spiraling distortions of wheat foliage; C. mature dark purplish-colored seed galls and developing greenish-colored seed galls, as compared to a healthy wheat kernel; D. central cavity of seed galls containing masses of 2nd-stage juveniles; E. eggs, 2nd-stage juveniles and adult life stages. Photographs: Adnan Tulek, Turkey.

PART 1

GLOBAL STATUS

FAO ACTIVITIES ON PLANT PRODUCTION AND PROTECTION IN CENTRAL ASIA

H. MUMINJANOV*

Dr Hafiz Muminjanov, FAO Sub-regional Office
for Central Asia, Ankara, Turkey,
*Corresponding author email: Hafiz.Muminjanov@fao.org

Central Asia is a sub-region in which most countries are in transitions from planned to market economies. In general, these countries are predominately agricultural economies and they have high potential for development and for achieving food security.

Trends on population growth in the Central Asia countries demonstrates that the sub-regional challenges are more serious than overall global challenges. During the last 50 years the population increased by 2.5 times and the birth rate in the most of the countries still remains high. With the purpose of responding to the food demand, an intensive land reclamation has been taking place and has resulted in expansion of irrigated crop areas of up to three times, and has led to environmental problems. However the area is limited and further expansion of irrigated lands is not possible. The area under arable land per capita is declining, and competition and conflicts for land and water are raising.

Based on official statistics, crop production in general is increasing, but the focus of this paper is on production of wheat, which is growing only slowly. Wheat is the main staple crop in all countries in the sub-region. The consumption rate of wheat is therefore the highest in the world, at over 200 kg/year/capita. On the other hand, production of wheat per capita is slightly declining and wheat yield is remaining very low.
In addition to this, the serious infestation of quarantine and transboundary

* Muminjanov H (2015) FAO activities on plant production and protection in Central Asia. In 'Nematodes of Small Grain Cereals: current status and research.' (AA Dababat, H Muminjanov and RW Smiley) pp. 3-6. (FAO: Ankara, Turkey)

pests and diseases severely damage crop yields. Every year the farmers observe damage caused by locusts, rusts, nematodes, gypsy moth, American white fly, and other dangerous pests and diseases.

To control pests and diseases the farmers apply pesticides but the registration and quality control system of pesticides and pesticide application equipment is not in place. That imposes serious risks to the environment and to human health. Thus, very often illegal and low-quality pesticides are used. Another problem is obsolete pesticides. The countries in the sub-region mostly inherited from the former Soviet Union a huge amount of obsolete pesticides; about 200,000 tones or 40% of world reserves. These pesticides require proper management and elimination.

The sub-region counts as one of the centers of origin and diversity of several crop species. We are proud that the sub-region is really blessed with a rich diversity of plant species, however the efficiency of conservation and utilization of plant genetic resources for food and agriculture is very low. An informal seed system dominates over a formal system, and use of high-quality seed of superior varieties requires further development of seed quality control and certification.

In addition to the problems stated above, the prices of inputs (fuel, fertilizer, seed, pesticides, etc.) are rising, the yields of main crops are affected by climate change, there is a lack of institution capacity and legislation, there is a generation gap and a lack of qualified experts, there is a lack of modern knowledge and technology, and there are also other challenges the farmers are currently facing.

To respond to these challenges, the FAO has developed a paradigm "Save and Grow" promoting the theme of growing more with less. This is a guideline for policymakers that uses a holistic approach for solving the problems, and developing efficient and sustainable utilization of ecosystem services and inputs while conserving and enhancing natural resources and reducing environmental pollution. "Save and Grow" provides the systems that are adaptable to specific conditions, locations and scales.

With the purpose of responding to the challenges on crop production in the sub-region "Save and Grow" is translated in four areas.

1. The first area is intensification and diversification of cropping systems that foresees the provision of technical assistance to the countries in developing their policies and strategies on sustainable crop intensification and diversification of cropping systems, organic farming, preparedness for drought and climate change mitigation, promotion of conservation agriculture, and sustainable pasture and grassland management.
2. The second area for cooperation is strengthening capacity of the National Plant Protection Organizations. In this regard FAO can cooperate with the countries and provide support in strengthening policies on plant protection, implementation of international conventions and standards (e.g., ISPM) and harmonization of regional phytosanitary legislation. Promotion of integrated pest management (IPM) by developing Farmer Field Schools is a comparative advantage of FAO that was implemented in different countries around the world. FAO will continues support for developing capacities to control transboundary pests and diseases (locusts, wheat rusts) and carrying out the monitoring, surveillance and control of wheat rust (e.g. SMS monitoring). The key activities in both areas will be the training of young plant breeders and pathologists in cooperation with the CGIAR centers (CIMMYT and ICARDA) and the development of training programs and curriculums.
3. The third area is focused on strengthening national frameworks for pesticide management, covering improvement of the national systems for pesticide registration, testing, quality control, management, inventory and disposal of obsolete pesticides, adoption and promotion of integrated pest management IPM, enhancing the membership of countries in the international organizations, and implementation of international conventions like IPPC and Rotterdam Conventions and the International Code of Conduct on Pesticide Management.
4. The fourth direction is providing assistance in better management of plant genetic resources (PGR) and seed systems, focusing on improving conservation, efficient utilization of plant genetic resources, and seed sector development, especially formulation and implementation of seed policies and harmonization of the national seed legislation with the internationally accepted norms and standards.

The above-listed activities are implemented intensively under the regular program. In addition, a number of crop related projects at the sub-regional and

national levels were implemented focusing on Country Programming Frameworks (CPFs) of Central Asian countries, built with the cooperation with FAO. Besides, strong cooperation and partnerships have been established between the CGIAR Centers and other public and civil society organizations in successfully implementing the projects and the program in the sub-region.

The cornerstone of FAO's technical assistance in plant production and protection is safeguarding and intensifying crop production. In this sense, for a strong and healthy harvest, FAO-SEC helps farmers incorporate innovative techniques, such as conservation agriculture and integrated pest management, into traditional farming practices. Other priorities include preventing and responding to transboundary pest outbreaks, as well as mitigating the damage caused by outdated pesticides.

There are several FAO projects on conservation agriculture, organic agriculture, and integrated pest management. Most of the projects in these topics are based on strengthening the capacities of the national plant protection organizations in developing their policies and strategies.

During the implementation of these projects, the majority of the activities consisted of providing technical assistance for drafting national legislation and national strategy plans for the countries, for organizing several trainings, study tours, workshops, and field days for farmers and/or technical staff, and for establishing demonstration fields of identified new crops using innovative techniques and new technologies for the promotion of intensified crop production, organic and conservation agriculture in Central Asia. FAO also places a high importance on supporting participation of the national specialists from the sub-region to attend the international trainings and conferences and on improving their capacities and developing translations of books, manuals, guidelines and normative documents on conservation agriculture and organic agriculture in Russian or in national languages of Central Asian countires.

USING FACTOR ANALYSIS AND LATENT REGRESSION PLOTS TO UNDERSTAND GENOTYPE BY ENVIRONMENT INTERACTION IN FIELD AND GLASSHOUSE EXPERIMENTS TO DETERMINE THE RESISTANCE OF VARIETIES TO *PRATYLENCHUS* IN AUSTRALIA

BEVERLEY J. GOGEL*

University of Adelaide, School of Agriculture, Food and Wine,
PMB 1, Glen Osmond SA 5064, Australia.
*Corresponding author email: beverley.gogel@adelaide.edu.au

SUMMARY

In this paper we describe a multi-environment trial (MET) data set for varietal resistance to *Pratylenchus thornei* that spans both the agronomic regions of interest and a series of glasshouse experiments. The aim of this work is to determine the extent of genotype by environment interaction (GxE) both within and between the different trial types (field and glasshouse). We describe the factor analytic (FA) mixed model approach that is widely used to model GxE interaction in Australian plant breeding programs. The key feature of the approach is its utility at identifying and explaining GxE, through the link of FA models with principal component analysis. Exploiting this link leads to the use of so-called latent regression plots which have recently been used for the analysis of the National Variety Trial (NVT) system data-sets in Australia (Smith *et al.* 2015). We present preliminary results for an FA analysis of the example data set.

* Gogel BJ (2015) Using factor analysis and latent regression plots to understand genotype by environment interaction in field and glasshouse experiments to determine the resistance of varieties to *Pratylenchus* in Australia. In 'Nematodes of Small Grain Cereals: current status and research.' (AA Dababat, H Muminjanov and RW Smiley) pp. 7-16. (FAO: Ankara, Turkey).

INTRODUCTION

Pratylenchus thornei and *Pratylenchus neglectus* are the two most important root lesion nematodes (RLN) affecting broad acre crops in the southern cropping region of Australia. Reliable information on the affect of new or emerging cereal varieties on RLN population densities (resistance) and their ability to yield in the presence of significant population densities (tolerance) is essential to assist growers in making informed decisions on varietal selection and in planning their cropping rotations.

Resistance classifications have traditionally been determined using glasshouse data. In these trials, environmental factors such as temperature and soil moisture are controlled, resulting in greater consistency in the ranking of cultivars even across diverse RLN resistance screening procedures (Sheedy *et al.* 2014). Recently resistance ratings have been determined for a series of field trials established in the southern growing region to evaluate varieties for tolerance to RLN. We therefore have the opportunity to compare resistance ratings in a MET analysis where the environments span both field and glasshouse trials across sites and seasons.

In Australia, most plant breeding MET data are analyzed using the FA mixed model approach of Smith *et al.* (2005). This model allows a separate spatial covariance structure and error variance for each site (synonymous with trial and environment). It also accommodates a separate genetic variance for each site (GxE due to changes in the *scale* of the genetic effects across sites) and heterogeneity of genetic covariance between pairs of sites (GxE due to changes in the ranking, or *crossover*, of the genetic effects across sites). The FA model has a multiplicative form that has strong links with principal component analysis and has been found to be particularly effective in explaining GxE in plant breeding data. The key outputs of an FA analysis include accurate predictions of the GxE effects, the estimated genetic correlation matrix with a heat-map representation for visualizing the GxE and a set of latent regression plots. These are a graphical display of the rotated factors associated with the multiplicative model and offer an effective means of investigating varietal stability across environments. We use the combined glasshouse and field (GF) resistance data for *P. thornei* to demonstrate the FA technology.

METHODS

Motivating example: The GF MET dataset for *P. thornei* comprised 10 field (F) and 9 glasshouse (G) trials between 2010 and 2014. They were conducted in the 4 states New South Wales (NSW: 4F), Queensland (QLD: 5G), South Australia (SA: 4G, 3F) and Victoria (VIC: 3F), where the numbers in brackets indicate the number of field and/or glasshouse trials. A summary of the trials is presented in Table 1.

Trial designs: The SA and QLD glasshouse trials were designed as randomized complete blocks (RCBs) of either 3 or 4 replicates, with layout details as presented in the table. All soil samples that go through the South Australian Research and Development Institute (SARDI) Molecular Diagnostics Center (in this case all SA, NSW and VIC field and glasshouse samples) go through a three stage process: 1. Glasshouse/field trial, 2. DNA extraction & storage 3. Polymerase Chain Reaction (PCR) processing. Separate randomizations were generated for both the first and second stages for the two SA glasshouse trials in 2012. However, second stage randomization was not continued beyond 2012 as analyses of these first two trials indicated little random variation associated with this stage. The NSW field trials were also arranged in an RCB layout. The SA and VIC field trials were established to evaluate new varieties for tolerance to RLN but were also scored for initial and final nematode DNA. They were arranged as large split-plot trials in which varieties were sown to pairs of adjacent plots of high and low nematode densities produced by growing resistant and susceptible varieties in the previous year.

Crop type: For all but three of the four NSW field trials, the varieties tested at a site were a mix of barley (B), durum (D), triticale (T) and wheat (W) varieties. Specifically, the SA glasshouse trials were a mix of B, D, T and W, the QLD glasshouse trials contained mostly W but some B, D and T (no T in 2013 and 2014), CNSW2013NarWht included both W and D while the remaining three NSW field trials contained W only, and the SA and VIC field trials contained a mix of B, D and W. There was mostly reasonable varietal connectivity across sites.

Data collection: For the SA and VIC field trials a bulk of 20 cores per plot at a depth of 10 cm was processed through the SARDI MDC using a real-time TaqMan PCR system with a species specific primer. For the NSW trials, a bulk of either 10 cores per plot (depth 0-30 cm) or 20 cores per plot (depth

0-15 cm) was processed. For the SA glasshouse trials, individual pot data was processed through the SARDI MDC while for the QLD glasshouse trials actual nematode numbers in the roots and soil of each cultivar were recorded. The final analysis trait for all trials was *P. thornei* per gram soil.

Table 1. Summary of trials in the glasshouse and field MET data set for *P. thornei* resistance

State	Year	Trial name	G/F	Design layout	rows	cols	reps	repsB	vars	plots	mean
NSW	2012	CNSW2012Bumw	F	RCB	27	6	3	-	53	162	52.50
NSW	2012	CNSW2012NSmw	F	RCB	24	8	4	-	47	192	2.47
NSW	2013	CNSW2013NarWht	F	RCB	36	6	3	-	24	216	4.45
NSW	2013	CNSW2013Wong	F	RCB	21	6	3	-	38	126	11.76
QLD	2010	CQLD2010	G	RCB	36	13	3	-	156	468	275.69
QLD	2011	CQLD2011	G	RCB	12	39	3	-	156	468	97.96
QLD	2012	CQLD2012	G	RCB	11	39	3	-	143	429	11.15
QLD	2013	CQLD2013	G	RCB	12	36	3	-	144	432	86.50
QLD	2014	CQLD2014	G	RCB	10	33	3	-	110	330	57.04
SA	2012	CSA2012thorn1	G	RCB 2S	18	12	3	-	36	144	21.60
SA	2012	CSA2012thorn2	G	RCB 2S	18	12	3	-	36	144	13.22
SA	2013	CSA2013thorn	G	RCB	15	20	4	-	72	300	6.65
SA	2014	CSA2014thorn	G	RCB	15	20	4	-	72	300	10.64
SA	2011	R2011PTMinn	F	Split plot	68	5	5	-	33	340	50.45
VIC	2012	R2012PTBany	F	Split plot	56	6	6	-	28	336	54.66
SA	2012	R2012PTSBay	F	Split plot	66	5	5	-	33	330	21.49
VIC	2013	R2013PTBany	F	Split plot 2B	60	6	6	6	30	360	101.51
VIC	2014	R2014PTBany	F	Split plot 2B	60	5	5	5	30	300	49.32
SA	2014	R2014PTSBay	F	Split plot	80	5	5	-	40	400	15.13

G/F indicates if the trials were set up in the glasshouse (G) or in the field (F), rows = total number of rows, cols = total number of columns, reps = total number of blocks, repsB = total number of blocks when a second block structure was generated, vars = total number of varieties, plots = total number of plots (of pots for glasshouse trials) and mean = mean of the raw DNA/g soil. RCB indicates a randomized complete block design, 2S indicates when randomization was undertaken for both the glasshouse and DNA extraction stages and 2B indicates designs with 2 block structures.

Statistical methods: Use of the FA model for Australian plant breeding MET data is well documented (Smith *et al.* 2001, 2005, Kelly *et al.* 2007, Beeck *et al.* 2010 and Cullis *et al.* 2010). A more recent application is for the analysis of the NVT system in Australia, see Smith *et al.* (2015) who provide a comprehensive overview of the both the underlying theory and its application for NVT. Let $\boldsymbol{y}$ be the combined $(n \times 1)$ vector of data for the trait of interest across t trials, and consider the case of m varieties. The model for $\boldsymbol{y}$ can be written as

$$\boldsymbol{y} = \boldsymbol{X\tau} + \boldsymbol{Z_g u_g} + \boldsymbol{Z_p u_p} + \boldsymbol{e}$$

where $\boldsymbol{\tau}$ is a vector of fixed effects with associated design matrix $\boldsymbol{X}$ (assumed to have full column rank), $\boldsymbol{u_g}$ is the $(mt \times 1)$ vector of genetic effects with associated design matrix $\boldsymbol{Z_g}$, $\boldsymbol{u_p}$ is a vector of non-genetic (or peripheral) random effects with associated design matrix $\boldsymbol{Z_p}$ ($\boldsymbol{u_p}$ includes terms associated with the blocking structure of each trial) and $\boldsymbol{e}$ is the combined $(n \times 1)$ vector of residual errors. In the simplest case, $\boldsymbol{\tau}$ is the vector of t environment means. We assume that $\boldsymbol{u_g}$, $\boldsymbol{u_p}$ and $\boldsymbol{e}$ are mutually independent and have a multivariate normal distribution with zero means. The variance matrix for $\boldsymbol{u_p}$ is given by $\boldsymbol{G_p} = \oplus_{k=1}^{b} \sigma_{p_k}^2 \boldsymbol{I}_{q_k}$ where b is the number of components in $\boldsymbol{u_p}$ and q_k is the number of effects in $\boldsymbol{u}_{p_k}$. The variance matrix for the residuals is assumed to be $\boldsymbol{R} = \oplus_{j=1}^{t} \boldsymbol{R}_j$ where $\boldsymbol{R}_j$ is the variance matrix for the residuals for the j^{th} site. We assume that the variance matrix of the GxE effects is given by $\text{var}(\boldsymbol{u_g}) = \boldsymbol{G_e} \otimes \boldsymbol{I}_m$ where $\boldsymbol{G_e}$ is a $(t \times t)$ symmetric positive (semi)-definite matrix that is often referred to as the *between environment genetic variance matrix*. One of the simplest forms for $\boldsymbol{G_e}$ is a diagonal form, that is, $\boldsymbol{G_e} = \oplus_{j=1}^{t} \sigma_{g_j}^2$ where $\sigma_{g_j}^2$ is the genetic variance for environment j. In this case the variety effects are assumed to be independent between the environments. The most general model for $\boldsymbol{G_e}$ is the unstructured form that specifies a separate genetic variance for each site and a separate genetic covariance for each pair of sites. Unfortunately, this form is difficult to estimate for even moderately large numbers of trials and is rarely used in practice. The multiplicative mixed model of Smith *et al.* (2001) uses FA models for GxE interaction while adjusting for spatial field trend. It has been found to provide an effective and informative

approximation to the unstructured form for the genetic variance matrix (Kelly *et al.* 2007). The FA model accounts for the genetic covariances between environments in terms of a small number of hypothetical factors. If k denotes the number of factors, then the form of the model for the effect of variety i in environment j can as be written as

$$u_{ij} = \lambda_{1j} f_{1i} + \lambda_{2j} f_{2i} + \ldots + \lambda_{kj} f_{ki} + \delta_{ij}$$

where f_{ri} is the value (also called a *score*) of the r^{th} hypothetical factor $(r = 1, \ldots, k)$ for variety i and λ_{rj} is the coefficient (also called a *loading*) for environment j. The factors are usually assumed to be independent with unit variance so that $f_{ri} = 1$. This model is also of the form of a multiple regression of the variety effects for an environment on a set of environmental covariates (*loadings*) with a separate slope (*score*) for each variety. The feature that distinguishes the FA model from an ordinary regression is that both the covariates and the slopes are estimated from the data. The δ_{ij} are considered to be *genetic regression residuals* since they represent a lack of fit of the regression. The model is written more concisely as

$$\boldsymbol{u_g} = (\Lambda \otimes \boldsymbol{I_m})\boldsymbol{f} + \boldsymbol{\delta}$$

where Λ is the $(t \times k)$ matrix of loadings, $\boldsymbol{f}$ is the $(mk \times 1)$ vector of scores and $\boldsymbol{\delta}$ is the $(mt \times 1)$ vector of genetic regression residuals. The vectors of random effects $\boldsymbol{f}$ and $\boldsymbol{\delta}$ are assumed to be mutually independent and distributed as multivariate normal with zero means. The variance matrices are assumed to be $\text{var}(\boldsymbol{f}) = \boldsymbol{I}_{mk}$ and $\text{var}(\boldsymbol{\delta}) = \boldsymbol{\psi} \otimes \boldsymbol{I_m}$ where $\boldsymbol{\psi}$ is a $(t \times t)$ diagonal matrix with a variance (called a specific variance) for each environment. Finally, these assumptions lead to a variance matrix for $\boldsymbol{u_g}$ given by

$$\text{var}(\boldsymbol{u_g}) = (\Lambda\Lambda' + \boldsymbol{\psi}) \otimes \boldsymbol{I_m}$$

so that the between environment genetic variance matrix is

$$\boldsymbol{G_e} = \Lambda\Lambda' + \boldsymbol{\psi}.$$

We fit FA models using the ASReml-R software (Butler *et al.* 2009) within R (R Core Development Team, 2013). The variance parameters are estimated using residual maximum likelihood (REML). For the FA model,

the variance parameters are the estimated loadings and specific variances. This gives the estimated between environment genetic variance matrix

$$\widehat{\boldsymbol{G}}_{\boldsymbol{e}} = \widehat{\boldsymbol{\Lambda}}\widehat{\boldsymbol{\Lambda}}' + \widehat{\boldsymbol{\psi}}$$

which can be converted to the estimated between environment genetic correlation matrix $\widehat{\boldsymbol{C}}_{\boldsymbol{e}}$. This is a key output of the FA analysis and gives information on crossover GxE. A heat map representation of $\widehat{\boldsymbol{C}}_{\boldsymbol{e}}$ provides a visual representation to aid interpretation of the GxE. When $k > 1$, the matrix of environmental loadings ($\boldsymbol{\Lambda}$) is non-unique and the loadings are rotated to a principal components solution. Under this rotation the first factor accounts for the maximum amount of GxE in the data, the second factor represents the second largest amount of GxE, and so on. The latent regression plots present the contribution of each factor graphically and offer an effective means of investigating the response of each variety to changes in environment, that is, varietal stability across environments. The estimated variance parameters are used to form empirical best linear unbiased estimates of the fixed effects and empirical best linear unbiased predictors of the random effects. For the FA model, the random terms are the factor scores and genetic regression residuals. In practice, we commence with fitting an FA1 model for GxE and progress from there to higher order models. The residual maximum likelihood ratio test (REMLRT) can be used to compare a sequence of FA models. In practice we decide on k using the REMLRT in combination with a less formal assessment of the percentage of variance explained by the FAk, and we stop fitting higher order k when the percentage of genetic variance accounted for by the FA model has reached an acceptable level (typically > 85%).

RESULTS

The GF MET dataset for *P. thornei* was more complicated than a standard MET data set given the two stage structure of the two 2012 glasshouse trials for SA and the six SA and VIC split-plot field trials which were each considered to represent two (sub)environments characterized as having low and high initial nematode densities, respectively. We therefore constructed a new environment factor, *Enviro,* coded for the SA and VIC field trials as the trial

name appended with either *Low* or *High* for plots with low and high nematode densities respectively, and as the trial name for the remaining trials. We also constructed a *Type* factor with levels corresponding to the four crop types B, D, T and W. Using these factors, we fitted a linear mixed model that included a fixed environment by type interaction term, random model terms for the blocking structure at each site, an initial DNA density covariate for the 12 SA and VIC field environments and additional site-specific fixed and/or random model terms as required. A separate spatial covariance structure was fitted for the residual errors at each site for all but the two SA glasshouse trials in 2012. For these two trials, we fitted a spatial covariance structure to the glasshouse row by range interaction effects and we specified a separate residual (DNA plate well) error variance. We commenced with a diagonal variance structure for the GxE interaction effects which is equivalent to separate analyses for each environment. An FA1 analysis was a significant improvement over the diagonal model and we progressed from there to an FA4 analysis which accounted for 97% of the GxE variance. Figure 1 is a heat map representation of the estimated genetic correlation matrix ordered on the basis of a dendrogram constructed using agglomerative hierarchical clustering. In the heat map, environments that are more highly correlated are situated closer together while environments with lower genetic correlation are further apart. The colours indicate the level of correlation, from dark blue for low genetic correlation to orange/red for high positive correlation. There are two distinct groups of environments as indicated by the two predominantly orange/red blocks in the lower left and upper right partitions of the map and yellow/green off-diagonal blocks. With the exception of the QLD glasshouse trial in 2012 (CQLD2012), this represents a separation of the field trials (lower left partition) from the glasshouse trials (upper right partition). Latent regression plots provide further evidence of the distinction between the trial types (glasshouse and field). They reflect both types of GxE, that is, changes in the scale of the genetic effects across environments and cross-over GxE. They also offer an effective means of investigating varietal stability across environments. Further details will be presented in the talk.

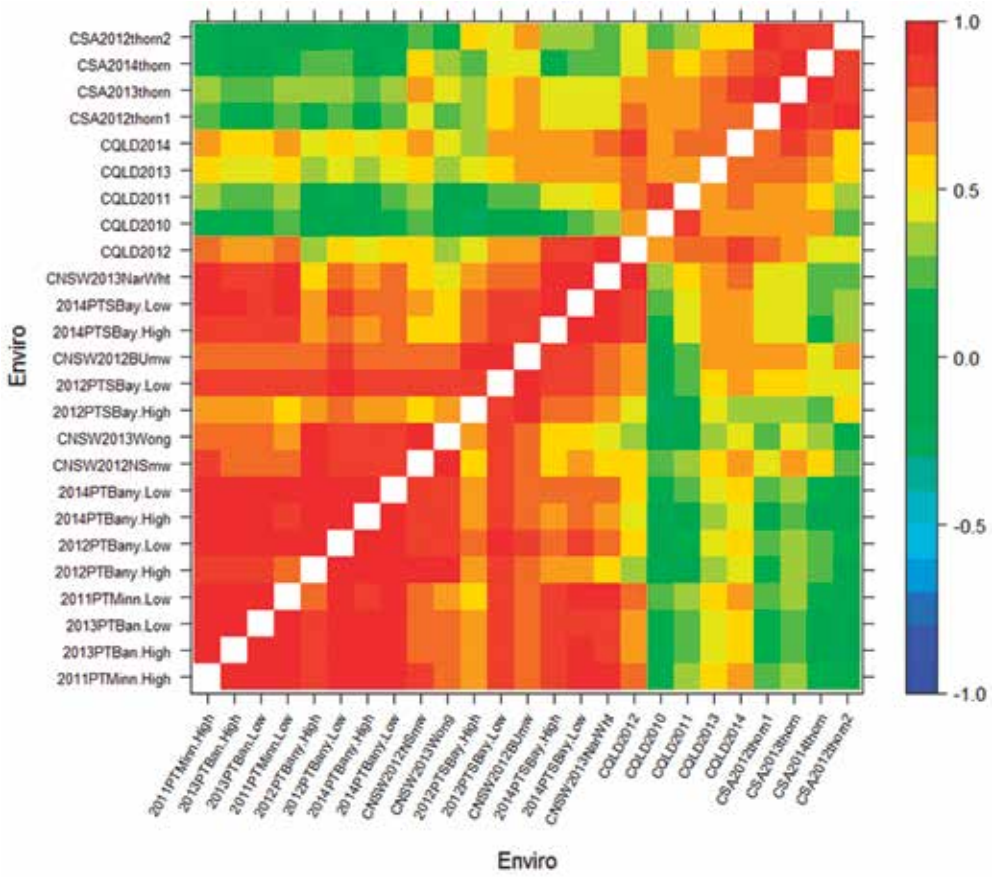


Figure 1. Heat map of the estimated between environment genetic correlation matrix for the combined glasshouse and field MET resistance data for P. thornei. Colours range from dark blue for high negative genetic correlation to red for high positive genetic correlation.

DISCUSSION

An FA4 analysis of the GF MET data set for resistance to *P. thornei* has revealed consistent ranking of varieties within both the glasshouse and the field, but significant crossover GxE between these two trial types. Strong estimated genetic correlation between the SA and QLD glasshouse trials is in keeping with the results of Sheedy *et al.* (2014) who report consistent ranking of cultivars using diverse resistance screening procedures in a glasshouse setting. Likewise, strong correlation between the SA and VIC field trials is in keeping with an earlier MET analysis of these sites as a part of a wider tolerance related project. Generally low levels of genetic correlation between glasshouse and field trial types may have implications for ongoing use of glasshouse data to form resistance ratings. This will be the focus of further research.

ACKNOWLEDGMENTS

Sincere thanks to Alan McKay (SARDI) and Grant Hollaway (VIC Department of Environment and Primary Industries) for initiating this research and to the Grains Research and Development Corporation of Australia (GRDC) for financial support. Thanks also to Jason Sheedy (University of Southern Queensland), Steven Simpfendorfer (NSW Department of Primary Industries), Sjaan Davies, Katherine Linsell and Danuta Pounsett (SARDI) and Josh Fanning (VICDEPI) for data collection. Sincere thanks to Sabela Munoz Santa for technical assistance in analyzing this data and to Brian Cullis and Alison Smith (University of Wollongong) for helpful discussions.

REFERENCES

- Beeck C, Cowling WA, Smith AB, Cullis BR (2010) Analysis of yield and oil from a series of canola breeding trials. Part I: fitting factor analytic models with pedigree information. *Genome*, 53, 992-1001.
- Cullis BR, Smith AB, Beeck C, Cowling WA BR (2010) Analysis of yield and oil from a series of canola breeding trials. Part II: exploring VxE using factor analysis. *Genome*, 53, 1002-1016.
- Kelly A, Smith A, Eccleston J, Cullis BR (2007) The accuracy of varietal selection using factor analytic models for multi-environment plant breeding trials. *Crop Science*, 47, 1063-1070.
- Murray GM, Brennan JP (2009) Estimating disease losses to the Australian wheat industry. *Australasian Plant Pathology*, 38, 558-570.
- Sheedy JG, McKay AC, Lewis J, Vanstone VA, Fletcher S, Kelly A, Thompson JP (2014) Cereal cultivars can be ranked consistently for resistance to root-lesion namatodes (Pratylenchus thornei & P. neglectus) using diverse procedures. *Australasian Plant Pathology*, 42(2), 175-182.
- Smith AB, Cullis BR, Thompson R (2001) Analyzing variety by environment data using multiplicative mixed models and adjustments for spatial field trend. *Biometrics*, 57, 1138-1147.
- Smith AB, Cullis BR, Thompson R (2005) The analysis of crop cultivar breeding and evaluation trials; an overview of current mixed model approaches. *Journal of Agricultural Science Cambridge*, 143, 449-462.
- Smith AB, Ganesalingam A, Kuchel H, Cullis BR (2015) Factor analytic mixed models for the provision of grower information from national crop variety testing programs. *Theoretical and Applied Genetics*, 128, 55-72.

OCCURRENCE, DISTRIBUTION AND INTEGRATED MANAGEMENT OF THE CEREAL CYST NEMATODES (*HETERODERA AVENAE* & *H. FILIPJEVI*) IN CHINA

DELIANG PENG*, HUAN PENG, AND WENKUN HUANG

State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, China. *Corresponding author email: pengdeliang@caas.cn

SUMMARY

The distribution of cereal cyst nematodes (CCN; *Heterodera avenae, H. filipjevi*) has been confirmed to occur in six new provinces and an Autonomous Region (including Jiangsu, Shanxi, Tianjing, Ningxia, Xizang (Tibet) and Xijiang Uygur Autonomous Region) based on the morphology and molecular characterization and species-specific primer diagnosis. CCNs have therefore been confirmed to be distributed in 16 provinces in China. *Heterodera filipjevi* was first reported from Henan province in 2010. The population densities found were much higher than those in published reports where CCN was recognized as being economically damaging on wheat. Preliminary yield losses were determined with inoculation and field trials, in Henan, Hebei, Beijing suburb, and Qinghai. The results showed that yield losses reached up to 18-35% in Henan, 15-20% in Hebei, 11-18% in Beijing Suburb, and 10-28% in Qinghai. The results confirmed that only one generation of *H. avenae and H. filipjevi* occur per year in Beijing, Hebei, Jiangsu and Shandong, and this is in agreement with other published reports around the world. Four diagnostic methods based

* Peng DL, Peng H, Huang WK (2015) Occurrence, distribution and integrated management of the cereal cyst nematodes (*Heterodera avenae* & *H. filipjevi*) in China. In 'Nematodes of Small Grain Cereals: current status and research.' (AA Dababat, H Muminjanov and RW Smiley) pp. 17-24. (FAO: Ankara, Turkey).

on SCAR-PCR and LAMP were developed to detect *H. avenae* and *H. filipjevi* from infested fields. One new pathotype of *H. avenae*, named Ha91, was found from Beijing and Qinghai. A cDNA library from the second stage juveniles of *H. avenae* was constructed for exploring more candidate parasitism genes. We generated 5800 ESTs and obtained 2568 unigenes. Three β-1,4-endoglucanase genes (*Ha-eng-1a, Ha-eng-2* and *Ha-eng-3*) expressed in the pharyngeal glands of *H. avenae* were cloned and functions were tested with RNAi.

OCCURRENCE AND DISTRIBUTION OF *H. AVENAE* AND *H. FILIPJEVI*

Cereal cyst nematode (*Heterodera avenae*) is the most important plant-parasitic nematode on cereal crops in wheat producing areas of China. *H. avenae* was first reported in Hubei Province, China in 1989 (Chen *et al.* 1992). It was subsequently reported in Henan, Hebei, Beijing, Inner Mongolia, Qinghai, Anhui, Shandong, Shaanxi and Gansu (Peng *et al.* 2009. A survey of cereal cyst nematodes (CCN) were conducted in China from 2009 to 2013. Based on the morphology and molecular characterization and species-specific primer diagnosis, the CCNs *H. avenae* and *H. filipjevi* have been confirmed to exist in six new provinces and Autonomous Region, including Jiangsu, Shanxi, Tianjing, Ningxia, Xizang (Tibet), and XijiangUygur Autonomous Region (Li 2009, Peng *et al.* 2009, Hunag *et al.* 2010, Peng *et al.* 2012, Li *et al.* 2012, Zhao *et al.* 2013). The survey confirmed that the CCN's are distributed in 16 provinces in China, and are found at high level of prevalence. *H. filipjevi* was first reported from Henan province (Peng *et al.* 2010) and now it is distribution has expanded into three provinces, including Henan, Qinghai and Ningxia. Therefore, there are two species, *H. avenae* and *H. filipjevi*, occurring in the wheat production areas of China, with *H. avenae* being the dominant species. The population densities found were much higher than those in published reports in which CCN is recognized as economically damaging to wheat. The wheat growing area of these 16 provinces and Autonomous Region represents about 90% of the total for China, around 40 million ha, with average yields ranging from 3 to 6 t/ha depending on the agro-ecological region.

MOLECULAR DIAGNOSIS

Four diagnostic methods based on species-specific sequence characterized amplified regions (SCAR-PCR) and loop-mediated isothermal amplification (LAMP) were developed to detect and identify *H. avenae* and *H. filipjevi* from infested wheat roots and field soil.

The SCAR marker system had been established to detect cysts and the J2 of *H. avenae*. The species-specific primers were designed according to the randomly amplified polymorphic DNA (RAPD) markers amplified with random primer. The SCAR marker system has the ability to detect several stages of *H. avenae*, and the 1010 bp DNA fragment could be clearly identified when the dilution was 1/2000 of a cyst or 1/80 of a J2 for all replicates. The establishment of the SCAR marker system for rapid molecular detection of *H. avenae* is fast for detecting *H. avenae* from mixed nematode samples, and is accurate and highly sensitive (Qi *et al.* 2012).

The species-specific primers were designed according to the randomly amplified polymorphic DNA (RAPD) markers amplified with random primer OPK16. A 646 bp specific fragment of sequence was generated, which characterized amplified regions in *H. filipjevi*. The detection limit of the PCR assay was as low as 0.125 µl second-stage juvenile (J2) lysate, 3.9×10-3µl adult female lysate and 10-3 µl cyst lysate. The method was able to detect the various stages (J2, J3, J4 and female) of *H. filipjevi*, and a single nematode in 0.5 g of soil. *H. filipjevi* was detected by the method in two of six field samples and one of those samples contained a mixed population of *H. filipjevi* and *H. avenae*. This study is the first to provide a definitive diagnostic assay for *H. filipjevi* in wheat roots and soil (Peng *et al.* 2013).

A novel, simple, rapid and highly sensitive assay and diagnostic tool for *H. avenae* was developed using LAMP. This method had been established to diagnose and detect this species. The LAMP assay targeted on the RAPD fragments of *H. avenae*. Three LAMP primers were designed and specificity of the LAMP assay was confirmed using 10 different plant nematode species including *H. filipjevi*, *H. goettingiana*， *H. elachista*, *H. glycine*, *H. latipons*, *Meloidogyne javanica*， and *Meloidogyne arenaria*. The detection limitation of the LAMP assay was as low as 10^{-2} and 10^{-4} of single juvenile and cyst DNA, and the detection sensitivity of the LAMP method for *H. avenae* DNA is 100 times higher than normal PCR-based detection methods. The LAMP amplifications could be observed directly by eye by adding SYBR Green I and the lateral flow dipstick (LFD). LAMP assay could be used for nematode detection from field-grown wheat roots infected by *H. avenae*. This is a practical and useful diagnostic tool for early diagnosis of diseased wheat infested by *H. aveane*. The LAMP assay developed in this study is highly effective, easy to perform and readily adaptable for diagnosis and monitoring of *H. avenae* in an early stage of seedling development in the field (Peng, unpublished data).

BIOLOGY AND YIELD LOSSES

Preliminary yield losses were determined with inoculations and in the field, in Henan, Hebei, Beijing suburb, Qinghai. Yield losses reached up to 18-35% in Henan, 15-20% in Hebei, 11-18% in Beijing Suburb, and 10-28% in Qinghai. The population dynamics and life cycle of *H. avenae* were investigated in Beijing, Hebei, Jiangsu and Shandong from 2010 December to 2012 December. The results showed that there is one generation of *H. avenae* per year in the areas mentioned above.

The pathotypes of 20 populations of *H. avenae* from Beijing, Hebei, Jiangsu and Shandong were tested and identified by using the International Test Assortment (provided by CIMMYT). Two new pathotypes were identified and reported, and were named Ha43 and Ha91. Yuan *et al.* (2009) reported a new pathotype Ha43 from Zhengzhou. The pathotypes of two populations of *H. avenae*, one each from Xushui and Xingyuang, villages near Zhengzhou, Henan were tested using 23 standard international differentials. The pathotype of Xushui populations were found to be a previously undescribed pathotype which was named as Ha43. This pathotype differs from the most similar pathotype Ha13 by being avirulent in oat cv. Sivan and wheat cv. Loros and Iskamish K-2-light.

The pathotypes of Daxing and Huangyuan populations were characterized by tests on 23 entries of the standard "International Test Assortment". Tested materials were grouped by virulence of three nematode populations on resistant genes (*Rha1*, *Rha2*, *Rha3*, *Cre1*) and on nonresistant genes, varieties and lines. Both Daxing and Huangyuan populations were avirulent to Ortolan (*Ha1*). Barley cvs. Ortolan, Siri, Morocco, Bajo Aragon 1-1, and Martin 403-2 were all resistant to both populations. Cultivars Herta, Harlan 43 and wheat Iskamish-K-2-light were all susceptible to the Huangyuan population. All of them, however, were resistant to the Daxing population. The other five oats were all resistant to the two tested CCN populations. Except for Iskamisch K-2-light, all the other wheat cultivars (Capa, Loros×Koga, AUS 10894 and Psathias) were susceptible to the Daxing population. Because the pathotypes of the two CCN populations in Beijing and Qinghai were not identical as any of the 13 pathotypes previously reported and named, we identified the new pathotype as Ha91 (Cui *et al.* 2015).

More than 200 cultivars were tested and evaluated for resistance to *H. avenae* and *H. filipjevi* in the greenhouse or in the field, respectively. The results showed that no immune cultivars were found to the any of the two species.

Five cultivars including VP1620, BATAVIA, SUNR23, AUS4930 6.5/GS50a and Taikong 6 were highly resistant to the CCN Beijing populations (Peng, unpublished data).

MOLECULAR CHARACTERIZATION, PUTATIVE EFFECTORS AND TRANSCRIPTOMES

A cDNA libarary from the J2-stage of *H. avenae* was constructed for exploring more candidate parasitism genes. We generated 5,800 ESTs and obtained 2,568 unigenes. The transcriptomes of different developmental stages of *H. avenae* isolated from Taian were sequenced by HiSeq 2,000 and were analyzed. Over 49, 46 and 47 million high-quality reads were obtained from pre-parasitic J2s, J4s, and adult females, respectively. These resulted in 66962 transcripts with average transcript length of 1,546 bp, 43,953 transcripts with average transcript length of 1,029 bp, and 62,697 transcripts of average transcript length of 1,068 bp with Trinity assemblers, respectively. There were 45.42%, 46.64% and 43.10% of transcripts of J2, J4, and adult females successfully annotated in Nt, KEGG, Swiss-Prot and Nr databases, respectively (Peng, unpublished).

Six parasitism genes of *H. avenae*, including three β-1,4-endoglucanase genes (Ha-eng-1a, Ha-eng-2 and Ha-eng-3), one expansin gene (Ha-exp-1), one cellulose binding protein new gene (Ha-cbp-1), and a new acid phosphatase gene (Ha-acp1).

The cDNA of Ha-eng-1a encoded a deduced 463-amino acid sequence containing a catalytic domain and a cellulose binding module separated by a linker. The genomic DNA of Ha-eng-1a is 2,129-bp long, containing eight introns ranging from 56 bp to157 bp and nine exons ranging from 70 bp to 299 bp. Southern blot analysis revealed that two copies of the Ha-eng-1a gene are present in *H. avenae*. In-situ hybridization showed that the Ha-eng-1a transcripts specifically accumulated in the two subventral gland cells of the second-stage juveniles (Long *et al.* 2012). Two new β-1,4-endoglucanase genes (Ha-eng-2 and Ha-eng-3) of *H. avenae* were cloned. Both of the predicted proteins have a putative signal peptide for secretion and a catalytic domain. Neither peptide linkers nor cellulose binding domains were present. In-situ hybridization showed that the transcripts of Ha-eng-2 and Ha-eng-3 accumulated specifically in the two subventral gland cells of *H. avenae*. RT-PCR analysis confirmed that their transcriptions were strong in the pre-parasitic and early parasitic second-stage juveniles, and were undetectable at the late parasitic stages of the nematode. Cellulase activities of the recombinant proteins HA-ENG-2 and

HA-ENG-3 were confirmed in vitro. Knocking down Ha-eng-2 using RNA interference reduced nematode infectivity by 40%. The results indicate that these β-1,4-endoglucanases can be secreted into plant tissues and play an important role in the wall degradation of plant cells during penetration and the migration of J2s in host roots (Long *et al.* 2013).

A new expansin gene (Ha-expb1) of *H. avenae* was cloned. Southern blot analysis suggested that Ha-expb1 is a member of a multigene family. The deduced protein Ha-EXPB1 consists of a signal peptide, a CBM II and an expansin domain, and was significantly similar to expansins and expansin-like proteins from *Globodera rostochiensis* and *Bursaphelenchus* spp. In-situ hybridisation showed that the Ha-expb1 transcript specifically accumulated in the two subventral gland cells of the J2s. Developmental expression confirmed that its transcript abundances were high in the motile juvenile stages and low in the sedentary stage of the nematode (Long *et al.* 2012).

The cDNA sequence of Ha-cbp-1 (GenBank accession GQ178086) was cloned by RACE kit based on the homologous cloning method. The results showed that the cDNA sequences of Ha-cbp-1 contained an open reading frame, which encoded 131 amino acids with a predicted signal peptide sequence for secretion and a cellulose-binding domain. The DNA sequence of Ha-cbp-1 contained two introns with the length of 932 bp. The predicted HA-CBP-1 amino acid sequence had 60% identity and 75-76% similarity with HS-CBP-1 and HG-CBP-1 (Gu *et al.* 2011).

A new acid phosphatase gene (Ha-acp1) of *H. avenae* was cloned and the characteristics of the gene were analyzed. Results showed that the gene had a putative signal peptide for secretion and in-situ hybridization showed that the transcripts of Ha-acp1 accumulated specifically in the subventral gland cells. Southern blot analysis suggested that Ha-acp1 belonged to a multigene family. RT-PCR analysis indicated that this transcription was strong in the pre-parasitic juveniles. Knocking down Ha-acp1 using RNA interference technology could reduce nematode infectivity by 50%, and suppress the development of cysts (Liu *et al.* 2014).

INTEGRATED MANAGEMENT CEREAL CYST NEMATODES

The CCNs *Heterodera avenae* and *H. filipjevi* are the most economically important plant-parasitic nematodes on cereal crops in the wheat production

area of China. These CCNs have been confirmed to be distributed in 16 provinces in China. Yield losses estimated were 18-35% in Henan, 11-18% in Hebei, and 15-28.24% in Qinghai. The population dynamics and life cycle of *H. avenae* was one generation.

Dababat *et al.* (2015) reviewed the management strategies to control cereal cyst nematodes in wheat in Turkey including cultural practices, chemical, biological and resistant germplasm. In our study of control practices for CCN on wheat in China, six kinds of seed-coatings treatments were used; Gannong seed coating I, Gannong seed coating II, Gannong seed coating III, abamectin seed coating AV1, abamectin seed coating AV2, and 5.76% emamectin benzoate. Wheat seeds were coated with eight different treatments before sowing and the changes in number of cysts in soil and wheat yield were compared after harvesting. The results showed that the numbers of cysts in soil collected from different treatments were clearly reduced after seed-coating. The highest reductions in cysts were 56%, 53% and 47% obtained with the Gannong seed coating III (1:35), Gannong seed coating I (1:50), and Gannong seed coating II (1:35), respectively. The yields were increased when compared to the control. The wheat yields were increased with Gannon seed coating III (1:35), Gannon seed coating I (1:50), Gannon seed coating II (1:35), and Gannon seed coating I (1:35), by 37.6%, 19.4%, 17.9% and 17.7%, respectively. The self-patented Gannong seed coating III not only has the better efficacy on controlling CCN, but also has characteristics of environmental safety, lower toxicity, labor and cost saving, which is suitable for wide application in practical disease control.

ACKNOWLEDGMENTS

This study was supported by the Special Fund for Agro-scientific Research in the Public Interest (No. 201503114, 200903040) and the National Natural Science Foundation of China ((31171827)) and the National Key Basic Research Program of China (973 Program, 2013CB127502).

REFERENCES

- Cui JK, Huang WK, Peng H, Liu SM, Wang GF, Kong LA, Peng DL (2015) A new pathotype characterization of Daxing and Huangyuan populations of cereal cyst nematode (*Heterodera avenae*) in China. *Journal of Integrative Agriculture*, 14(4), 708–715.

- Dababat AA, Imren M, Erginbas-Orakci G, Ashrafi S, Yavuzaslanoglu E, Toktay H, Pariyar SR, Elekcioglu H, Morgounov A, Mekete M (2015) The importance and management strategies of cereal cyst nematodes, *Heterodera* spp., in Turkey. *Euphytica*, 202, 173-188.
- Hou SY, Wang AL, Zhang G, Huang LL (2012) Effect of cereal cyst nematode on agronomic characteristics and yield losses of spring wheat. *Journal of Agriculture*, 2, 33–36. (In Chinese)
- Li HM, Wang X, Pei S, Le XH, Liang Z (2010) A preliminary survey of the cereal cyst nematode *Heterodera avenae* on wheat in Jiangsu Province of China. *Plant Protection*, 36, 172–175. (in Chinese)
- Li HX, Liu YE, Wei Z, Li MQ (2012) The detection of *Heterodera avenae* from the cereal field in Autonomous Regions of Tibet and Xinjiang. *Nematology Research in China*, 4, 164–165. (in Chinese)
- Nicol JM, Rivoal R (2008) Global knowledge and its application for the integrated control and management of nematodes on wheat. In. 'Integrated Management and Biocontrol of Vegetable and Grain Crops Nematodes.' (Eds. A Ciancio, KG Mukerji) pp. 243–287. (Springer Academic Publishing, The Netherlands).
- Peng DL, Huang WK, Sun JH, Liu CJ, Zhang HM (2012) First report of cereal cyst nematode (*Heterodera avenae*) in Tianjin, China. *Nematology Research in China*, 4, 162–163. (in Chinese)
- Peng DL, Li HX, Wang XF, Riley IT, Nicol JM, Hou SY, Ou SQ (2008) New distribution of cereal cyst nematode (*Heterodera avenae*) in China. *Nematology Research in China*, 2, 344–345. (in Chinese)
- Peng DL, Nicol JM, Li HM, Hou SY, Li HX, Chen SL, Ma P, Li HL, Riley IT (2009) Current knowledge of cereal cyst nematode (*Heterodera avenae*) on wheat in China. In. 'Cereal cyst nematodes: Status, Research and Outlook.' (Eds. IT Riley, JM Nicol, AA Dababat). pp. 29–34. (CIMMYT, Ankara, Turkey).
- Peng DL, Subbotin S, Moens M (2003) rDNA restriction fragment length polymorphism of *Heterodera avenae* in China. *Acta Phytopathologica Sinica*, 33, 323–329. (in Chinese)
- Qi XL, Peng DL, Peng H, Long HB, Huang WK, He WT (2012) Rapid molecular diagnosis based on SCAR marker system for cereal cyst nematode. *Scientia Agricultura Sinica*, 45, 4388–4395. (in Chinese)
- Yuan HX, Sun JW, Yang WX, Xing XP, Wang ZY, Riley IT, Li HL (2010) New pathotypes of *Heterodera avenae* (cereal cyst nematode) from winter wheat in Zhengzhou, Henan, China. *Australasian Plant Pathology*, 39, 107–111.

CURRENT STATUS OF CYST AND ROOT LESION NEMATODES ATTACKING CEREALS IN JORDAN

LUMA AL-BANNA[1,*], ADEL AL ABDED[2], ISAM FATTASH[3], WAFA KHRFAN[1], HAMZEH LAFI[1], TOUJAN ABU SHWEIMEH[1], DUA MAZRAWI[1], AND ISAAF ABU AL RAGHEB[1]

[1]Department of Plant Protection, University of Jordan: University of Jordan Amman-11942, Jordan; [2]National Centre for Agri Res and Ext (NCARE), Ministry of Agriculture, Amman, Jordan; [3]American University of Madaba, Department of Biology and Biotechnology, Madaba, Jordan; *Corresponding author email: lalbanna@ju.edu.jo

SUMMARY

In Jordan, 50% of the utilized agricultural area is grown with cereals. Barley and wheat have occupied 70 and 20% of the total cereals sowing area in Jordan, respectively. Cereal cyst nematodes (CCN) and root lesion nematodes (RLN) are the most common nematodes attacking cereals and causing serious damage worldwide. Therefore we aimed to review the current status and impacts of those two nematodes on cereals in Jordan. The earliest report of nematodes attacking cereals in Jordan was in 1978, by Bridge, who recorded the presence of RLN attacking wheat. Since then and up to now several surveys of nematodes associated with roots of cultivated crops were conducted and revealed the presence of RLN on wheat and barley roots. However these surveys were limited and primitive since not all cereal production areas were sampled to detect RLN. An intensive survey of CCN was conducted on barley growing regions and showed that the Mediterranean cereal cyst nematode (MCCN), *Heterodera latipons,* is the only

* Al-Banna L, Al Abded A, Fattash I, Khrfan W, Lafi H, Abu Shweimeh T, Mazrawi D, Abu Al Ragheb I (2015) Current status of cyst and root lesion nematodes attacking cereals in Jordan. In 'Nematodes of Small Grain Cereals: current status and research.' (AA Dababat, H Muminjanov and RW Smiley) pp. 25-34. (FAO: Ankara, Turkey).

CCN species in Jordan so far. The MCCN was detected in 66% of the barley fields sampled in Northern and Southern Mediterranean regions and Eastern Desert, with moderate to severe infestation levels. The MCCN was also detected on sites of wheat growing areas. Etiological and epidemiological studies were performed including life cycle, cloning parasitism genes, virulence of geographical isolates, and effect of environment on the biology and pathology of this MCCN. Cereals genotypes were screened against MCCN with collaboration with experts in international organizations (ICARDA and CIMMYT). The current status of RLN on cereals in Jordan is primitive and much work is needed on the distribution, epidemiology and on the management issues. In contrast, the current status of MCCN in Jordan showed good initiatives and future research must be continued in the laboratory and in field experiments.

INTRODUCTION

For the last twenty years, a total area of 130 thousand hectares is sown annually with field crops which comprise 50% of the utilized agricultural area in Jordan (Annual Agriculture Statistics, 1994-2013). Barley and wheat have occupied 70 and 20% of the total field crops cultivated areas in Jordan, respectively (Annual Agriculture Statistics, 1994-2013). The production of those two cereal crops is considered relatively low, which can be related to environmental stress including pests. Plant-parasitic nematodes alone cause 10% annual cereal crop losses (Nicol 2002, Whitehead 1998). Cereal cyst nematodes (CCN) and root lesion nematodes (RLN) are recorded to be the most common nematodes attacking cereals and causing serious damage (Franklin 1969, Nicol *et al.* 2003, Philis 1995, Safari *et al.* 2005). In this context, we aimed to overview the history, current status and impacts of CCN and RLN on cereals in Jordan. Certainly, this overview will help us to identify the gaps to be filled by future research.

HISTORY

Cereals were already grown in the Fertile Crescent, which includes part of Jordan, as long as 9,000 years ago. Plant-parasitic nematodes associated with cereals may have coevolved with cereals in this region or they might have been introduced. The first record of nematodes associated with cereals was reported in 1970's. Hashim (1979) reported the presence of the RLN *Pratylenchus thornei* on roots of wheat grown in Northern phytogeographical region at one site in the Irbid and Jordan valley area. Bridge (1978) recorded undescribed species of *Pratylenchus* associated with wheat roots in the east-

ern rainfed area. Several general surveys were conducted since 1994 till now to investigate the presence of plant parasitic nematodes attacking cultivated crops in Jordan (Yousef and Jacob 1994, AL-Abed *et al.* 2004, Karajeh and Al-Ameiri 2010, AL Banna *et al.* unpublished data). Only AL-Abed *et al.* (2004) conducted a survey on CCN in barley production areas. Tables 1-4 show the distribution of CCN and RLN in Jordan so far.

STATUS AND IMPACT

Root Lesion Nematodes: The general surveys of plant parasitic nematodes revealed the presence of the RLN '*P. neglectus*' on cultivated barley roots at the AL Karak area in the Southern Mediterranean phytogeographical region. Individuals of *Pratylenchus* sp. were found in the roots of wild barley in the eastern desert at Safawi site (Table 1). Wheat surveys revealed the presence of RLN (*Pratylenchus* sp. and *P. thornei*) on sites of the Northern Mediterranean phytogeographical region and the Northern Jordan Valley region (Table 2). However, it was noticed that RLN were found in high numbers in certain fields while in other fields, they were present in few numbers. Results showed that surveys were preliminary and did not cover all cereal production areas or even the wild species of cereals such as oats, barley and goat grass. Not only that but also, to our knowledge, no other epidemiological or management studies have been performed on RLN attacking cereals in Jordan.

Cereal Cyst Nematodes: Surveys of CCN on barley and wheat were more specific (Tables 3 and 4). The cyst nematode species was identified as the Mediterranean cereal cyst nematode (MCCN) and belongs to the species *H. latipons*. The identification to the species level was based on morphological characters and was confirmed later using the D2-D3 and ITS of rRDNA sequences (AL-Abed *et al.* 2004, Khrfan 2012). AL-Abed *et al.* (2004) showed that *H. latipons* was detected in 66% of the barley fields sampled in the Northern Mediterranean and Southern Mediterranean regions and in the Eastern Desert, but it was not found in the Northern Jordan Valley or in the Southern Desert area (Table 3). The incidence of MCCN in the two Mediterranean areas ranged from 50% in Jarash and At-Tafilah, to 100% in Ar-Ramtha, with moderately severe to severe infested fields. In the Eastern Desert the MCCN was found in only 30% of fields sampled, but infestation was severe.

On the other hand, surveys of wheat were less intensive and all regions were sampled. Those general or preliminary surveys showed that the MCCN was

Table 1. Distribution of root lesion nematodes (Pratylenchus sp.) in barley producing regions in Jordan

Phytogeographical region	Collection sites	Pre-sent[1]	RLN species	References
Northern Mediterranean	Ar-Ramtha	NS		
	Irbid	NS		
	Madaba and Amman	-		AL Banna, and others unpublished
	Jarash	-		AL Banna, and others unpublished
Southern Mediterranean	AL Karak	+ +	P. neglectus, Pratylenchus spp.	Karajeh and Al-Ameiri (2010)
	At-Tafilah	NS		
	Ash-Shawbak	NS		
Northern Jordan Valley	Dier Allah	NS		
	North Shuna	NS		
Eastern Desert	Mafraq	NS		
	Safawi	+	Pratylenchus spp.	AL Banna and Abu Shweimeh, unpublished
Southern Desert	AL Mudawwarah	NS		

[1]Present or absent: + = present, - = absent, NS = not sampled

Table 2. Distribution of root lesion nematodes (Pratylenchus sp.) in wheat producing regions in Jordan

Phytogeographical region	Collection sites	Pre-sent[1]	RLN species	References
Northern Mediterranean	Ar-Ramtha	+	Pratylenchus spp.	AL Banna and Khrfan, personal observation
	Irbid	+	P. thornei	Hashim (1979)
	Irbid	+	Pratylenchus spp.	AL Banna and Fattash, unpublished
	Madaba and Amman	+	Pratylenchus spp.	Bridge J (1978)
	Jarash	-		AL Banna and Fattash, unpublished
Southern Mediterranean	AL Karak	NS1		
	At-Tafilah	NS		
	Ash-Shawbak	NS		
Northern Jordan Valley	Karameh	NS		
	Dier Allah	-		AL Banna, and others unpublished
	North Shuna	+	P. thornei	Hashim (1979)
	Baqura	-		AL Banna and Fattash, unpublished
Eastern Desert	Mafraq	NS		

[1]Present or absent: + = present, - = absent, NS = not sampled

detected on different sites of the Northern Mediterranean phytogeographical region and the Northern Jordan Valley region with variation in infestation levels (Table 4).

Our observation also showed that the MCCN completed only one cycle during the growing season in all areas in Jordan, as reported by other workers in Cyprus and other countries (Mor *et al.* 1992, Philis 1999).

Virulence studies of three Jordanian geographical isolates of the MCCN showed that these isolates varied on their virulence on the two cultivars of barley (Abed *et al.* 2004). Moreover, morphometric studies were conducted to differentiate those isolates (AL Abed *et al.* 2004, Lafi *et al.* 2015). *In vitro* epidemiological studies of MCCN were performed. Such studies included the effect of temperature and moisture on hatching and penetration of the second stage juveniles, on the development of the MCCN inside roots of barley and wheat, and on the production of cysts (AL Abed *et al.* 2004, Khrfan 2012). Other epidemiological studies included the study of soil texture and structure on the production of cysts of the MCCN (AL Abed *et al.* 2004).

The parasitism of MCCN at the molecular level was investigated. Lafi and his colleagues (2009) were able to clone candidate parasitism genes from local populations of *H. latipons*. These targeted genes encode secretory protein products. The 18 clones were analyzed and they showed similarities to putative proteins such as digestive gland protein, glutamyl transpeptidedase and putative ubiquitin. The cloned ubiquitin sequence from the Jordanian isolate of MCCN has a high similarity to ubiquitin from the sugar beet cyst nematode, *H. schachtii*, and the soybean cyst nematode, *H. glycine*s.

Management of MCCN was studied by Wafa Khrfan (MSc. Student) with the collaboration of Dr. Francis Ogbonnaya, the previous head of the Biotechnology Research Section at the International Center of Agricultural Research in the Dry areas (ICARDA) who served as her co-advisor. She evaluated 56 wheat genotypes comprised of landraces of hard (durum), common bread wheat cultivars, synthetic hexaploid wheat and wheat-related species for their resistance against the Jordanian isolate of MCCN. The study showed that the tested wheat genotypes varied in their reactions to the MCCN. Results also revealed that genotypes carrying the *Cre3* resistance gene expressed a high level of resistance to MCCN while the genotypes carrying the *Cre1* resistance gene varied in their levels of resistance to MCCN. On the other hand, other commercial cultivars

Table 3. Distribution of the MCCN (Heterodera latipons) in barley producing regions in Jordan

Phytogeographical region	Collection sites	Present[1]	References
Northern Mediterranean	Ar-Ramtha	+	Abu Gharbieh and AL Banna, personal observation; AL-Abed et al. (2004); AL Banna and Abu Shweimeh, unpublished
	Irbid	+	AL-Abed et al. (2004)
	Madaba and Amman	+	AL-Abed et al. (2004)
	Jarash	+	AL-Abed et al. (2004)
Southern Mediterranean	AL Karak	+	AL-Abed et al. (2004)
	At-Tafilah	+	AL-Abed et al. (2004); AL Banna and Mazrawi, unpublished
	Ash-Shawbak	+	AL-Abed et al. (2004)
Northern Jordan Valley	Karameh	-	AL-Abed et al. (2004
	Dier Allah	-	AL-Abed et al. (2004
	North Shuna	NS[1]	
Eastern Desert	Mafraq	+	AL-Abed et al. (2004)
	Safawi	+	AL Banna and Abu Shweimeh, unpublished
Southern Desert	AL Mudawwarah	+	AL-Abed et al. (2004)

[1]Present or absent: + = present, - = absent, NS = not sampled

Table 4. Distribution of the MCCN (Heterodera latipons) in wheat producing regions in Jordan

Phytogeographical region	Collection sites	Pre-sent[1]	References
Northern Mediterranean	Ar-Ramtha	+	AL Banna and Mazrawi, unpublished AL Banna and Abu Al Rhageb, unpublished
	Irbid		
	Madaba and Amman	+	AL Banna and Mazrawi, unpublished AL Banna and others, unpublished
	Jarash	+	AL Banna and Mazrawi, unpublished AL Banna and Abu Rhageb, Unpublished
Southern Mediterranean	AL Karak	-	AL Banna and Mazrawi, unpublished AL Banna and Abu Al Rhageb, unpublished
	At-Tafilah	NS1	
	Ash-Shawbakbak	NS	
Northern Jordan Valley	Karameh	NS	
	Dier Allah	NS	
	North Souna	+	Yousef and Jacob (1994)
Eastern Desert	Mafraq	NS	

[1]Present or absent: + = present, - = absent, NS = not sampled

and improved genotypes were resistant to RJHL and do not have *Cre3* or *Cre1*. They may posses other uncharacterized *Cre* genes against MCCN and thus they represent potentially new sources of resistance genes that could be used for wheat improvement against *H. latipons*.

Screening more wheat genotypes as well as barley and oat cultivars against MCCN was continued with the collaboration with Dr Abdelfattah Dababat, the leader of the Soil Borne Pathogens Program at the International Maize and Wheat Improvement Centre (CIMMYT), and the Jordanian team from the University of Jordan and the Ministry of Agriculture. Results of the screening showed that resistance was noticed in some cultivars that are also resistant to other CCN species (Jaabari *et al.* Unpublished).

The search for bioagents against MCCN is still in its prelimary stages. Only one fungus was isolated from MCCN cysts and was fully characterized. This fungus was a good candidate for biological control of MCCN but further bio-assays are needed.

No field studies were conducted to assess yield loss or even the management demonstration to farmers.

PROSPECTS AND RECOMENDATION

The current status of RLN and MCCN enabled us to identify gaps for future research. These gaps and future research to overcome such gaps are summarized as follows:

RLN: There is a lack of knowledge of the distribution and pathology of RLN species attacking cereals in Jordan. Thus, initially intensive surveys of RLN species on cultivated and wild cereals in Jordan should be conducted. Consequently the pathology of RLN and other epidemiological studies should be investigated to understand the impact of such nematodes on cereals in Jordan. Once these epidemiological studies are investigated, management strategies can be set and control means can be employed. Such means include the screening of international and local cereal genotypes against RLN.

MCCN: There is a lack of complete information about the MCCN and maybe also other CCN species attacking wheat. Thus, a more intensified survey is needed to include all wheat growing areas. More studies are needed to un-

derstand the parasitism of MCCN to employ management to this nematode. Yield loss and control means at the field level are not yet assessed and they should be performed urgently. The staff at the Ministry of Agriculture and the researchers at the universities should conduct field studies and demonstrations to help farmers suppress the effect of this nematode. There should be continued screening tests of more international genotypes.

In Jordan, wheat and barley production is small compared to other countries due to severe abiotic stresses presented by drought, heat, and low precipitation per year. However, the understanding of the occurrence, distribution, and the population dynamics of the cereal nematodes attacking those two economically important crops are considered the main biotic stresses in Jordan and their control could increase the two crop's productivity. Moreover, and given that in Jordan a standard environment for abiotic stresses affecting wheat and barley, results from that area could be of high value to other research groups in the region and around the world.

All of this future research is needed to integrate efforts of all local researchers at the Ministry of Agriculture, University of Jordan, the private sectors, and with the collaboration with experts of international organizations.

ACKNOWLEDGMENT

This review and works was supported by the University of Jordan, The National Center for Agricultural Research and Extension (NCARE), Ministry of Agriculture, Jordan, and the Soil Borne Pathogens Program at the International Maize and Wheat Improvement Center (CIMMYT), Turkey.

REFERENCES

- AL-Abed A, Al-Momany A, Al Banna L (2004) *Heterodera latipons* on barley in Jordan. *Phytopathologia Mediterranea*, 43, 311–317.
- Annual Agricultural Statistics: 1994-2013. Department of Statistics. Amman, Jordan.
- Bridge J (1978) Plant Nematology in Jordan. A report on the visit to Jordan April 3-15,1978. Submitted to ODM, UK.
- Franklin MT (1969) *Heterodera latipons* n. sp., A cereal cyst nematode from the Mediterranean region. *Nematologica*, 15, 535–542.

- Hashim Z (1979) A preliminary report on the plant-parasitic nematodes in Jordan. *Nematologia Mediterranea*, 7, 177-186.
- Karajeh MR, Al-Ameiri NS (2010) A Survey of Plant-Parasitic Nematodes in Karak Province of Jordan. *Dirasat, Agricultural Sciences*, 37, 77–81.
- Khrfan W (2012) Characterization of genetic resistance against Mediterranean cereal cyst nematode (*Heterodera latipons*) in wheat. *PhD Thesis, University of Jordan, Amman, Jordan.*
- Lafi HA, Sadder M, AL-Banna L (2009) Cloning of parasitism genes from the Mediterranean cereal cyst nematode, *Heterodera latipons*. In: 'Cereal Cyst Nematodes: Status, Research and Outlook.' (Eds. IT Riley, JM Nicol, AA Dababat) pp. 208-212. *(CIMMYT: Ankara, Turkey).*
- Lafi HA, Al Banna L, Sadder M, Migdadi HM (2015) Morphological and morphometrical analysis of *Heterodera* spp. populations in Jordan. *Saudi Journal of Biological Sciences*, DOI: 10.1016/j.sjbs.
- Mor M, Cohn E, Spiegel Y (1992) Phenology, pathogenicity and pathotypes of cereal cyst nematodes, *Heterodera avenae* and *Heterodera latipons* (Nematoda: Heteroderidae) in Israel. *Nematologica*, 38, 494–501.
- Nicol JM (2002) Important nematode pests of cereals. In: 'Bread Wheat: Improvement and Production.' (Ed. BC Curtis) pp. 345-366. *FAO Plant Production and Protection Series, Rome, Italy.*
- Nicol J, Rivoal R, Taylor S, Zaharieva M (2003) Global importance of cyst *(Heterodera* spp.) and lesion nematodes (*Pratylenchus* spp.) on cereals: yield loss, population dynamics, use of host resistance and integration of molecular tools. *Nematology Monographs and Perspectives*, 2, 1-19.
- Philis J (1995) An updated list of plant parasitic nematodes from Cyprus and their economic importance. *Nematologia Mediterranea*, 23, 307–314.
- Philis J (1999) The life cycle of the mediterranean cereal cyst nematode *Heterodera latipons* in Cyprus. *Nematologia Mediterranea*, 27, 43-46.
- Safari E, Gororo NN, Eastwood RF (2005) Impact of *Cre1*, *Cre8* and *Cre3* genes on cereal cyst nematode resistance in wheat. *Theoretical and Applied Genetics*, 110, 567–572.
- Scholz U, Sikora RA (2004) Hatching behavior and life cycle of *Heterodera latipons* Franklin under Syrian agro-ecological conditions. *Nematology*, 6, 245–256.
- Yousef DM, Jacob JJ (1994) A nematode survey of vegetable crops and some orchards in the Ghor of Jordan. *Nematologia Mediterranea*, 22, 11–15.
- Whitehead AG (1998) Plant Nematode Control. (Wallingford, U.K.: CAB International).

INTERNATIONAL WINTER WHEAT IMPROVEMENT PROGRAM BREEDING MODERN GERMPLASM, LANDRACES AND SYNTHETICS FOR SOIL BORNE PATHOGENS

ALEXEI MORGOUNOV[1,*], BEYHAN AKIN[1], ABDELFATTAH A. DABABAT[1], GUL ERGINBAŞ-ORAKÇI[1], MESUT KESER[2], AND FATIH OZDEMIR[3]

[1]International Maize and Wheat Improvement Center (CIMMYT), Ankara, Turkey, [2] International Center for Agricultural Research in Dry Areas (ICARDA), Ankara, Turkey, [3] Bahri Dagdas International Agricultural Research Institute, Konya, Turkey, *Corresponding author email: a.morgounov@cgiar.org

International Winter Wheat Improvement Program (www.iwwip.org) is a partnership between Ministry of Food, Agriculture and Livestock of Turkey, CIMMYT and ICARDA to develop new winter wheat varieties for the region of Western and Central Asia. The main breeding priorities are broad adaptation, rust resistance and grain quality. Soil borne pathogens are also a high priority. Annually 800-1000 crosses are made which are subjected to conventional multi-locational breeding framework in Turkey. Around 500 new varieties and breeding lines are submitted to IWWIP by its collaborators for evaluation in Turkey and distribution through the international nurseries. The best advanced lines as well as the best introduced lines are annually distributed through FAWWON (Facultative and Winter Wheat Observation Nursery) to more than 100 cooperators in around 50 countries. Till now, around 60 varieties were released in the region occupying more than two million hectares. Cereal cyst nematodes (CCN) represent a high priority for IWWIP breeding

* Morgounov A, Akin B, Dababat AA, Erginbaş-Orakçi G, Keser M, Ozdemir F (2015) International Winter Wheat Improvement Program: Breeding modern germplasm, landraces and synthetics for soil borne pathogens. In 'Nematodes of Small Grain Cereals: current status and research.' (AA Dababat, H Muminjanov and RW Smiley) pp. 35-36. (FAO: Ankara, Turkey).

especially for rainfed conditions. Several thousands of genotypes have been screened and around 100 CCN-resistant entries have been identified and evaluated for a number of traits. Most of them are higher yielding with resistance to stripe rust. The breeding system is based upon utilization of proven sources of resistance with widely grown varieties and advanced high-yielding lines, development of the segregating populations up to F_2 without nematode pressure, the selection of the best plants under CCN pressure in Yozgat, and consequent testing and retesting of resistant progenies. The best resistant germplasm is offered to IWWIP cooperators globally.

(A full-length version of this paper was not available at the time of printing).

HISTORY AND CURRENT STATUS OF THE WHEAT GALL NEMATODE [*ANGUINA TRITICI* (STEINBUCH) FILIPJEV] ON WHEAT IN TURKEY

ADNAN TULEK[1]*, ILKER KEPENEKCI[2], ABDELFATTAH A. DABABAT[3], TUGBA HILAL ÇIFTÇIGIL[1], IRFAN OZTURK[1], AND ALEXEI MORGOUNOV[3]

[1]Thrace Agricultural Research Institute, 22100 Edirne, Turkey; [2]Gaziosmanpaşa University, Faculty of Agriculture, Department of Plant Protection, Taşlıçiftlik - 60250, Tokat, Turkey; [3]The International Maize and Wheat Improvement Center (CIMMYT), Ankara, Turkey. *Corresponding author email: adnantulek@gmail.com

SUMMARY

Wheat is one of the most important agricultural commodities in Turkey, and the country ranks among the top ten producers in the world. It is a staple and strategic crop, and an essential food in the Turkish diet. Wheat seed gall nematode, *Anguina tritici*, was the first plant parasitic nematode to be observed and described. Wheat gall nematode attacks plants and move up to the spikes via a film of moisture to affect new grains. Damage and yield loss are dependent on the initial nematode population. Wheat gall nematode cause significant yield losses and affect grain quantity and quality, yet they remain under-researched. Wheat gall nematode is one of the sporadically important pests of cereals particularly for wheat in Turkey. In Turkey, the first documented study wheat gall nematode were from surveys conducted in the mid-sixties. The infestation rate estimates were 0.2% in the Eastern Anatolia, 1.6 to 55.2% in the Western part, 25.4% in the Marmara, 3.6% in the South East Anatolia, and none at Aegean and Eastern Mediterranean region. The yield loss estimates were 0.2% in the

* Tulek A, Kepenekci I, Dababat AA, Çiftçigil TH, Ozturk I, Morgounov A (2015) History and current status of the wheat gall nematode [*Anguina tritici* (Steinbuch) Filipjev] on wheat in Turkey. In 'Nematodes of Small Grain Cereals: Current Status and Research.' (AA Dababat, H Muminjanov and RW Smiley) pp. 37-44. (FAO: Ankara, Turkey).

Eastern Anatolia and up to 60.0% in Central Anatolia. However a study approximately ten years later, checking in the seed lots of the same region in Central Anatolia, showed the losses of only 0.01%. In 2012, the seed gall nematode was detected in wheat fields of the Thrace region of Turkey. Yield losses in the four wheat varieties Kate-A, Pehlivan, Selimiye, and Glibolu were estimated as 51.3%, 53.2% 56.6%, 59.6%, respectively, with seed infection levels by *A. tritici* of 21.4%, 20.9%, 24.4%, and 18.3%, respectively. In Turkey, wheat gall nematode has been seen and has constituted losses in areas where seed cleaning systems and certificated seedlings are not used.

Key words: Wheat gall nematode, *Anguina tritici*, wheat, *Triticum aestivum* L. yield losses, distribution, prevalence

INTRODUCTION

Wheat (*T. aestivum* and *T. durum*) is the third largest cereal staple with production of 713Mt each year. The three largest producers are China, India and the USA. It is considered the key crop of importance for food security in the regions of West Asia and North Africa. Wheat is a particularly important crop in Turkey; it is considered an essential basic food and is cultivated on an area of 7.8 million ha, producing 22 million tons with an average grain yield of 2.8 t/ha (FAO 2014). Globally, wheat yields are affected by various biotic and abiotic factors (McDonald and Nicol 2005, Nicol *et al.* 2006, Dababat *et al.* 2014). The seeds are the most prevalent form of exchanged or traded agricultural commodity and seed-transmitted pests use this to their advantage and spread.

Anguina tritici [Steinbuch] causes ear-cockle disease of wheat, turning the seed into seed galls. Other species of *Anguina* attacks other crops. Needham, in 1743, observed soft white fibrous wool on brushing blackened wheat and, when separated in a drop of water, observed larvae within the galls. It is a large nematode, ranging from 3-5 mm in length. *Anguina tritici* has a three-part esophagus and the esophageal glands do not overlap with the intestine. The female body tends to be thickened and curved ventrally. It has a short stylet (8-11 µm). Females have one ovary and the vulva located posterior. Males possess small spicules and small bursae or alae (Tiwari and Khare 2000).

Common hosts are wheat (*Triticum aestivum*), emmer (*Triticum monococcum*), rye (*Secale cereale*), spelt (*T. spelta*), and *Lolium temulentum*. Barley (*Hordeum vulgare*) is a very poor host. There is no evidence that this nematode reproduces on oats (*Avena sativa*) and other grasses.

The symptoms are characterized by enlargement and swelling of the basal part of the stem, near the soil base, visible in 20-25 days old infested seedings. The leaves emerging from such seeding are twisted and crinkled. Frequently, some leaf sheaths are observed to be folded or twisted with their tips held near the growing point. Such leaves, however, recover almost too normal, 30 to 45 days after they have emerged from the growing point. In severe infections, all seeds in an ear may turn into green and soft galls in the beginning but become hard and brown to black in colour on crop maturity. The infected culms either die in the young stage of growth or may grow until the heading stage. In the latter case, either the emerging spike is narrow or short with grains partially or completely replaced by the bacterial mass or it may fail to emerge from the boot leaf. The glumes are spread out in the diseased ears. The stalk is always distorted when such infected ears show the bacterial infection. One of the common observations for the nematode infested wheat plants is that they are dwarfed, have more tillers and in the initial stages, the growth rate is comparatively more than in the healthy plants (Tiwari and Khare 2000).

Anguina tritici acts as both an ecto- and endo-parasite during growth of wheat plants. Perpetuation of the nematode takes place in seed galls. Nematode galls which fall in soil along with the contaminated seed at the time of sowing or may be present already from the previous season, absorb moisture and become soft, facilitating the release of the second stage larvae. Germinated wheat seed starts sprouting within three days after planting and the earliest release of larvae from galls is on the 4th day, which is always preceded by release of the juveniles in the soil. Juveniles (J2) emerge from the seed galls in the soil and move onto the newly germinated seedlings. These galls may fall into the soil at harvest and may serve as the source of new infections in the following season, if wheat is produced in the same field. However, a majority of the galls remain as contaminants of the harvested seed and serve as the source of new infestation with the use of the contaminated seed for sowing (Tiwari and Khare 2000). The J2 stage penetrates flower primordia and develops through the third and fourth stages to adulthood. The final molt to adulthood occurs only after the seed gall has formed. Galls can develop from undifferentiated flower buds, stamen tissues, and various other tissues. Galls contain up to 80 adults in a 1:1 sex ratio. Reproduction is amphimictic; mating occurs and females produce up to 2000 eggs per individual over several weeks. Thousands of J2s are present in seed galls. Galls fall to the ground, absorb water, and release juveniles in spring time, or galls may be harvested and stored with seed. Juveniles within drying galls can enter a cryptobiotic state; viable juveniles have been recovered for up to periods as long as 38 years. Galls appear darker, shorter, and thicker than

seed kernels. When galls become wet and absorb water, juveniles are activated. For nematodes forming seed galls, one generation is produced per year; more than one generation per year can be produced for nematodes that form leaf galls and more stages may be found in the gall. Ectoparasitic feeding of *A. tritici* may cause leaf rolling, curling, and spiraling. Plants mature more slowly, and produce smaller seed heads.

Crop rotation for 1 to 2 years with a non-host eliminates *A. tritici* from the soil. The nematode does not survive by feeding on fungi. Seed can be cleaned by placing it in a 20% brine solution; galls float to the surface where they can be separated. The seed is then rinsed and dried (heat treated). Mechanical separation is also effective in removing galls from seed. So far, there are no resistant varieties of wheat to *A. tritici.*

Generally known as ear cockle, *A. tritici* has been reported in West Asia, North Africa (Sikora 1988), and some regions of eastern Europe (Tesic 1969, Swarup and Sosa-Moss 1990), as well as specifically in Australia, Brazil, China, Egypt, England, Ethiopia, France, Germany, India, Iraq, Italy, Hungary, Netherlands, New Zealand, Pakistan, Romania, Russia, Sweden, Switzerland, Syria, Turkey, Turkmenistan, USA, and Yugoslavia (Yüksel *et al.* 1980, Maqbool 1988, Stephan 1988, Esser *et al.* 1991, McDonald and Nicol 2005). Generally, infection by *A. tritici* is more common in fields lacking modern agronomic practices and/or certificated seed, and can cause significant economic losses.

Anguina tritici was the first plant parasitic nematode to be described in the literature in 1743. Yield losses up to 70% have been reported, which ranged from 30-70%. Threshold of 10,000 juveniles /kg soil develop disease and are considered a damaging level. The damage is negligible in countries adopting modern mechanical and cleaning procedures to separate the nematode galls from wheat seeds. The use of high quality pathogen-free seeds has nearly eradicated this nematode from developed countries. The nematode also causes severe crop losses to rye 35- 65% (Anwar *et al.* 2001, Leukel 1929, 1957) in 3rd world countries, where poor agricultural practices, monoculture, and the use of poor quality seeds are used in sowing. Byars (1919) reported 30-80% yield loss due to cockle disease of wheat and the annual monetary loss was estimated as 2-3 crores rupees in India (Dhawan and Pankaj 1998).

In Turkey, the first documented study wheat gall nematode were from surveys conducted in the mid-sixties. A survey was conducted by Öztüzün (1970) after

harvesting in the storages, in the Southeastern part of Turkey in the year of 1967. According to the survey; 1 out of 12 seed samples were found contaminated in Hilvan district of Urfa province, 10 out of 11 seed samples in Suruç district of Urfa Province, 4 out of 19 seed samples in central district of Urfa Province, 1 out of 5 seed samples in Akçakale district of Urfa Province, 1 out of 5 seed samples in Nusaybin district of Mardin Province, 1 out of 3 seed samples in Tatvan district of Bitlis Province, and 3 out of 3 seed samples in Erciş district of Van Province.

Another survey was carried out in Black Sea region in the years of 1961-1968. The Tokat Province and its district were found contaminated with *Anguina tritici* (Bora 1970)

During 1976-1980, samples were collected from 1126 villages of 78 counties in eastern Anatolia (Yüksel *et al.* 1980). It was reported that levels of gall-infected wheat grains (caused by *A. tritici*) were highest in Erzurum and Kars plateaus (0.58% grain infection) and lowest in the Samsun-Amasya basin (0.03% grain infection). In the Eastern Part of Anatolia an average more than 0.20% of the wheat yield is lost by wheat gal nematode. Besides the other factors, the damage caused by wheat gall nematode fluctuates according to the sowing season and of wheat being irrigated or not. The destructiveness of the nematode on winter wheat is two or three times more than spring wheat and also the damage on wheat grown in irrigated conditions is more than the wheat grown under dry conditions.

In another study investigating the spread and severity of *A. tritici* infections in the wheat fields of Bilecik and its districts during 1983-84, Ağdacı and Efe (1986) found that 74 of 291 tested areas were infected with *A. tritici*, with percentage of infected fields being 25.4%. In storages, 294 tons of crops were inspected and wheat gal nematode contamination was found to be 1-15%. At the result of the surveys 292 samples were examined from nine provinces in the region and only eight samples were found contaminated with nematode. Six of the eight samples belong to Bilecik and the one to Kütahya and the other one belongs to Kırklareli.

More recently, Elmali (2002) investigated the wheat growing areas of 27 provinces and found that 22 of them (81.58%) were infected with *A. tritici.* Percentage of infected seed samples was highest in Aksaray (55.22%) and lowest in Bursa (1.6%).

Ozberk *et al.* (2011) studied on the effects of *A. tritici* on grain yield, quality and marketing price of durum wheat in the region of Viranşehir, Anatolia, Turkey during the 2008-2009 growing season. Grain yield losses due to *A. tritici* were reported up to 32%. Seed-gall also reduced the number of grains spike^{-1}, grain weight spike^{-1}, test weight (kg hl^{-1}), and 1000-kernel weight (g), but had no effect on SDS protein content (%). This effect on quality impacts the price of wheat; when levels of *A. tritici* contamination rose from 5% to 20%, the price of durum wheat fell from US $335 to US $297 per ton.

Another study in Turkey evaluated effects of *A. tritici* on four bread wheat varieties widely cultivated in Turkey, and found larger than expected losses in yields. Yield losses caused by wheat seed-gall nematode infection were found to be significant. For example, variety Gelibolu averaged grain losses of 59.6% and seed infection ratios of 18.3%. The 215 spikes of the Pehlivan variety selected at harvest for signs of nematode damage (folds and leaf shape deformations) were threshed separately. For these spikes, grain infection ratios ranged from 0-100%, with an average of 61.81% (Tulek *et al.* 2015).

FUTURE PERSPECTIVES

As a consequence, it is inevitable that breeding for resistance and perhaps tolerance is the major strategy for long-term and environmentally sound control of this pathogen. Hence there is a great need for global collaborative research programmes. Furthermore, the adoption of molecular tools to assist both in pathogen identification and plant breeding will become an integral part of future research developments and ultimate control of these important pathogens.

REFERENCES

- Ağdacı M, Efe E (1986) The preliminary study on the extend of distribution and the degree of contamination of wheat gall nematode (*Anguina tritici* Filipjev, 1936) in wheat growing areas in Marmara Region. *Bitki Koruma Bülteni Cilt*, 26, No: 1-2.
- Anwar SA, McKenry MV, Riaz A, Khan MSA (2001) Evaluation of wheat cultivars for *Anguina tritici* resistance, development and influence of nematode on wheat growth. *International Journal of Nematology*, 11, 150-156
- Bora A (1970) Investigations on the determination of plant parasitic nemas on Black Sea Region and their distribution and chemical control possibilities. *Plant Protection Bulletin*, 10, 53-71

- Dababat AA, Imren M, Erginbas-Orakci G, Ashrafi S, Yavuzaslanoglu E, Toktay H, Pariyar SR, Elekcioglu HI, Morgounov A, Mekete T (2014) The importance and management strategies of cereal cyst nematodes, *Heterodera* spp., in Turkey. *Euphytica*, 202, 173-188.
- Dhawan SC, Pankaj (1998). Nematode pests of wheat and barley. In: 'Nematode Diseases in Plants.' (Ed. PC Trivedi). *CBS Publishers and Distributors, New Delhi, India*.
- Elmali M (2002) The distribution and damage of wheat gall nematode [*A. tritici* (Steinbuch)] (Tylenchida: Tylenchidae) in western part of Anatolia. *Turkish Journal of Entomology*, 26 (2), 105-114.
- Esser RP, O'Bannon, JH, Clark RA (1991) Procedures to detect wheat seed gall nematode (*Anguina tritici*) should an infestation appear in Florida. *Gainesville, FL, USA, Nematology Circular No. 186.*
- *FAO, Food and Agricultural Organization, 2014. http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor*
- Leukel RW (1924) Investigation on the nematode disease of cereals caused by *Tylenchulus tritici*. *Journal of Agricultural Research*, 27, 925-955
- Leukel RW (1929) The nematode disease of wheat and rye. *U.S. Department of Agriculture Farmers' Bulletin* 1607:11.
- Leukel RW (1957) To treat seed grain. U.S. Department of Agriculture Misc. Publ. 218, Washington DC.
- Maqbool MA (1988) Plant status of research on plant parasitic nematodes in cereals and food legumes in Pakistan. In: 'Nematodes Parasitic to Cereals and Food Legumes in Temperate Semi-arid Regions. (Eds. MC Saxena, RA Sikora, JP Srivastava) pp. 173-180. (ICARDA, Aleppo, Syria).
- McDonald AH, Nicol JM (2005) Nematode parasites of cereals. In: 'Plant Parasitic Nematodes in Subtropical and Tropical Agriculture'. (Eds. M Luc, RA Sikora, J Bridge) pp. 131-192. (CAB International, Wallingford, UK).
- Nicol JM, Bolat N, Bagci A, Trethowan RA, and 20 others. (2006) CIMMYT and Turkey international shuttle breeding program to develop wheat lines with *Fusarium* crown rot and other soil borne pathogen resistances. In: 'The Global *Fusarium* initiative for International Collaboration.' (Eds. T Ban, JM Lewis, EE Phipps) pp 110-116. (CIMMYT, El Batán, Mexico).
- Ozberk I, Yolcu S, Yücel A, Köten M, Nicol JM (2011) The impact of seed gall nematode on grain yield, quality and marketing prices on durum wheat in Anatolia, Turkey. *African Journal of Agricultural Research*, 6 (16), 3891-3896.
- Öztüzün N (1970) Doğu ve Güneydoğu Anadolu Bölgesi Kültür Bitkilerine Arız Olan Bitki Parazit Nematodları Üzerinde Sürvey Çalışmaları. *Bitki Koruma Bülteni*, 10 (3), 180-198. (Turkish summary)

- Sikora RA (1988) Plant parasitic nematodes of wheat and barley in temperate semi-arid regions - a comparative analysis. In: 'Nematodes Parasitic to Cereals and Legumes in Temperate Semi-arid Regions.' (Eds. MC Saxena, RA Sikora, JP Srivastava) pp. 46-68. (ICARDA, Aleppo, Syria).
- Stephan ZA (1988) Plant parasitic nematodes on cereals and legumes in Iraq. In: 'Nematodes Parasitic to Cereals and Legumes in Temperate Semi-arid Regions.' (Eds. MC Saxena, RA Sikora, JP Srivastava) pp. 155-159. IICARDA, Aleppo, Syria).
- Swarup G, Sosa-Moss C (1990) Nematode parasites of cereals. In: "Plant Parasitic Nematodes in Sub-tropical and Tropical Agriculture.' (Eds. M Luc, RA Sikora, J Bridge) pp. 109-136. (CAB International, Wallingford, UK).
- Tesic T (1969) A study on the resistance of wheat varieties to wheat ealworm (*A.tritici* Stein). *Savrenema Poljoprivieda*, 17, 541-543.
- Tiwari SP, Khare MN (2000) Ear - cockle: an important seed borne disease of wheat. In: 'Glimpses in Botany.' (Eds. KG Mukherji, BP Chamola, AK Sharma) pp. 349-361. (AK APH Publishing Corporation, New Delhi, India).
- Tulek A, Kepenekci I, Ciftcigil TH, Ozturk I, Akin K, Seidi M, Yıldırım M, Dababat AA (2015) Effects of seed-gall nematode (*Anguina tritici)* on bread wheat grain characteristics and yields in Turkey. *Nematology*, 17, (in press).
- *Yüksel* H, Güncan A, Döken MT (1980) The distribution and damage of bunts (*Tilletia* spp.) and wheat gall nematode [*Anguina tritici* (Steinbuch) Chitwood] on wheat in the eastern part of Anatolia. *Journal of Turkish Phytopathology*, 9, 77-88.

THE STATUS OF CEREAL NEMATODES IN PAKISTAN

MUHAMMAD AMJAD ALI[1,2,*], AMJAB ABBAS[1], NAZIR JAVED[1], SHAHBAZ TALIB SAHI[1], MUHAMMAD IMTIAZ[3], AND ABDELFATTAH A. DABABAT[4]

[1]Plant Nematology Lab, Department of Plant Pathology, University of Agriculture, Faisalabad; [2]Centre of Agricultural Biochemistry and Biotechnology, University of Agriculture, Faisalabad; [3]CIMMYT Representative for Pakistan, CIMMYT, Int., NARC, Park Road Islamabad, Pakistan; [4]CIMMYT, ICARDA-CIMMYT Wheat Improvement Program, Ankara, Turkey; *Corresponding author emails: amjad.ali@uaf.edu.pk, amjad.ali2001@gmail.com

SUMMARY

The cereal cyst nematodes (CCNs), *Heterodera avenae*, *H. mani* and *H. zeae* were first reported in spring wheat (*Triticum aestivum*) and maize (*Zea mays*) in 1981 in Pakistan. A new CCN species, *Heterodera pakistanensis* specific to wheat was also reported from Pakistan in 1986. Several surveys were carried out to assess the geographic distribution and estimate yield losses caused by CCNs. In 1986, a survey documented 40 nematode species associated with wheat and 22 with barley. The survey reported crop losses caused by various CCNs with 15-20% of the losses by *H. avenae* alone, which has emerged as a serious menace for wheat production worldwide. In the 2003-2004 survey, 39% of the surveyed area was found to be infested with cyst nematodes with relatively high distribution and host range of corn cyst nematode, *H. zeae*. To date, no molecular identification and phylogenic studies have been carried out for characterization of CCNs and their pathotypes in various geographical zones of Pakistan. However, recently at the plant nematology lab at University of Agriculture, Faisalabad started preliminary work on the molecular

* Smiley RW (2015) Effects of cropping systems on dominance of individual species of *Pratylenchus*. In 'Nematodes of Small Grain Cereals: current status and research.' (Eds AA Dababat, RW Smiley) pp. 45-56. (FAO: Ankara, Turkey).

characterization of cyst nematodes associated with cereals and other crops. The genetic diversity for cereal cyst nematode resistance was assessed in spring wheat genotypes using RAPD and SSR markers by Erum *et al.* (2013). Moreover, the association of *XGWM 301* locus with the presence of *Cre3* gene was also worked out. Additionally *Meloidogyne graminicola* has also been isolated from rice and wheat plants from the rice growing areas of Central Punjab. It has been estimated that this nematode can cause 17-20% yield losses in rice. It is now being reported as an emerging threat to cereal crops in the areas where the rice-wheat-cropping system has existed for decades. This paper covers the status of cereal nematodes research in Pakistan and provides the prospects and recommendations for future research on nematodes to protect wheat and other cereals.

INTRODUCTION AND HISTORY

Bread wheat (*T. aestivum*) is the most important cereal crop in Pakistan. Pakistan is the 7th leading producer of wheat after EU, China, India, USA, Russia and Canada in 2013 (Economic Research Service, USDA). Wheat is grown on a large area in the country and it covers the most of the cultivated land as compared to any other crop, and is followed by cotton and rice (Table 1).

The area under wheat cultivation in the world during 2007-2008 was 192 million ha, with production of 726 million tones and average yield 2823 kg ha^{-1} (USDA, 2014), where as in Pakistan area under this crop was 9.199 million ha, and production was 25.98 million tones with grain yield of 2824 kg ha^{-1} (Pakistan Bureau of Statistics) (Table 2). However, there is a very big gap between New Zealand (which is giving the highest production in terms of per unit area) and Pakistan (32nd position in terms of production per unit area) (USDA, 2014).

The wheat grain is being used as staple food for almost all populations of the country. Wheat breeders in Pakistan have been striving to enhance its production with the passage of time since the dawn of so-called "Green Revolution" in the 1960s. However, on the other hand, the country's population also increased at the same rate with the passage of time (Figure 1). So it is very important to devise the strategies to enhance wheat production in the country to feed this ever-increasing population. This could be achieved by developing varieties with high productivity and incorporation of resistance against various abiotic and biotic agents including cereal cyst nematodes.

Table 1. Area under important crops as per each decennial agricultural census (million ha)*

Crops / Year	1972	1980	1990	2000
Wheat	6.479	7.260	8.166	9.469
Rice	1.789	2.238	2.420	2.918
Cotton	2.388	2.319	2.679	3.201
Sugarcane	0.498	0.652	0.720	0.882
Maize	0.680	0.542	0.826	0.874
Oil Seed	0.542	0.490	0.449	0.457
Pulses	1.611	1.465	1.052	1.275
Fodders	2.728	2.719	2.756	2.485
Potato	0.032	0.049	0.109	0.097
Vegetables	0.279	0.279	0.530	0.478
Orchard	0.166	0.235	0.384	0.380

*Taken from Pakistan Bureau of Statistics- Agriculture Census

Table 2. Area and production of important crops in Pakistan (Pakistan Bureau of Statistics, 2014)

Crops	2011-12		2012-2013		2013-2014	
	Area (000 ha)	Production (000 ton)	Area (000 ha)	Production (000 ton)	Area (000 ha)	Production (000 ton)
Wheat	8649.8	23473.4	8660.2	24211.4	9199.3	25979.4
Maize	1087.4	4338.4	1059.5	4220.1	1168.5	4944.2
Rice	2571.2	6160.4	2308.8	5535.9	2789.2	6798.1
Sugarcane	1057.5	58397.0	1128.8	63749.9	1172.5	67460.1
Cotton	2834.5	13595.0	2878.8	13030.7	2805.7	12768.9

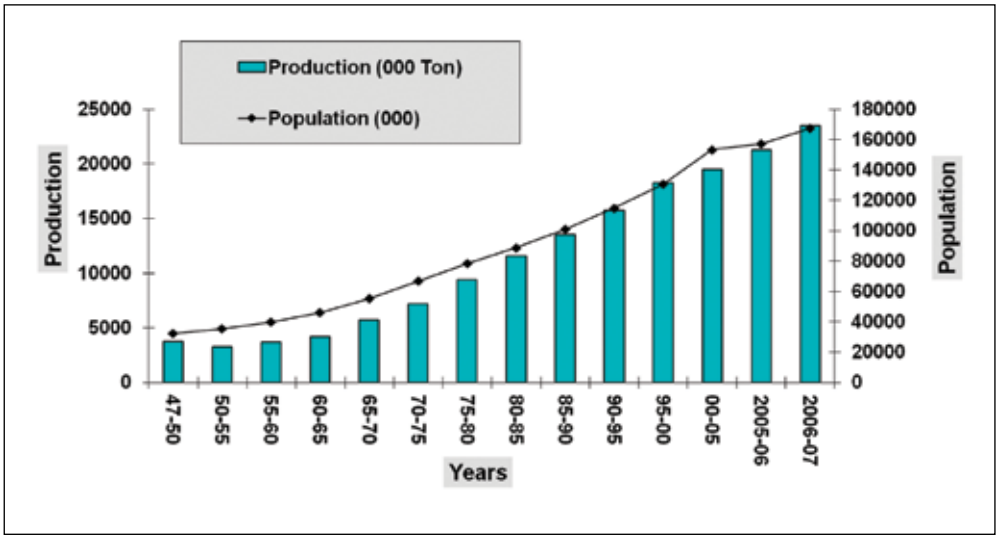


Figure 1. Comparison of wheat production and population growth in Pakistan (Courtesy: Wheat Research Institute, AARI, Faisalabad)

The per unit area production of wheat in Pakistan is lower than other countries due to several reasons; i.e., delayed planting, improper management, shortage of irrigation water, diseases and insect attack. There is a variety of devastating pathogens that attack wheat crops at different growth stages after planting and cause various diseases like rusts, smuts, bunts, bacterial leaf blight and crown rot. Wheat is also attacked by variety of plant parasitic nematodes; i.e., cereal cyst nematode (CCNs), root lesion nematodes (RLN), root-knot nematodes, and seed gall nematode. These nematodes are obligate biotrophic parasites which exert detrimental effects on agricultural production worldwide. Cereal cyst nematodes within the family *Heteroderidae* and root lesion nematodes (Family: *Pratylenchidae*) are especially dangerous for cereal crops.

Among these, CCNs are a serious threat to wheat crop by causing direct or indirect losses in Pakistan. Rice (*Oryza sativa*) and maize (*Zea mays*) are second and third most important and the most grown cereal crops after wheat respectively (Table 1 and 2). The production of these cereal crops is also affected by infestations of parasitic nematodes. There are several nematode species which affect cereal crops. Around 45 of the stylet bearing nematode species were recorded to be associated with wheat and barley; however, most of them have no impact on grain yield (Maqbool 1986). Ear-cockle disease caused by gall cyst nematode *Anguina tritici* in wheat, cereal cyst nematode (*H. avenae*) and corn cyst nematode (*H. zeae*) are major pests of cereals in the country (Maqbool 1997).

In 1917, seed gall nematode, *Anguina tritici* was firstly reported from the province of Punjab, however, after the foundation of Pakistan this species was reported in the districts of Dera Ghazi Khan, Muzaffargarh and Jhang in the Punjab Province (Maqbool 1997). Similarly, cereal cyst nematode (*H. avenae*) was first reported by Maqbool (1980, 1981) in the fields of wheat and maize in the province of NWFP (Now, Khyber-Pakhtunkhwa). In the same report, *H. mani*, *H. mothi* and *H. zeae* were reported in wheat and maize Maqbool (1981). Very recently, *M. graminicola* was reported by our research group from the rice growing districts of Central Punjab (Jabbar *et al.* 2015). It is now being reported as an emerging threat to cereal crops in the areas where the wheat-rice cropping pattern has prevailed for decades.

STATUS

The nematode problems are serious and complex in Pakistan. The main reason for this is the wide diversity of climatic conditions in the country, which

range from tropical to temperate. However, mainly, Pakistan lies in the tropical region where the climatic conditions are suitable for the activity and reproduction of nematodes throughout the year. Moreover, the soil type (i.e., sandy and warm), as found in the arid zone is most favorable for nematode development and infestation. Similarly, cultivation of perennial crops in irrigated areas and monoculture year after year has resulted in huge nematode development in these areas. The climatic changes over the last two decades are so diverse that they could lead from economical losses to sudden epidemic in the country and might be a big issue of the future that interrupts a sustainable supply of this commodity on which entire nation relies for their primary food needs.

In a very early survey done to determine the infestation and occurrence of cereal cyst nematodes, several nematode species were found to be associated with wheat and barley. Forty five stylet bearing nematode species were found to be associated with wheat and barley; however, most of them have no impact on grain yield (Maqbool 1986). This survey demonstrated 45 nematode species were associated with wheat and 22 with barley.

Cereal cyst nematodes, *Heterodera avenae*, *H. mani*, *H. mothi* and *H. zeae* are quite prevalent in wheat and barley fields in Pakistan (Maqbool 1980, 1981, 1986, 1997). Similarly, *Aphelenchus avenae*, *Hirschmanniella oryzae* and *Ditylenchus angustus* were predominant in rice fields. *Helicotylenchus* sp., *Hoplolaimus* sp., *Pratylenchus zeae* and *Heterodera zeae* were widespread and abundant plant-parasitic nematodes on maize. Maqbool (1997) reviewed the occurrence of cereal nematodes and provided a list of the 60 new nematode species associated with cereal crops in Pakistan.

According to a survey done in 2003-2004, 39% of the surveyed area was found to be infested with cyst nematodes with relatively wide distribution and host range of corn cyst nematode, *H. zeae* (Shahina and Erum 2007). In many articles, it is considered that the host range of *H. zeae* is limited to the family *Graminae*. On the other hand, in Pakistan, the presence of *H. zeae* has also been reported on plants of economic importance belonging to other important families such as sweet pepper (*Capsicum annuum*), citrus, jute (*Corchorus capsularis*), pear (*Pyrus communis*), almond (*Prunus dulcis*), small radish (*Raphanus sativus*) and tomato (*Solanum lycopersicon*) (Shahzad and Ghaffar 1986, Shahina and Erum 2007).

Similarly, the host range of root lesion nematode; *Pratylenchus zeae*, was studied in Pakistan and it was demonstrated that this nematode invades the roots of a variety of plant species from different families (Hashmi and Hashmi 1989). It was reported that this nematode species infects the roots of radish, carrot, tomato, chickpea, wheat, potato, onion, barley and different varieties of maize. Anwar *et al.* (1993) provided a comprehensive report on the nematode species infesting rice plants and weeds in rice field. They concluded that there was massive occurrence of *Aphelenchoides besseyi*, *Hirschmanniella* sp., and *Pratylenchus* sp. in these fields.

The prevalence of *Meloidogyne graminicola* is now alarming the producers of wheat and rice in the wheat-rice cropping zone. It is now being reported as an emerging threat to cereal crops in the areas where the wheat-rice cropping pattern has prevailed for decades (Jabbar *et al.* 2015).

The incorporation and evaluation of resistance against cereal nematodes in wheat is priority research subject in Pakistan for plant breeders and pathologists. The microsatellite marker XGWM 301 was found to be associated with the presence of *Cre3* gene. On the basis of this information, screening of 120 wheat cultivars and lines against cereal cyst nematodes was carried out (Erum and Shahina 2010). This study revealed that out of these 120 wheat lines, 30 were found to be resistant due to the presence of the XGWM 301 marker. However, recently the genetic diversity for cereal cyst nematode resistance was assessed in spring wheat genotypes using RAPD and SSR markers (Erum *et al.* 2013). On the basis of RAPD markers, 6 CCN resistant wheat genotypes (TD-1, SD-8006, Marvi-2000, Moomal-2002, Inqilab-91 and Bhittai) were found to be genetically diverse. However, Marvi-2000 was found the most diverse on the basis of 46 SSR primers and was identified as the most resistant genotype against CCN (Erum *et al.* 2013). Erum *et al.* (2013) recommended that the potential of Marvi-2000 should be utilized for breeding resistant lines against CCN. The strategies like chemical control, biological control and use of resistant wheat cultivars are being employed to manage nematodes in Pakistan.

Unfortunately, to this date, very little work has been carried out on molecular identification and phylogenic studies for characterization of CCNs and their pathotypes in various geographical zones of Pakistan. Now our group at the Plant Nematology Lab, in the Department of Plant Pathology at the University of Agriculture, Faisalabad, is establishing the facilities for molecular identification and characterization of CCNs and other nematode species.

IMPACTS

Plant-parasitic nematodes cause serious reduction in crop yields resulting in annual crop losses worth over $150 billion worldwide (Abad *et al.* 2008). Altogether, plant-parasitic nematodes can cause up to 20% yield losses in individual crops, which can be overwhelming for low-income farmers in the developing countries (Atkinson *et al.* 1995). Crop losses caused by CCNs rely heavily on environmental conditions and could be up to 92% in some environments (Nicol *et al.* 2011). The *CCN H. avenae* is a dangerous species resulting in the yield *losses worldwide up to 92%. However, this nematode causes 15*-20% yield losses in wheat in *Pakistan* (Maqbool 1988). On the other hand, minute economic losses (2-3%) are caused by the seed gall nematode, *A. tritici*, in bread wheat in Pakistan (Maqbool 1987). The corn cyst nematode, *H. zeae*, results in 13-73% suppression of maize growth and yield (reviewed by McDonald and Nicol 2005). In Pakistan, the yield losses caused by *H. zeae* are around 15% in barley (Maqbool 1987). Recently we have estimated that *M. graminicola* can cause 17-20% yield losses in rice (Jabbar *et al.* 2015). Figure 2 indicates the rice field infested with *M. graminicola*. On the right side in Figure 2, hook like knots (arrows) caused by *M. graminicola* are quite visible. The host range and crop losses caused by this nematode are being worked out at our department. Rao and Biswas (1973) estimated that after the addition of every 1,000 *M. graminicola* larvae, rice yield decreased at the rate of 2.6%. Corn cyst nematode *H. zeae* has shown a wide host range, which could result in serious crop losses due to this nematode especially in hot and dry areas. Similarly, lesion nematodes like *Pratylenchus brachyurus*, *P. thornei*, *P. zeae* and *P. penetrans* have wide host ranges. These lesion nematodes also infect weed hosts that highly influence the development of nematode inoculum in infested fields. Wide host range of nematode species associated with cereal crops is very devastating because the nematodes are able to perpetuate on other crops/weeds and are ready to infect the next season's crop. *P. thornei* has been widely studied worldwide and has caused crop losses up to 75% (Nicol and Rivoal 2008), but the yield losses due to this species are not still estimated in Pakistan.

PROSPECTS

From the previous reports and from our own findings, it is quite evident that nematodes are a serious threat to cereal crops, and specifically to wheat. But the knowledge regarding diversity, virulence, alternative host crops and prev-

Figure 2. A. Above-ground symptoms in rice field infested with M. graminicola; **B.** Roots infected with M. graminicola, the hook-like knots (arrows) caused by M. graminicola are quite visible (reproduced from Jabbar et al. 2015).

alence of these plant parasites is not established to such a stage as to draw a logical consequence regarding the local conditions of the Pakistan. To answer such questions, and keeping in mind the diversity of nematodes worldwide, comprehensive surveys in the core areas of cereals along with noncore areas in Pakistan are important. Such surveys will provide very basic information about the diversity if these surveys are based on the modern sophisticated molecular techniques and on controlled-virulence testing. Since the nematodes need hosts to grow in the lab, stock keeping and maintaining the cultures is still a challenge. The study of plant-nematode interactions at the molecular level to find the basis of resistance in plants and various defense pathways involved in the incompatible interactions is still to be worked out for CCNs. If the resistance against nematode is supported by different set of genes or gene families, then in the future gene pyramiding would be a tool to address the problem in a more aggressive way. In this regard, conventional breeding and molecular biology could be valuable approaches. A lack of expertise and knowledge for identification and recognition of CCNs is an important factor that limits wheat production potential, coupled with improper breeding strategies and slow screening processes that reduce genetic gains for resistance to CCNs (Dababat *et al.* 2014). So the available wheat germplasm and advanced lines of wheat could be challenged against CCNs (especially *H. avenae*) for characterization in response to CCN, which up to the date, has not been performed in Pakistan. Recently, Dababat *et al.* (2014) developed management strategies against *H. avenae* and *H. filipjevi,* two important CCNs, by using wheat cultivars and their wild relatives in Turkey. This kind of strategy must be explored and utilized in Pakistan for the management of CCNs.

National and international collaboration will be helpful to further explore the basic and applied issues in nematology research in Pakistan. Such basic understandings of nematode virulence and diversity will provide a platform for the selection of more resistant plant types from our germplasm and will boost our yield in future.

ACKNOWLEDGEMENTS

The authors would like to thank CIMMYT-Turkey and CIMMYT-Pakistan for financial support to attend this workshop in Ankara, Turkey. Moreover, the authors extend thanks to the Higher Education Commission for supporting the studies. The authors also acknowledge Endowment fund, UAF for financial support. The authors acknowledge the scientific support from Wheat Research Institute, AARI, Faisalabad.

REFERENCES

- Abad P, Gouzy J, Aury JM, Castagnone-Sereno P, Danchin EGJ, Deleury E, Perfus-Barbeoch L, Anthouard V, Artiguenave F, Blok VC *et al.* (2008) Genome sequence of the metazoan plant-parasitic nematode *Meloidogyne incognita*. *Nature Biotechnology*, 26, 909-915.
- Anwar SA, Dorsi S, Rauf CA (1993) Nematode diseases of rice in Punjab, Pakistan. *Pakistan Journal of Agricultural Research*, 14(2&3), 184-191
- Atkinson HJ, Urwin PE, Hansen E, Mcpherson MJ (1995) Designs for Engineered Resistance to Root-Parasitic Nematodes. *Trends in Biotechnology*, 13, 369-374.
- Dababat A, Imren M, Erginbas-Orakci G, Ashrafi S, Yavuzaslanoglu E, Toktay H, Pariyar SR, Elekcioglu HI, Morgounov A, Mekete T (2015) The importance and management strategies of cereal cyst nematodes, *Heterodera* spp., in Turkey. *Euphytica*, 202, 173-88.
- Erum YI, Shahina F (2010) Validation of XGWM 301 microsatellite marker against cereal cyst nematode gene. *Pakistan Journal of Nematology*, 28, 143-144.
- Erum YI, Kazi AM, Kazi AG, Shahina F (2013) Genetic diversity assessment of cereal cyst nematode resistant wheat genotypes using different molecular marker systems. *Pakistan Journal of Nematology*, 31, 139-152.
- Hashmi S, Hashmi G (1989) Host status of several economic plants to *Pratylenchus zeae* from Pakistan. *Nematropica*, 19, 195-197.

- Jabbar A, Javed N, Khan SA, Ali MA (2015) *Meloidogyne graminicola* an emerging threat to rice and wheat in Punjab province in Pakistan. *Pakistan Journal of Nematology*, 33(2), 227-228
- Maqbool MA (1981) Occurrence of root-knot and cyst nematodes in Pakistan. *Pakistan Journal of Nematology*, 13, 448. [abstract]
- Maqbool MA (1988). Present status of research on plant parasitic nematodes in cereals and food and forage legumes in Pakistan. In: 'Nematodes Parasitic to Cereals and Legumes in Temperate Semiarid Regions.' (Eds. MC Saxena, RA Sikora, JP Srivastava) pp. 173-180. (ICARDA, Aleppo, Syria).
- Maqbool MA (1997) Plant nematode problems and their control in the near east region. *FAO Plant Production and Protection Paper* 144. FAO, 1997 (http://www.fao.org/docrep/v9978e/v9978e0j.htm).
- Maqbool MA, Shabina F (1986) New species of cyst nematode *Heterodera pakistanensis* (Nematoda: *Heteroderidae*) attacking wheat in Pakistan. *Journal of Nematology*, 18, 541-548.
- Maqbool MA (1980) Occurrence of eight cyst nematodes on some agricultural crops in Pakistan. *Journal of Science*, 8, 103-108.
- Maqbool MA (1986) Classification and distribution of plant parasitic nematodes in Pakistan. National Nematological Research Centre, University of Karachi, Karachi-75270, Pakistan.
- Maqbool MA (1987) Present status of research on plant parasitic nematodes in cereals, food and forage legumes in Pakistan. *Proc*eedings of the *Workshop Nematode Parasitic to Cereals Legumes in Temperate Semi-arid Regions*, *Cyprus*, pp. 173-180
- McDonald AH, Nicol JM (2005) Nematode parasites of cereals. In: 'Plant Parasitic Nematodes in Subtropical and Tropical Agriculture. (Eds. M Luc, RA Sikora, J Bridge) pp. 131-191. (CABI Publishing, Wallingford, UK).
- Shahina F, Erum YI (2007) Distribution of cyst nematodes in Pakistan. *Pakistan. Journal of Nematology*, 25, 29-35.
- Nicol JM, Rivoal R (2008) Global knowledge and its application for the integrated control and management of nematodes on wheat. In 'Integrated Management and Biocontrol of Vegetable and Grain Crops Nematodes.' (Eds. A Ciancio, KG Mukerji) pp. 243-287. (Springer Academic Publishing)
- Nicol JM, Turner SJ, Coyne DL, den Nijs L, Hockland S, Tahna Maafi Z 2011. Current nematode threats to world agriculture. In: Genomics and Molecular Genetics of Plant-Nematode Interactions. (Eds. J Jones, G Gheysen, C Fenoll) pp. 21-43. (Springer, Heidelberg, Germany).

- Rao YS, Biswas H (1973) Evaluation of yield losses in rice due to the root-knot nematode *Meloidogyne incognita*. *Indian Journal of Nematology*, 3, 74
- Shahzad S, Ghaffar A (1986) Tomato, a natural host of *Heterodera zeae* in Pakistan. *International Nematology Network Newsletter* (USA), 3, 38.

PART 2

ECONOMIC IMPORTANCE AND POPULATION DYNAMICS





CURRENT OCCURRENCE OF CEREAL CYST NEMATODES IN SOME FIELDS OF NORTHERN ALGERIA

FATIMA HADDADI[1,*] AND AISSA MOKABLI[2]

[1]Faculty of Biological Sciences, USTHB, Algiers, Algeria. [2]Faculté des Sciences de la Nature et de la Vie et des Sciences de la Terre, Université de Khemis Miliana, Ain Defla, Algeria. *Corresponding author email: fatima.haddadi@yahoo.fr

SUMMARY

The occurrence and distribution of the cyst nematodes belonging to the *Heterodera avenae* Group in the cereal growing areas of Algeria was studied during 2009-2011. Forty soil samples collected from cereal plots spread over 22 localities throughout the country were analyzed. The survey included wheat and barley fields. The results showed that cereal cyst nematodes (CCNs) are widely distributed and were present in 92.5% of the soil samples. Population densities of CCN in soil varied widely between localities and even within plots at an incidence of 3.6 - 281 cysts per kg soil. The highest levels of infestation are noted in areas with cereal vocation, located in the highlands as at Bouandas (Sétif) and Tamlouka (Guelma) where they were about 281 and 267 cysts/kg of soil, respectively.

* Haddadi F, Mokabli A (2015) Current occurrence of cereal cyst nematodes in some fields of northern Algeria. In 'Nematodes of Small Grain Cereals: current status and research.' (AA Dababat, H Muminjanov and RW Smiley) pp. 59-66. (FAO: Ankara, Turkey).

Keywords: Occurrence, cereal cyst nematodes, Algeria, *Heterodera* spp.

INTRODUCTION

Cereals are the most important food source in the world. About 70% of the land intended for food crops are devoted to cereal crops (Riley *et al.* 2009). In Algeria, cereal food is of interest and is important socio-economically. Winter cereals (durum wheat, soft wheat and barley) are of strategic importance in human nutrition and animal feed. Cereals and their derivatives provide more than 60% of the caloric content and 75 to 80% of protein intake in the diet. Cereal products consumption is around 205 kg/capita/year (Chehat 2007). The grain land (fallow included) occupies about 80% of the agricultural area. The area sown to cereals is between 3 and 3.5 million ha annually. But only a third of the acreage is located in the bioclimatic floor that has an average annual rainfall greater than 450 mm (Djermoun 2009).

National production reached 49.12 million quintals in 2013, representing a yield of 18.11 t/ha (OAIC, 2013). It remains low and does not cover the needs of the population, hence the recourse to imports. Grain farming in Algeria is facing the combined effects of abiotic (including drought and poor crop management technology) and biotic (fungal diseases and pests) stresses. Among these, the cereal cyst nematodes (CCNs) *Heterodera avenae* Group are the main limiting factor for grain production (Rivoal and Cook 1993). This group contains at least 12 species that affect cereals and grasses. Three main species (*H. avenae, H. filipjevi*, and *H. latipons*) are the most economically important cyst nematodes attacking cereals (Rivoal and Cook 1993, Nicol 2002, Dababat *et al.* 2015).

Since the discovery of *H. avenae* in 1962 by Scotto La Massese, many unpublished works were carried out on cyst nematodes and that work demonstrated the wide distribution of these pests through the cereal growing areas other than the irrigated areas in the Adrar region in the south of the country (Haddadi 1999).

Therefore, the objectives of this study were to: 1) determine the current distribution of cereal cyst nematodes in cereal growing areas by surveying and collecting cyst nematodes from infested fields of the north and, 2) investigate population densities of cyst nematodes and their distribution.

MATERIALS AND METHODS

Soil sampling was conducted during the summer period at the diapause of the parasite. It begins in the formation of cysts and extends to the emergence of the larvae at the end of autumn. About 1.5 kg of soil was taken from each field at the depth of 30 cm and consisting of several subsamples of 300 g soil collected across the two diagonals of the field. Their number depends on the surface of the treated field. It is about 20 subsamples per ha.
In the laboratory, the soil samples were dried and weighed and cysts were extracted from 300 g of soil using the Fenwick can method (Fenwick 1940). The cysts were harvested separately using a brush under a dissecting microscope (G × 20) and placed in petri dishes. The total number of full and empty cysts is calculated to estimate the average infestation of the plot (Abidou *et al.* 2005), which is expressed by cyst per kg of soil.

RESULTS

Field infestation and population densities: The nematode analysis of soil samples shows that 37 of 40 surveyed cereal plots are infested with *Heterodera* spp., representing a rate of 92.5% infested fields. Only three fields were free of cysts; these fields were located in the localities of Isser (Boumerdes), P2 Lamtar (Sidi Bel Abbes) and El Maleh (Ain Temouchent).

The infestation levels are highly variable between localities and even between the fields of the same locality. They ranged from 3.6 cysts/kg soil in the Tadmait plot to 281 cysts/kg of soil at Buouandas (Setif). The infestation levels are noted in fields cropped with wheat and barley especially when their preceding crops were cereals. This case is found in Ouled Rahmoun, ITGC Khroub, Bouandas, P1of Tipaza and Tamlouka, where the total numbers exceeded 100 cysts/kg of soil.

The respective fields of Draa El Mizene, P1 of Tadmait, Lamtar, Oum Bouaghi, Oued Tlilet and P1 of El Ghomri showed infestation levels under to 10 cysts/kg of soil. In most of the remaining plots, the cyst numbers ranged from 10 to 99 cysts/kg of soil.

The highest levels of infestation were noted in areas with cereal vocation, located in the highlands (as Setif and Guelma) where they were around 300 cysts/kg of soil and exceeded 100 cysts/kg of soil at Constantine and Tlemcen.

Other fields have expressed relatively high levels of infestation (more than 50 cysts) as Tleghma (Mila), (P2 and Brahimi Belkacem (Tipaza), Mansoura (Borj Bouariridj) Djendel (Ain Defla) Bechloul, Ain Bessam (Bouira) and Kais (Khenchela) (Table 1).

Species involved: Cyst nematodes were identified on the basis of morphological and morphometric characters of the cyst vulval cone (Wouts and Baldwin 1998). The collected cysts were typically ovoid to lemon-shaped but had different sizes. Preliminary results showed that *H. avenae* was the dominant species in 52% of samples. It was found alone or in mixture with *H. latipons* and *H. mani*.

The results of this survey allowed us to draw a geographical distribution map of cereal cyst nematodes in the investigated areas, as shown in Figure 1.

Figure 1. Geographical distribution of cereal cyst nematodes in northern surveyed localities of Algeria

DISCUSSION

Our investigations have shown that cyst nematodes belonging to the *H. avenae* Group are present in almost all cereal plots surveyed, including those regions with high potential for grain production of Algeria. Although suggestive because of the unsystematic sampling procedures related to some circumstances of sampling (method and number of samples) and to losses during extraction, this study underestimates the levels of infestation of surveyed fields.
Considering the number of eggs (up to 600) contained in a cyst (Siddiqui 2000), infestation levels recorded in most of the plots can be considered as

Table 1. Population densities in different localities infested by cereal cyst nematodes

Wilaya and geography*	Locality / field	Climate*	Current crop	Cyst/kg of soil
Blida 36°28′00″ N; 2°49′00″ E	Boufarik	Sub-humid	Cereal straw	28.72
Boumerdes 36°46′00″ N; 3°29′38″ E	Isser	Sub-humid	Cereal straw	00
Constantine 36°22′02″ N; 6°37′08″ E	Ouled Rahmoun	Semi-arid	Wheat	134
	ITGC Khroub P1	Semi-arid	Wheat	111
	ITGC Khroub P2		Wheat	33.4
Tizi Ouzou 36°43′ N; 4°03′00″ E	Draa Benkheda	Sub-humid	Oat	23.3
	Draa Elmizene	Sub-humid	Cereal straw	20.3
	Tadmaït	Sub-humid	Cereal straw	03.6
Sétif 36°9′29”N ; 5°26′34” E	Ain roua	Semi-arid	Cereal straw	281
	Bouandas	Semi-arid	Wheat	26
	Ain Kebira		Cereal straw	28.3
Tipaza 36°35′31″ N; 2°26′00″ E	Ferme B.Belkacem	Sub-humid	Cereal straw	71.9
	P1	Sub-humid	Wheat	100.4
	P2	Sub-humid	Wheat	98
	P3	Sub-humid	Barley	41
Bouira 36°22′48″ N; 3°53′5″ E	Ain Bessem	Sub-humid	Barley	65.7
	Bachloul	Sub-humid	Barley	68.4
	El Asnem	Sub-humid	Wheat	145
	Mechedellah	Sub-humid	Barley	17.7
	Route Tikejda	Sub-humid	Barley	31.4
Alger 36°42′30″ N; 3°3′34″ E	ITGC Oued Smar P1	Sub-humid	Wheat	24.5
	ITGC Oued Smar P2	Sub-humid	Barley	14.6
Tlemcen 34°52′42″ N; 1°18′54″ E	Ain Khedra	Sub-humid	Wheat	105.5
Mila 36°27′0″N; 6°16′0″ E	Tleghma	Sub-humid	Barley	99
Batna 35°33′ N ; 6°10″ E	Timgad	Semi-arid	Cereal straw	14.2
Guelma 36°28′58″ N; 7°26′2″ E	Ain Makhlouf	Semi-arid	Wheat	29.3
	Tafira	Semi-arid	Barley	43.7
	Tamlouka	Semi-arid	Wheat	267
Ain Defla 36°15′55″N; 1°58 ‘13″ E	Djendel	Semi-arid	Wheat	69.4
Sidi Bel Abes 35°11′38″ N; 0°38′29″ E	Lamtar P1	Sub-humid	Oats	04.6
	Lamtar P2	Sub-humid	Oats	00
Mostaganem 35°5′59″ N; 0°5′25″ E	Mostaghanem	Sub-humid	Wheat	25.4
AinTemouchent 35°4′0″ N; 1°08′28″ E	El Maleh	Sub-humid	Cereal straw	00
Oran 35°42′27″ N; 0°38′57″ E	Oued T′lilet	Sub-humid	Wheat	15.5
Borj Bouariridj 36°04′00″ N; 4°46′00″ E	Mansoura	Semi-arid	Wheat	71.2
Khenchela 35°25′55″ N; 7°8′40″ E	Kaïs	Semi-arid	Wheat	59
Souk Ahras 36°17′15″ N; 7°57′15″ E	Ain Dalia	Sub-humid	Barley	88.8
OumBouaghi 35°52′39″ N; 7°06′49″ E	Oum Bouaghi	Semi-arid	Cereal straw	06.3
Mascara 35°23′00″ N; 0°09′00″ E	El Ghoumri P1	Semi-arid	Barley	21.7
	El Ghoumri P2	Semi-arid	Wheat	18.8

high and can have a negative impact on yields. Smiley and Yan (2010) reported that the decline in wheat yields can occur when the number of eggs and larvae of *H. avenae* contained in cysts plus larvae already present in the soil exceeds five nematodes per gram of soil. These high infestations are due to several factors such as monoculture (cereal after cereal) practiced for several consecutive years, the absence of fallow and the rotations. Indeed, repeated cultivation of cereals on the same plot contributes to the outbreak of cyst nematodes populations (Sharma *et al.* 2007, Renco and Cereukoua 2008).

The low levels recorded Tadmait M'chedellah (Bouira), P1 Oued Smar, Oued Tlilet (Oran), Timgad (Batna) and P2 El Ghoumri (Mascara) would probably be due to previous crops that are either fallow or non-host plants for the nematodes, which significantly lower their population numbers in the soil (Brown 1984, Griffin 1988, Bourdon and Rivoal 2005, Singh *et al.* 2009). However, this observation does not apply to the plot Ain Roua where the density of *Heterodera* is low in spite of monoculture commonly adopted.

The absence of cysts in some samples does not necessarily mean that the plots are free of these parasites. Various factors may cause of this absence include: 1) The recent introduction of cereal crops in these fields, 2) The clay soils such as in Batna and Tadmait because soil nature has a considerable influence on the numbers of nematodes in the soil (Trigiano *et al.* 2004) and Sikora (1987) reported that CCNs attacks are more severe in light sandy soils than on heavier soils, 3) The host quality: Some crops limit the development of CCNs, such as oats which are considered a poor host for *H. avenae* in Mediterranean countries, unlike some countries of northern Europe, where they are considered as preferential hosts (Nicol 2002, Ireholm 1994). These crops could explain the low densities registered in the localities of Ain Roua (Setif), Ain Makhlouf (Guelma), El Maleh (Ain Temouchent) and P1 Lamtar.

Also, the plots of P2 ITGC Khroub, Ain Kebira (Sétif), Mechedelah (Bouira) and Oued T'lilet (Oran) showed a low infestation which is probably related to the rotation cereal/legume (especially chickpea) practiced in these areas. This crop is non-host for CCNs therefore it is recommended to introduce it into the crop system for a year or more if infestations are strong in order to reduce the densities of these pests below damaging levels (Parker *et al.* 2011). At higher densities, the potential for infection could constitute a real threat to subsequent crops, particularly if no control measures are taken for proper management of these nematodes in time and in space.

REFERENCES

- Abidou H, El-Ahmed A, Nicol JM, Bolat N, Rivoal R. and Yahyaoui A (2005) Occurrence and distribution of species of the *Heterodera avenae* group in Syria and Turkey. *Nematologia Mediterranea*, 33, 197- 203.
- Chehat F (2007) Analyse macroéconomique des filières: la filière blés en Algérie. Projet PAMLIM « *Perspectives agricoles et agroalimentaires Maghrébines Libéralisation et Mondialisation* » Alger 9-7 April.
- Dababat AA, Imren M, Erginbas-Orakci G, Ashrafi S, Yavuzaslanoglu E, Toktay H, Pariyar SR, Elekcioglu HI, Morgounov AI, Mekete T (2015) The importance and management strategies of cereal cyst nematodes, *Heterodera* spp., in Turkey. *Euphytica*, 202,173-188.
- Djermoun A (2009) La production céréalière en Algérie: les principales caractéristiques. *Nature et Technologie*, 01, 45-53.
- Fenwick DW (1940) Methods for the recovery and counting of cysts of *Heterodera schachtii* from soil. *Journal of Helminthology*, 18, 155-172.
- Haddadi F (1999) Aperçu sur l'état d'infestation de quelques régions céréalières d'Algérie par les nématodes à kyste des céréales. *Proceeding du deuxième symposium régional sur les maladies des céréales et des légumineuses Alimentaires.* Nabeul, Tunisie, 33-39.
- Ireholm A (1994) Characterization of pathotypes of cereal cyst nematodes *Heterodera* spp. in Sweden. *Nematologica,* 4, 399–411.
- Nicol JM (2002) Important nematode pests of cereals. In:'Bread Wheat: Improvement and Production.' (Eds. BC Curtis, S Rajaram, G Macpherson) pp. 345-366. *Plant Production and Protection Series, No. 30*. (FAO, Rome).
- OAIC (Office Algérien Interprofessionnel des Céréales), (2013) Note de Conjoncture 3ème Trimestre 2013. Documents internes OAIC, 11p.
- Parker W, Pritchard I, Mac Leod B, Vanstone V, Thomas G (2011) Using break crops to manage wheat diseases. Farm note No 464, Department of Agriculture and Food. Western Australian Agriculture Authority 4p.
- Riley IT, Nicol JM, Dababat AA (2009) Cereal Cyst Nematodes: Status. Research and Outlook. (CIMMYT, Ankara, Turkey).
- Rivoal R, Cook R (1993) Nematodes pests of cereals. In: 'Plant Parasitic Nematodes in Temperate Agriculture'. (Eds. K Evans, DL Trudgill, JM Webster) pp. 259-303. (CAB International, Wallingford, UK).
- Sharma SM, Sain RS, Mathur BN, Sharma GL, Bhatnagar VK, Singh H, Midha RL (2007) Development and validation of the first cereal cyst nematode (*Heterodera avenae*) resistant wheat CCNRV1 for Northern India. *SABRAO. Journal of Breeding and Genetics,* 39 (1), 1-16.

- Siddiqui MR (2000) Tylenchida Parasites of Plants and Insects, 2nd Ed. pp. 387- 413. (CABI Publishing, New York).
- Sikora RA (1987) Plant parasitic nematodes of wheat and barley in temperate semi-arid region – a comparative analysis. In: 'Nematodes Parasitic to Cereals and Legumes in Temperate Semi-arid Regions.' (Eds. MC Saxena, RA Sikora, JP Srvastava) pp. 46-48. (ICARDA, Aleppo, Syria).
- Singh AK, Sharma AK, Shoran J (2009) *Heterodera avenae* and its management on wheat in India. In: 'Cereal Cyst Nematodes: Status, Research and Outlook.' (Eds. IT Riley, JM Nicol, AA Dababat) pp. 17-22. (CIMMYT, Ankara, Turkey).
- Smiley RW, Yan GP (2010) Cereal cyst nematodes: Biology and management in Pacific Northwest wheat barley and oat crops. Pacific Northwest Extension Bulletin 520. Oregon State University, Corvallis, Oregon.
- Trigiano RN, Windham MT, Windham AS (Eds.) (2004) Plant Pathology: Concepts and Laboratory Exercises. (CRC press, Washington).
- Wouts WM, Baldwin JC (1998) Taxonomy and identification. In: 'The Cyst Nematodes.' (Ed. SB Sharma) pp. 83-122. (Kluwer Academic Publishers, London, UK).

POPULATION DYNAMICS OF CEREAL CYST NEMATODE (*HETERODERA AVENAE*) IN BEIJING AREA

SHU-SEN LIU[1,2], JIAN-LONG ZHAO[1], CHANG-LONG CHEN[1], IAN RILEY T[2], QIAN LIU[1], REN-DE QI [2,3], AND HENG JIAN[1]

[1]Department of Plant Pathology, China Agricultural University, Beijing 100193, China; [2]Institute of Plant Protection and Agro-products Safety, Anhui Academy of Agricultural Sciences, Hefei 230031, Anhui, China; [3]Scientific Observing and Experimental Station of Crop Pests in Hefei, Ministry of Agriculture, Hefei 230031, Anhui, People's Republic of China.

SUMMARY

Cereal cyst nematode, *Heterodera avenae* is now recognized as a highly destructive pathogen of wheat in China. Investigations of vertical distribution of second-stage juveniles (J2s) in soil, population dynamic of J2s, and the infection cycle of *H. avenae* were carried out under field conditions in the Beijing area during the 2010-2012 wheat growing seasons. The results showed that J2s were distributed in 0-20cm soil, and the emergence of J2s was bimodal with a small peak in late autumn and a main peak in spring. The initial infection occurred in October and the severe invasion occurred in the following spring with the main emergence of juveniles. Although the egg hatching and infection occurred twice in one wheat growing season, only one cohort of adults are developed in the Beijing area. The hatching and infection experiments confirmed

* Liu SS, Zhao JK, Chen CL, Riley IT, Liu Q, Qi RD, Jian H (2015) Population dynamics of cereal cyst nematode (*Heterodera avenae*) in Beijing area. In 'Nematodes of Small Grain Cereals: current status and research.' (AA Dababat, H Muminjanov and RW Smiley) pp. 67-68. (FAO: Ankara, Turkey).

that low temperature stimulates egg hatching and emergence of J2s from the new season's cysts. It is also postulated that the nematodes that penetrated in roots before winter come from the residual cysts that have experienced one or more winters. These findings help us to better understanding the biology and ecology of *H. avenae* and to take effective approach to manage this nematode.

(A full-length version of this paper was not available at the time of printing).

INVESTIGATION OF CEREAL CYST NEMATODE DISTRIBUTION IN A NATURALLY INFESTED WINTER WHEAT FIELD

HE QIONG[1], HAI YAN WU[1], AND DE LIANG PENG[2]

[1]Agricultural College of Guangxi University, Nanning 530004, China; [2]State Key Laboratory for Biology of Plant Diseases and Insect Pests, Chinese Academy of Agricultural Sciences, 100193 Beijing, China

SUMMARY

The Cereal Cyst Nematode (CCN; *Heterodera avenae*) is found in at least 16 provinces in China. CCN often damages wheat and barley, causing severe economic losses. The Shandong winter wheat region is the second largest wheat production area in China. CCN was investigated in Binzhou, Jinan, Dezhou, Heze, Taian and Zaozhuang cities of Shandong Province in 2008, 2009 and 2010. We collected 269 samples from wheat roots and rhizosphere soils. CCN were found in 11 counties/districts of the six cities. The cyst was not detected in Shanting and Taierzhuang districts of Zaozhuang city. Occurrence of CCN was 36.4% in this study. CCN density showed significant differences in different regions. There was a higher cyst density in Dawenkou and Hutun town in Taian city than in other areas. We explored feasible management approaches in this location. A field in Dawu village, Dawenkou County (35°57´N, 117°06´E), as a typical naturally-infested soil, was investigated to determine the horizontal and vertical distributions of the CCN population under local climatic and planting pattern conditions. The spatial distribution in and between planting rows was also determined. The results showed that the distribution of cysts was uneven at the horizontal scale. When the row spacing was 20 cm, higher numbers of cysts were observed in the planting row and midway between two

* Qiong H, Wu HY, Peng DL (2015) Investigation of cereal cyst nematode distribution in a naturally infested winter wheat field. In 'Nematodes of Small Grain Cereals: current status and research.' (AA Dababat, H Muminjanov and RW Smiley) pp. 69-70. (FAO: Ankara, Turkey).

planting rows. Moreover, 79% cysts were distributed in the 0–20 cm soil layer. These results suggested that the specific site for the effective application of chemicals or biological control agents was at a 20-cm depth in and between planting rows. This information can serve as a reference for the development of an effective and mechanized site-specific management strategy for the control of CCN in large wheat fields.

This research was sponsored by the Special Fund for Agro-scientific Research in the Public Interest (201503114).

(A full-length version of this paper was not available at the time of printing)

RESISTANCE IDENTIFICATION OF DIFFERENT WHEAT VARIETIES FROM CIMMYT TO DAXING POPULATION OF *HETERODERA AVENAE*

JIANG-KUAN CUI[1], DE-LIANG PENG[1,*], WEN-KUN HUANG[1], HUAN PENG[1], ABDELFATTAH A. DABABAT[2], GUL ERGINBAS-ORAKCI[2], WEN-TING HE[1], LING-AN KONG[1], LI TING[1], AND QING-SONG WU[1]

[1]State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, China; [2]International Maize and Wheat Improvement Centre (CIMMYT) P.K.39 06511, Emek, Ankara, Turkey. *Corresponding author email: dlpeng@ippcaas.cn

INTRODUCTION

The Cereal Cyst Nematode (CCN, *Heterodera avenae*) is a destructive nematode pest on cereal crops worldwide and causes significant economic yield losses in many countries (Cook & Noel 2002, Smiley and Nicol 2009, Dababat *et al.* 2015). *Heterodera avenae* is considered to be one of the most important cyst nematodes of cereal crops (Rivoal & Cook 1993) and the losses caused by this species range from 30 to 100%. In Europe, more than 50% of the fields in major cereal-growing areas are infested by this nematode (Rivoal & Cook 1993), resulting in staggering annual yield losses reaching £3 million (Nicol and Rivoal 2008). In Oregon, Smiley *et al.* (2005) have indicated losses on spring wheat of up to 24%. In 1989, *H. avenae* was first reported from Hubei Province (Chen *et al.* 1992). Subsequent surveys revealed that this species is widely distributed

* Cui JK, Peng DL, Huang WK, Peng H, Dababat AA, Erginbas-Orakci G, He WT, Kong LA, Ting L, Wu QS (2015) Resistance identification of different wheat varieties from CIMMYT to Daxing population of *Heterodera avenae*. In 'Nematodes of Small Grain Cereals: current status and research.' (AA Dababat, H Muminjanov and RW Smiley) pp. 71-78. (FAO: Ankara, Turkey).

and has now been found in more than in 16 provinces including Henan, Hebei, Beijing, Inner Mongolia, and Qinghai (Peng 1995), Anhui (Zheng *et al.* 1996) Shandong (Liu *et al.* 2009), Shaanxi and Gansu (Peng *et al.* 2008), Jiangsu (Li *et al.* 2010) and Tianjin (Peng *et al.* 2012), and Tibet and Xinjiang (Li *et al.* 2012). More than 4,000,000 ha of the major wheat producing regions were infested (Peng *et al.* 2007, Peng *et al.* 2009). The yield losses of wheat induced by this nematode can reach 40% in some particularly heavily infested fields (Peng *et al.* 2007). In 2013, resistance identification of different wheat varieties from CIMMYT to *H. avenae* of the Beijing Daxing population was performed.

MATERIALS AND METHODS

Nematode collection and molecular identification: The soil and roots samples of *H. avenae* were collected from wheat fields of Daxing district, Beijing at the end of the growing season in 2012. Cysts were extracted from the soil by Cobb's sieving gravity method (Persmark *et al.* 1992). Nematode genomic DNA extraction was as described by Peng *et al.* (2003). Twenty four individual cysts were selected randomly for further molecular identification with specific primers which were described previously (Qi *et al.* 2012);
HaF1 (5′-TGACGAGAACATATGATGGGGAT-3′), and
HaR1 (5′-GAGGGGGTGGGAATGAAATGGAT-3′).

Resistance testing: The determined cysts were incubated at 4°C for 60 days, then at 16°C to stimulate the hatching of pre-parasitic second-stage juveniles (J2s). The seed were germinated in Petri dishes and transplanted into the PVC tubes (3-cm diam. × 30-cm length) with 200 cm^3 sterile soil mixture (compost: sand = 7:3). Five hundred *H. avenae* juveniles were inoculated into each tube immediately after seed was planted and again three hundred J2 at three-day intervals, for a final inoculum density of about 800 J2/tube. Plants were grown in a glasshouse for nine weeks at 15-18°C and with a 16-hour light period. Plants were irrigated, fertilized and treated with chemicals to control disease and insect whenever needed to give normal plant growth. Seventy days after the second inoculation, plants and soil were washed in a gentle stream of tap water over 250 µm sieves. White females attached to the roots and those collected from the 250 µm sieve were counted under a stereoscopic microscope, and the mean number of females per plant was calculated.

The cultivars were classified as immune, resistant, moderately resistant, moderately susceptible, susceptible and highly susceptible to CCN accord-

ing to the Nicol *et al.* (2009) scale which is based on the number of cysts per root system: M = Immune (0 females), R = Resistant (0.1-5.0 females), MR = Moderately Resistant (5.1-10.0 females), MS = moderately susceptible (10.1-15.0 females), S = susceptible (15.1-25.0 females), and HS = highly susceptible (>25.0 females).

RESULTS

Using specific sequence characterized amplified region (SCAR) primers, the species of the Beijing populations were identified by PCR. The results showed that one single fragment of 1010 bp were amplified from 24 cysts randomly picked from Beijing Daxing population (Fig. 1). This species-specific fragment is unique for *H. avenae* according to the molecular diagnosis method developed by Qi *et al.* (2012). Morphology combined with SCAR marker method significantly clarified that the species of Beijing Daxing populations were *H. avenae*.

In the present study, there were five wheat entries MILAN, CROC_1/AE.SQUARROSA(224)//OPATA,CROC_1/AE.SQUARROSA(224)//OPATA, VP1620 and MIRZABEY2000 that showed resistance to the population. Two wheat lines FRAME and KATE A-1 were moderately resistant. Additionally, there were 17 wheat lines that were highly susceptible, one wheat lines showed moderately susceptible (Table 1). The highly susceptible wheat lines included a considerable proportion (68%) of the wheat varieties, while the resistant lines (20%) were a very tiny part.

DISCUSSION

CCN is acknowledged as a global economic problem on cereal production systems. Key results indicate that CCN is widely distributed in the dryland and rain-fed wheat production systems and causes significant yield loss on common cultivars. Global warming will enhance dramatically the noxiousness of these pathogens in both dryland and rain-fed production of cereals as well as in intensive production systems in Western Europe. Cultural practices represent efficient methods based on rotational combinations of non-hosts crops or cultivars and clean fallows, although this option is limited in the winter wheat production areas of China. One study indicated that nematode population densities decreased by 70% with continued rotation with non-host crops (Singh *et al.* 2009). In Australia, over 50% of current cultivars with moderate to

Table 1. The reaction different wheat varieties from CIMMYT to Heterodera avenae of Beijing Daxing population

Code	Line Name	SW/WW[1]	Mean[2]	Reaction[3]
CCNHD 1112-01	6R(6D)	SW	33.8	HS
CCNHD 1112-02	FRAME	SW	9.7	MR
CCNHD 1112-03	SILVERSTAR	SW	45.4	HS
CCNHD 1112-04	VP5053	SW	31.7	HS
CCNHD 1112-05	T-2003	SW	25.7	HS
CCNHD 1112-06	RAJ 1	SW	32	HS
CCNHD 1112-07	ID-2150	SW	73.6	HS
CCNHD 1112-08	MILAN	SW	5.0	R
CCNHD 1112-09	AUS 4930.7/2*PASTOR	SW	39.5	HS
CCNHD 1112-10	AUS GS50AT34/SUNCO//CUNNINGHAM	SW	38.9	HS
CCNHD 1112-11	VL411R	SW	42.8	HS
CCNHD 1112-12	CROC_1/AE.SQUARROSA(224)//OPATA	SW	0.95	R
CCNHD 1112-13	CROC_1/AE.SQUARROSA(224)//OPATA	SW	0.7	R
CCNHD 1112-14	VP1620	SW	2.7	R
CCNHD 1112-15	F130L1.12/ATTILA	WW	54.1	HS
CCNHD 1112-16	SONMEZ	WW	30.1	HS
CCNHD 1112-17	CPI133859	WW	66.0	HS
CCNHD 1112-18	CPI133872 [two plots in WNT05]	WW	110.5	HS
CCNHD 1112-19	KATE A-1	WW	8.3	MR
CCNHD 1112-20	PRINS	WW	39.6	HS
CCNHD 1112-21	MIRZABEY2000	WW	2.8	R
CCNHD 1112-22	AU/CO652337//2*CA8-155/3/F474S1-1.1	WW	73.2	HS
CCNHD 1112-23	F372	WW	83.3	HS
CCNHD 1112-24	TAIKONG	WW	46.1	HS
CCNHD 1112-25	ZHONGYU	WW	17.4	MS

[1]SW, Spring Wheat; WW, Winter Wheat. [2]Data are the means of 10 replicates. [3]HS = highly susceptible; MS = moderately susceptible; MR = Moderately Resistant; R = Resistant

strong resistance to *H. avenae* has been deployed and *H. avenae* has generally been contained markedly (Riley & McKay 2009).

Integrated management, based primarily on genetic host resistance, seems to be most effective when two or more soil borne pathogens occur in the soil at same time (Nicol and Rivoal 2007). According to the identification of thousands of wheat lines, the resistant cultivars were relatively scarce (Cui, unpublished data). The present study also showed similar results. The highly susceptible wheat lines include a considerable proportion (68%) of the wheat varieties, while the resistant wheat lines (20%) are a very tiny part. Wang *et al.* (2012) reported that the agricultural machinery operation across different areas, and running water, might be main ways of CCN diffusion. Application of fertilisers and soil amendments may compensate the reducing effect of nematodes on

wheat yields but their use is frequently limited by financial constraints (Rivoal and Nicol 2009). Many different control options such as chemical, cultural, genetic (resistance/tolerance) and biological control are available and their net effect should be aimed at decreasing and maintaining CCN population densities below damage thresholds, so as to maintain or reach the attainable yield.

Heterodera avenae caused a reduction of wheat yield valued at 1.9 billion RMB in China (Peng *et al.* 2009) and has received considerable attention in China (Peng *et al.* 2009, Yuan *et al.* 2010). With the use of resistant cultivars, the CCN virulence pathotype could change rapidly, and the population purity also affects the pathotype (Cook & Rivoal 1998). Cui *et al.* (2014) studied the pathotype characterization of Beijing Daxing and Qinghai Huangyuan populations of *H. avenae* in China; the Beijing Daxing population pathotype was Ha91. Nematode biotypes that can infect resistant plants have been identified (Valerie 1999). With the focus upon host resistance, there are a series of resistance genes recognized and inserted into cultivars available to farmers (Nicol 2002). To exploit resistant cultivars better, we have to know the nematode population pathotype and work on morphological and molecular identification and host resistance tests. The results of the current work can provide a data reference for resistance breeding to improve wheat productions in Northern and Northwest China.

ACKNOWLEDGMENTS

This study was supported by the National Natural Science Foundation of China (No. 30921140411) the Special Fund for Agro-scientific Research in the Public Interest (No. 201503114, 200903040) and the National Key Basic Research Program of China (973 Program, 2013CB127502),

REFERENCES

- Chen PS, Peng DL, Wang MZ (1992) Cereal cyst nematode disease of wheat. *Plant Protection*, 18, 37–38. (in Chinese)
- Cook R, Noel GR (2002) Cyst nematodes: *Globodera* and *Heterodera* species. In: 'Plant Resistance to Parasitic Nematodes.' (Eds. JL Starr, R Cook, J Bridge) pp. 71–105. (CABI Publishing, Wallingford, UK).
- Cook R, Rivoal R (1998) Genetics of resistance and parasitism. In: 'The Cyst Nematodes.' (Ed. SB Sharma) pp. 322–352. (Chapman and Hall, London, UK).

- Cui JK, Huang WK, Peng H, Liu SM, Wang GF, Kong LA, Peng DL (2015) A new pathotype characterization of Daxing and Huangyuan populations of cereal cyst nematode (*Heterodera avenae*) in China. *Journal of Integrative Agriculture*, 14, 724-731.
- Dababat AA, Imren M, Erginbas-Orakci G, Ashrafi S, Yavuzaslanoglu E, Toktay H, Pariyar SR, Elekcioglu H, Morgounov A, Mekete M (2015) The importance and management strategies of cereal cyst nematodes, *Heterodera* spp., in Turkey. *Euphytica*, 202, 173-188.
- Li HM, Wang X, Pei S, Le XH, Liang Z (2010) A preliminary survey of the cereal cyst nematode *Heterodera avenae* on wheat in Jiangsu Province of China. *Plant Protection*, 36, 172–175. (in Chinese)
- Li HX, Liu YE, Wei Z, Li MQ (2012) The detection of *Heterodera avenae* from the cereal field in Autonomous Regions of Tibet and Xinjiang. *Nematology Research in China*, 4, 164–165. (in Chinese)
- Liu J, Wu HY, Zhang GM, Yu GL, Peng DL (2009) Observations and morphometrics of cereal cyst nematode from Binzhou, Shandong, China. In: 'Cereal Cyst Nematodes: Status, Research and Outlook.' (Eds. IT Riley, JM Nicol, AA Dababat) pp. 124-129. (CIMMYT, Ankara, Turkey).
- Nicol JM, Rivoal R (2007) Global knowledge and its application for the integrated control and management of nematodes on wheat. In: 'Integrated Management and Biocontrol of Vegetable and Grain Crop Nematodes.' (Eds. A Ciancio, KG Mukerji) pp. 251–294. (Springer, Dordrecht, The Netherlands).
- Nicol JM, Rivoal R (2008) Global knowledge and its application for the integrated control and management of nematodes on wheat. In: 'Integrated Management and Biocontrol of Vegetable and Grain Crops Nematodes.' (Eds. A Ciancio, KG Mukerji) pp. 243–287. (Springer, Dordrecht, The Netherlands).
- Nicol JM (2002) Important nematode pests. In: 'Bread Wheat: Improvement and Production.' (Eds: BC Curtis, S Rajaram, HG Macpherson) pp. 345–366. (Food and Agriculture Organization of the United Nations, Rome, Italy).
- Peng DL, Huang WK, Sun JH, Liu CJ, Zhang HM (2012) First report of cereal cyst nematode (*Heterodera avenae*) in Tianjin, China. *Nematology Research in China*, 4, 162–163. (in Chinese)
- Peng DL, Li HX, Wang XF, Riley IT, Nicol JM, Hou SY, Ou SQ (2008) New distribution of cereal cyst nematode (*Heterodera avenae*) in China. *Nematology Research in China*, 2, 344–345. (in Chinese)

- Peng DL, Nicol JM, Li HM, Hou SY, Li HX, Chen SL, Ma P, Li HL, Riley IT (2009) Current knowledge of cereal cyst nematode (*Heterodera avenae*) on wheat in China. In: 'Cereal Cyst Nematodes: Status, Research and Outlook.' (Eds. IT Riley, JM Nicol, AA Dababat) pp. 29–34. (CIMMYT, Ankara, Turkey).
- Peng DL, Subbotin S, Moens M (2003) rDNA restriction fragment length polymorphism of *Heterodera avenae* in China. *Acta Phytopathologica Sinica*, 33, 323–329. (in Chinese)
- Peng DL, Zhang DS, Qi SH, Chen PS (1995) Distribution and control of cereal cyst nematode (*Heterodera avenae*) in China. In: '*Advances of Chinese Plant Protection*.' pp. 53–56. (China Science and Technology Press, China). (in Chinese)
- Peng DL, Zhang D, Nicol JM, Chen SL, Waeyenberge L, Moens M, Li HL, Tang WH, Riley IT (2007) Occurrence, distribution and research situation of cereal cyst nematode in China. In: 'Proceedings of the XVI International Plant Protection Congress.' 15–18 October, 2007. Glasgow, Scotland, UK. pp. 350–351. (British Crop Protection Council, Alton, Hampshire, UK).
- Persmark L, Banck A, Andersson S (1992) Evaluation of methods for extraction of nematodes and endoparasitic fungi from soil. *Nematologica*, 38, 520–530.
- Qi XL, Peng DL, Peng H, Long HB, Huang WK, He WT (2012) Rapid molecular diagnosis based on SCAR marker system for cereal cyst nematode. *Scientia Agricultura Sinica*, 45, 4388–4395. (in Chinese)
- Riley IT, McKay AC (2009) Cereal cyst nematode in Australia: Biography of a biological invader In: 'Cereal Cyst Nematodes: Status, Research and Outlook.' (Eds. IT Riley, JM Nicol, AA Dababat) pp. 23–28. (CIMMYT, Ankara, Turkey).
- Rivoal R, Cook R (1993) Nematode pests of cereals. In: 'Plant Parasitic Nematodes in Temperate Agriculture.' (Eds. K Evans, DL Trudgill, JM Webster) pp. 259–303. (CAB International, Wallingford, UK).
- Rivoal R, Nicol JM (2009) Past research on the cereal cyst nematode complex and future needs. In: 'Cereal Cyst Nematodes: Status, Research and Outlook.' (Eds. IT Riley, JM Nicol, AA Dababat) pp 3–10. (CIMMYT, Ankara, Turkey).
- Singh AK, Sharma AK, Shoran J (2009) *Heterodera avenae* and its management on wheat in India. In: 'Cereal Cyst Nematodes: Status, Research and Outlook.' (Eds. IT Riley, JM Nicol, AA Dababat) pp. 17-22. (CIMMYT, Ankara, Turkey).

- Smiley RW, Nicol JM (2009) Nematodes which challenge global wheat production. In: 'Wheat Science and Trade.' (Ed. BF Carver) pp. 171–187. (Wiley-Blackwell, Ames Iowa, USA).
- Smiley RW, Whittaker RG, Gourlie JA, Easley SA, Ingham RE (2005) Plant-parasitic nematodes associated with reduced wheat yield in Oregon: *Heterodera avenae*. *Journal of Nematology*, 37, 297–307.
- Valerie MW (1999) Plant nematode resistance genes. *Current Opinion in Plant Biology*, 2, 327–331.
- Wang T，Qi RD，Wu XH，Zhao W，Gao TC，Zhang L，Wu HP (2012) A preliminary study on diffusion ways of the cereal cyst nematode. *Plant Protection*, 38, 98-100.
- Yuan HX, Sun JW, Yang WX, Xing XP, Wang ZY, Riley IT, Li HL (2010) New pathotypes of *Heterodera avenae* (cereal cyst nematode) from winter wheat in Zhengzhou, Henan, China. *Australasian Plant Pathology*, 39, 107–111.
- Zheng JW, Lin M, Cheng H, Fang Z (1996) Identification of two cyst nematode populations parasitizing wheat in Anhui, China. *Jiangsu Journal of Agricultural Sciences*, 12, 31–35. (in Chinese).

INFLUENCE OF POPULATION DENSITY OF CEREAL CYST NEMATODE POPULATIONS (*HETERODERA AVENAE* WOLLENWEBER) ON THE NEMATODE REPRODUCTION AND DAMAGE TO WHEAT CULTIVARS UNDER GREENHOUSE CONDITIONS

MOHAMED BAKLAWA[1,3,*], BJÖRN NIERE[2], AND SAMIA MASSOUD[3]

[1]Julius Kühn-Institut, Institute for Epidemiology and Pathogen Diagnostics, Messeweg 11/12, 38104 Braunschweig, Germany. [2]Julius Kühn-Institut, Institute for Plant Protection in Field Crops and Grassland, Messeweg 11/12, 38104 Braunschweig, Germany. [3]Suez Canal University, Faculty of Agriculture, Agricultural Botany Department, Ismailia, Egypt. *Corresponding author email: mohamed.baklawa@jki.bund.de

SUMMARY

The cereal cyst nematode (CCN) *Heterodera avenae* Wollenweber, is an important nematode pest of wheat. The extent of damage caused by cereal cyst nematodes depends among others on population densities in the soil. The objective of this work was to determine the influence of initial nematode population density of *H. avenae* from Egypt on i) grain yield and plant growth parameters of different wheat cultivars and ii) on nematode reproduction. Plant growth (grain yield, spike weight, shoot dry weight and root dry weight) was negatively affected by increasing the initial population (P_i) density of *H. avenae*. The reduction in the grain yield of the Egyptian wheat cultivars by *H. avenae* ranged between 16-28% at a P_i -value of 5 J2/ml soil,

* Baklawa M, Niere B, Massoud S (2015) Influence of population density of cereal cyst nematode populations (*Heterodera avenae* Wollenweber) on the nematode reproduction and damage to wheat cultivars under greenhouse conditions. In 'Nematodes of Small Grain Cereals: current status and research.' (AA Dababat, H Muminjanov and RW Smiley) pp. 79-90. (FAO: Ankara, Turkey).

20-34% at a P_i –value of 10 J2/ml soil and 24-40% at a P_i -value of 20 J2/ml soil. The local wheat cultivar 'Sakha 93' showed tolerance in spite of his high relative susceptibility to the nematode at a P_i -value of 5 and 10 J2/ml soil as there was no significant reduction in grain yield. The final nematode population density was positively correlated with the initial population density on all the tested wheat cultivars. A negative relationship between the initial population density and the rate of reproduction was observed. This study indicates that Egyptian populations of *H. avenae* are serious pests of Egyptian wheat cultivars in the production of wheat in Egypt.

INTRODUCTION

The cereal cyst nematode (CCN) *Heterodera avenae* Wollenweber, causes significant economic losses in cereal crops. In Egypt, *H. avenae* has been reported in wheat fields for the first time by Ibrahim *et al.* (1986) on barley and wheat in the Nile Delta and other localities of Northern Egypt. In 2007, Ibrahim and Handoo reported the occurrence of *H. avenae* on Egyptian wheat in Alexandria and El-Behera governorates. Recently, *H. avenae* was detected in wheat fields of Abu Khalifah, Abu Suwayr, El Kasasen, El Shark (West Sinai), and Serabeum regions in Ismailia province (Baklawa *et al.* 2015).

Reductions of wheat yields by *H. avenae* have been reported from different regions of the world. In Morocco, *H. avenae* caused wheat grain yield losses of about 40-50% (Rammah 1994), up to 90% in Spain (Romero *et al.* 1988) and as high as 96% in Tunisia (Namouchi-Kachouri *et al.* 2009). In Turkey, significant yield losses (average 42%) in several rain-fed winter wheat locations have been reported (Nicol *et al.* 2005). Reductions of wheat yield by *H. avenae* have been reported from Libya (Siddiqui and Khan 1986), France (Rivoal and Sarr 1988) and Italy (Greco *et al.* 1993). In Egypt, since the distribution of CCN in the other wheat growing regions is still unknown, and the local wheat cultivars which are susceptible to the nematode, are grown in monoculture by most local growers; the problem of *H. avenae* is becoming more serious.
In Australia, *H. avenae* decreased the yield of wheat by 20% at an initial population density (Pi) of 2 eggs and juveniles/g soil, and 40% at a Pi -value of 16 eggs and juveniles/g soil (Meagher and Brown 1974). In Asia, the damage threshold of *H. avenae* in the temperate semi-arid regions of India is considered to be 5-20 eggs and juveniles/g soil (Pi) for wheat (Gill and Swarup, 1971; Dhawan and Nagesh, 1987). Mathur *et al.* (1986) reported loss in wheat ranging from 32.4 to 66.5% in India, due to initial nematode populations densities

(Pi) of *H. avenae* varying from 4.6 to 10.6 eggs/ml soil. The objective of this work was to determine the influence of different initial nematode population densities (0, 5, 10, 20 eggs+ juveniles/ml soil) of *H. avenae* on plant growth and yield of wheat cultivars and on nematode reproduction.

METHODS

Nematode populations: One Egyptian population of *H. avenae* from El Shark (West Sinai) in Ismailia province was used to examine the effect of increasing initial population densities of *H. avenae* on plant growth parameters of wheat cultivars and on nematode reproduction. Nematode cysts were dried at room temperature (20±2°C) and kept at 7°C until further use. Nematodes were reared on the Egyptian wheat (*Triticum aestivum*) cultivar 'Sakha 93' and newly formed cysts were used in the experiment. Cysts were squashed according to Seinhorst and Den Ouden (1966) and the total numbers of eggs and second stage juveniles (J2) per cyst were counted to determine cyst contents. Cyst content was on average 131.3±12.1 eggs and J2/cyst.

Plant materials: Three standard wheat cultivars ('Aus 10894', 'Capa' and 'Iskamish K-2-Light') from the International Test Assortment in addition to three locally-grown bread wheat cultivars ('Gemmeza 9', 'Sahl 1' and 'Sakha 93') from Egypt were used in this study.

Experimental set-up: Plastic pots (500 ml) were filled with a sterilized soil mixture (2 loam: 1 field soil), fertilized with a granular fertilizer (Osmocote Exact Standard® 1.5 g/kg soil). Cysts of *H. avenae* were added to the pots to give initial population densities (P_i) of 0, 5, 10, 20 eggs and juveniles per ml soil. Five pots were used as replicates for each initial nematode population density (P_i). Each pot contained five seedlings of the respective wheat cultivar. For each cultivar, five pots of non-infested soil served as control. Plants were grown in a greenhouse at 15±3°C (16 h light / 8 h dark) and watered as necessary with tap water.

Data collection and analysis: After 4 months, the final nematode numbers (P_f) were determined. Cysts were extracted from the soil using the floatation technique (Shepherd 1986). Counting and separation of cysts from soil debris and other organic materials retained on the filter paper were carried out at 25x magnification under a stereoscopic binocular (Leica MZ8). Cysts were squashed according to Seinhorst and Den Ouden (1966)

and the number of eggs and juveniles were counted. The nematode reproduction factor (*Rf*) for each replicate was calculated as follows: $Rf = P_f/P_i$; where P_f = final population density of eggs and J2/ml soil; P_i = initial population density of eggs and J2/ml soil.

Resistance was assessed as relative susceptibility (*RS*) to the standard susceptible control cultivar 'Capa' (*RS*= P_f on the test cultivar/P_f on susceptible control 'Capa')*100, where *Pf*= final population density of eggs and J2/ml soil. A rating system based on the relative susceptibility was used to characterize the host response of different wheat cultivars (Lücke 1976). Cultivars with *RS* less than 5% were considered resistant. The cultivars were considered moderately resistant if *RS* was between 6-20%. Cultivars with 21-50% relative susceptibility were considered moderately susceptible while cultivars with *RS* > 51% were recorded as susceptible cultivars.

Growth parameters (shoot dry weight, root dry weight, spike weight and grain yield per pot) were recorded to determine the damage potential of *H. avenae* populations on wheat cultivars. The percentages of reduction in different plant growth parameters were calculated as follows: *Red* (%) = ((*CP-IP*)/*CP*)*100, where *Red* (%) = percentage of reduction, *CP*= growth parameters of control plant, *IP*= growth parameters of infested plant. The tolerance index of a cultivar at each nematode initial population density was calculated as follows: *TI*= ((*GCP-GIP*)/*GCP*)*100/P_f, where *TI*= tolerance index, *GCP*= grain yield of control plant, *GIP*= grain yield of infested plant, P_f= final population density of eggs and J2/ml soil. Wheat cultivars with *TI* less than 0.5 considered tolerant. Wheat cultivars were less tolerant when *TI* was between 0.5 – 1. Wheat cultivars with *TI* higher than 1, were considered sensitive to *H. avenae* populations (Dixon *et al.* 1990). Levene's test was used to test homogeneity of variances. Data were analyzed using ANOVA (SPSS version 19.0, IBM Corporation, New Orchard Road Armonk, New York, United States). Means were separated using Tukey HSD test at $P \leq 0.05$.

RESULTS

Effect of increasing initial population densities of *H. avenae* on the final population densities: The Egyptian *H. avenae* population (ES) reproduced on all tested wheat cultivars. As the P_i of *H. avenae* increased, the P_f *was* significantly higher on all the cultivars except 'Aus 10894' and 'Sakha 93' (Table 1). The P_f of *H. avenae* on the cultivars 'Aus 10894' and 'Sakha 93' were not signifi-

Table 1. Effect of different initial population densities (Pi) of Heterodera avenae on nematode final population density (Pf), nematode reproduction factor (Rf) and relative susceptibility (RS) of wheat cultivars.

Cultivars	Pi = 5 J2/ml soil				Pi = 10 J2/ml soil				Pi = 20 J2/ml soil			
	Pf 1	Rf [2]	RS[3]	RR[4]	Pf	Rf	RS	RR	Pf	Rf	RS	RR
Aus 10894	13.5±2.5a	2.7a	19.2	(R)	17.0±2.5a	1.7b	18.5	(R)	21.5±4.9a	1.1c	18.4	(R)
Capa	70.4±10.3a	14.1a	100	S	91.5±5.5b	9.1b	100	S	116.8±11.4c	5.8c	100	S
Gemmeza 9	33.1±6.3a	6.6a	47.0	(S)	56.2±8.7b	5.6ab	61.4	S	83.0±8.2c	4.2b	71.1	S
Iskamish K-2	40.4±4.9a	8.1a	57.4	S	48.4±4.2ab	4.8b	53.0	S	56.4±8.3b	2.8c	48.3	(S)
Sahl 1	22.4±4.3a	4.5a	31.8	(S)	43.7±4.5b	4.4a	47.8	(S)	74.3±4.0c	3.7a	63.6	S
Sakha 93	63.4±5.9a	12.7a	90.1	S	68.1±6.6a	6.8b	74.4	S	70.6±4.3a	3.5c	60.5	S

[1] P_f = final population density of eggs and J2/ml soil.
[2] R_f (Reproduction factor) = P_f (Final population density)/ P_i (Initial population density).
P_f and R_f means in a row followed by the same letter are not significantly different based on Tukey test ($P \leq 0.05$).
[3] RS (Relative susceptibility %) = P_f on the test cultivar/P_f on susceptible control 'Capa')*100.
[4] RR (Resistance ranking) according to Lücke (1976): R, resistant (0-5%); (R), moderately resistant (6-20%); (S), moderately susceptible (21-50%); and S, susceptible (>51%).

cantly different at all P_i levels and ranged between 13.5 – 21.5 and 63.4 – 70.6 J2/ml soil, respectively. At a P_i of 5 and 10 J2/ml soil, the P_f of *H. avenae* on the cultivar 'Iskamish K-2-Light' were not significantly different from each other, while the final population was significantly higher at a P_i of 20 J2/ml soil. The P_f on the cultivars 'Capa', 'Gemmeza 9' and 'Sahl 1' increased significantly with increasing P_i levels. The highest P_f was recorded at a P_i of 20 J2/ml soil on the cultivar 'Capa' with 117 J2/ml soil followed by the cultivars 'Gemmeza 9' and 'Sahl 1' with P_f of 83 and 74.3 J2/ml soil, respectively.

Effect of increasing initial population densities of *H. avenae* on the nematode reproduction factor: The reproduction factor of *H. avenae* decreased significantly on the tested wheat cultivars in response to increasing nematode P_i except on the cultivar Sahl 1 (Table 1). Nematode reproduction factor at a P_i of 5 and 10 J2/ml soil ranged between 2.7 – 14.1 and 1.7 – 9.1, respectively. The highest *Rf* at a P_i of 5 and 10 J2/ml soil was reported on the cultivar 'Capa' followed by the cultivar 'Sakha 93' while the lowest *Rf* was recorded on the cultivar 'Aus 10894' followed by the cultivar 'Sahl 1'. The reproduction factor of *H. avenae* on wheat cultivars ranged between 1.1- 5.8 at a P_i of 20 J2/ml soil. The highest *Rf* at a P_i of 20 J2/ml soil was recorded on the cultivar 'Capa' followed by the cultivar 'Gemmeza 9' while lowest *Rf* was recorded on the cultivars 'Aus 10894' and 'Iskamish K-2-Light'.

Effect of increasing initial population densities of *H. avenae* on the relative susceptibility of wheat cultivars: A positive correlation between the P_i and *RS*

was detected on the wheat cultivars 'Gemmeza 9' and 'Sahl 1', while a negative relationship between the P_i and *RS* was detected on the wheat cultivars 'Aus 10894', 'Iskamish K-2-Light' and 'Sakha 93'. At a P_i of 5 J2/ml soil, 'Gemmeza 9' and 'Sahl 1' were moderately susceptible cultivars to *H. avenae* with a *RS* of 47 and 31.8%, respectively (Table 1). While at a P_i of 10, Gemmeza 9 was classified as susceptible. The cultivar 'Sahl 1' changed from moderately susceptible to susceptible. The cultivar 'Aus 10894' was moderately resistant at all P_i levels of *H. avenae.* The cultivar 'Sakha 93' was susceptible at all P_i levels. Different response from the wheat cultivar 'Iskamish K-2-Light' was recorded at all P_i levels. This cultivar was susceptible at a P_i of 5 and 10 J2/ml soil, while it was classified as moderately susceptible at P_i of 20 J2/ml soil.

Effect of increasing the initial population density of *H. avenae* on grain yield of wheat cultivars: As the P_i of *H. avenae* increased, the grain yield of all the tested cultivars decreased (Table 2). Reduction in grain yield of the cultivar 'Aus 10894' was not significant at all P_i levels compared to the non-infested control and ranged between 4 – 11%. Grain yield of the cultivars 'Capa', 'Gemmeza 9' and 'Sahl 1' was significantly reduced at all P_i levels. The highest reduction in grain yield (55%) was recorded at a P_i of 20 J2/ml soil on the cultivar 'Capa' followed by the cultivars 'Gemmeza 9' and 'Sahl 1' with reduction of 40 and 39 %, respectively. At a P_i of 5 and 10 J2/ml soil, reduction in grain yield of cultivars 'Iskamish K-2-Light' (12-18%) and 'Sakha 93' (16-20%) was not significant compared to the non-infested control, while the reduction in grain yield was significant at P_i of 20 J2/ml soil.

Table 2. Effect of initial population densities (Pi) of Heterodera avenae on grain yield of wheat cultivars.

Cultivars	0	Pi = 5 J2/ml soil		Pi = 10 J2/ml soil		Pi = 20 J2/ml soil	
	Yield (g)1	Yield (g)	Red (%) 2	Yield (g)	Red (%)	Yield (g)	Red (%)
Aus 10894	2.9 ± 0.4 a	2.8 ± 0.5 a	03.8	2.8 ± 0.4 a	05.9	2.6 ± 0.3 a	11.0
Capa	3.2 ± 0.6 a	1.8 ± 0.3 b	43.7	1.6 ± 0.3 b	51.1	1.5 ± 0.3 b	54.7
Gemmeza 9	3.9 ± 0.3 a	2.8 ± 0.4 b	27.4	2.6 ± 0.4 b	34.3	2.4 ± 0.5 b	39.6
Iskamish K-2	3.5 ± 0.5 a	3.1 ± 0.5 ab	12.3	2.9 ± 0.4 ab	18.2	2.8 ± 0.4 b	21.4
Sahl 1	3.6 ± 0.4 a	2.9 ± 0.3 b	19.0	2.5 ± 0.4 bc	31.1	2.2 ± 0.4 c	39.3
Sakha 93	3.7 ± 0.6 a	3.1 ± 0.4 ab	15.9	3.0 ± 0.2 ab	19.9	2.8 ± 0.4 b	24.1

[1] Yield (g)= Means of grain yield/pot ± standard deviation.
Means in a row followed by the same letter are not significantly different based on Tukey test ($P \leq 0.05$).
[2] Red (%) = Percentage of reduction in grain yield compared to control (0).
Red (%)=((CP-IP)/CP)*100, where Red (%)= percentage of reduction, CP= grain yield of control plant, IP= grain yield of infested plant.

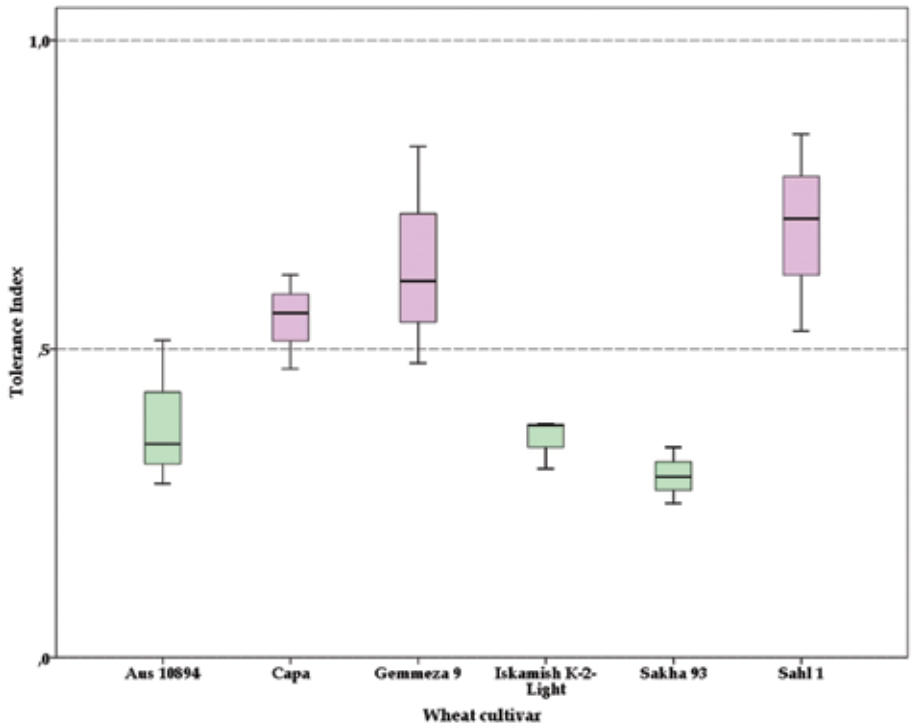


Figure 1. Box plot of the tolerance index of wheat cultivars to initial population densities of Heterodera avenae. Tolerance index (TI) = ((GCP-GIP)/GCP)*100/Pf, where, GCP= grain yield of control plant, GIP= grain yield of infested plant, Pf= final population density of J2/ml soil Tolerance ranking: tolerant (0-0.5); less tolerant (0.5-1) and sensitive (>1).

The tested wheat cultivars showed different degrees of tolerance at nematode P_i levels (Figure 1). The wheat cultivar 'Sakha 93' was the most tolerant cultivar at all P_i levels as the tolerance index (*TI*) ranged between 0.2-0.3. Wheat cultivars 'Iskamish K-2-Light' and 'Aus 10894' were less tolerance and their *TI* ranged between 0.3-0.4 and 0.3-0.5, respectively. Tolerance of wheat cultivars 'Capa' and 'Gemmeza 9' was low to all *H. avenae* P_i levels, as *TI* ranged between 0.5-0.6 and 0.5-0.8, respectively. The lowest tolerance to the nematode was recorded by the cultivar 'Sahl 1' and *TI* ranged between 0.6–0.9.

DISCUSSION

The nematode damage to host plants depends upon nematode population density in the soil as well as its reproduction in the host plant (Seinhorst 1965; Barker and Olthof 1976). A positive correlation between final population densities and increasing initial population densities of *H. avenae* was observed in this experiment. On the other hand, nematode reproduction was negatively

correlated with increasing initial population densities. This could be attributed to the competition for feeding sites and the greater damage of infected roots with increasing nematode initial density, which decreases the suitable area of the roots for nematodes to infect, establish and reproduce (Fisher and Hancock 1991). These results are in accordance with the previous report of Magi (1989) who found that the final number of eggs and juveniles increases with increasing initial density but the reproductive rate decreases. Studies on the relationship between initial population densities of *H. avenae* and nematode reproduction on wheat and barley showed significant negative correlations (Dhawan and Nagesh, 1987; Rivoal and Sarr 1988). Fisher and Hancock (1991) reported that the reproduction factor of *H. avenae* reproduced tenfold at low initial densities, while it decreased with increasing in initial population densities of *H. avenae*.

In this study, increasing the initial population densities of *H. avenae* led to increase in the relative susceptibility of the wheat cultivars 'Gemmeza 9' and 'Sahl 1' (Table 1). At a P_i of 5 J2/ml soil, these cultivars were moderately susceptible, while they were susceptible at a P_i of 20 J2/ml soil. This increase in susceptibility may due to the significant increase in the final population density of *H. avenae* on these cultivars following the increase in P_i. On the other hand, increasing the initial population densities of *H. avenae* led to decrease in the relative susceptibility of the wheat cultivar 'Iskamish K-2-Light' (Table 1). At a P_i of 5 J2/ml soil, this cultivar was susceptible, while it was moderate susceptible at a P_i of 20 J2/ml soil. This decrease in susceptibility may due to the constancy in the final population density of *H. avenae* on this cultivar in spite of the increase in P_i.

Negative relationship between the initial population density and different plant growth parameters (grain yield, spike weight, shoot dry weight and root dry weight) was detected in this study. Previous reports have concluded that losses in the grain yield of wheat caused by *H. avenae* are mainly due to the reduction of the number of spikes, number and weight of grains/spike (Goent 1982; Romero *et al.* 1988; Romero *et al.* 1991; Zancada and Althofer 1994).

This study indicates that Egyptian populations of *H. avenae* are serious pests of Egyptian wheat cultivars. The reduction in the grain yield of the Egyptian cultivars by *H. avenae* ranged between 16 - 40% under greenhouse conditions. The substantial reduction in the grain yield of the Egyptian wheat cultivars found in this study indicates that even the lowest P_i (5 eggs and J2/ml soil)

caused significant damage to wheat under greenhouse conditions. On the other hand, the local wheat cultivar 'Sakha 93' showed some degree of tolerance as the reduction in grain yield was not significant in spite of the high relative susceptibility to *H. avenae* at a P_i of 5 and 10 J2/ml soil. The grain yield of 'Sakha 93' was only significantly reduced at a P_i of 20 eggs and J2/ml soil.

ACKNOWLEDGEMENTS

The authors would like to express their gratitude to the Nordic Genetic Resource Center (Dr. Fredrik Ottosson), Alnarp, Sweden; for supplying seeds of the International Test Assortment. Many thanks to the Department of crops, Faculty of Agriculture, Suez Canal University, Egypt; for supplying seeds of the local Egyptian wheat cultivars.

REFERENCES

- Baklawa M, Niere B, Heuer H, Massoud, S (2015) Characterisation of cereal cyst nematodes in Egypt based on morphometrics, RFLP and rDNA-ITS sequence analyses. *Nematology*, 17, 103-115.
- Barker KR, Olthof THA (1976) Relationships between nematode population densities and crop responses. *Annual Review of Phytopathology*, 14, 327-353.
- Dhawan SC, Nagesh M (1987) On the relationship between population densities of *Heterodera avenae* growth of wheat and nematode multiplication. *Indian Journal of Nematology*, 17, 231-236.
- Dixon AGO, Bramelcox PJ, Reese JC, Harvey TL (1990) Mechanisms of resistance and their interactions in 12 sources of resistance to Biotype E Greenbug (Homoptera, Aphididae) in sorghum. *Journal of Economic Entomology*, 83, 234-240.
- Fisher JM, Hancock TW (1991) Population dynamics of *Heterodera avenae* Woll in South Australia. *Australian Journal of Agricultural Research*, 42, 53-68.
- Gill JS, Swarup G (1971) On the host range of the cereal cyst nematode, *Heterodera avenae* Woll. 1924, the causal organism of 'Molya' disease of wheat and barley in Rajasthan, India. *Indian Journal of Nematology*, 1, 63-67.
- Goent I, Goent Z (1982) The effect of frequency of growing spring barley and oats on the same field on grain yield and soil infestation by *Heterodera avenae. Pamietnik Pulawski*, 77, 49-62.

- Greco N, Daddabbo T, Brandonisio A, Elia F (1993) Damage to Italian crops caused by cyst-forming nematodes. *Journal of Nematology*, 25, 836-842.
- Ibrahim IKA, Hanoo ZA (2007) A survey of cyst nematodes (*Heterodera* sp.) in Northern Egypt. *Pakistan Journal of Nematology*, 25, 335-337.
- Ibrahim IKA, Rezk MA, Ibrahim AAM (1986) Occurrence of the cyst nematodes *Heterodera avenae*, *Heterodera daverti* and *Heterodera rosii* in Northern Egypt. *Journal of Nematology*, 18, 614-614.
- Lücke E (1976) Pathotype investigations with populations of *Heterodera avenae* 1966-1975 (German). *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz*, 83, 647-656.
- Magi E (1989) Relationships between the cereal cyst nematode population density, barley yield and nematode multiplication in field plots. *Eesti NSV Teaduste Akadeemia Toimetised Bioloogia*, 38, 189-194.
- Mathur BN, Handa DK, Swarup G, Sethi CL, Sharma GL, Yadav BD (1986) On the loss estimation and chemical control of Molya disease of wheat caused by *Heterodera avenae* in India. *Indian Journal of Nematology*, 16, 152-159.
- Meagher, JW, Brown, RH (1974) Microplot experiments on effect of plant hosts on populations of cereal cyst nematode (*Heterodera avenae*) and on subsequent yield of wheat. *Nematologica*, 20, 337.
- Namaouchi-Kachouri N, B'Chir MM, Hajji A (2009) Global importance of the main nematodes associated with cereals in Tunisia. In: 'Cereal cyst nematodes: status, research and outlook'. (Eds. IT Riley, JM Nicol, AA Dababat) pp. 244. *Proceedings of the First Workshop of the International Cereal Cyst Nematode Initiative, Antalya, Turkey, 21-23 October 2009.* Antalya, CIMMYT, Turkey.
- Nicol JM, Bolat N, Sahin E, Tulek A, Yildririm AF, Yorgancilar A, Kaplan A, Braun HJ (2005) The cereal cyst nematode is causing economic damage on rainfed wheat production systems of Turkey. *American Phytopathological Society, Pacific Division Annual Meeting.* Portland, Oregon.
- Rammah A (1994) Cereal cyst *nematode* (*Heterodera avenae*) in Morocco. *Arab and Near East Plant Protection Newsletter*, p.40.
- Rivoal R, Sarr E (1988) Field experiments on *Heterodera avenae* in France and implications for winter wheat performance. *Nematologica*, 33, 460-479.
- Romero MD, Valdeolivas A, Lacasta C (1991) Incidence of *Heterodera avenae* on the growth and yield of cereals in Spain. *Nematologia Mediterranea*, 19, 77-79.

- Romero MD, Valdeolivas A, Lacasta C, Duce A (1988) Effects of attack by *Heterodera avenae,* a parasitic nematode of cereals, and its repercussions on yields of wheat cv. Anza. In: 'Comunicaciones del III Congreso Nacional de Fitopatologia.' (Ed. LG Llobet). *Puerto de la Cruz.* La Laguna, Tenerife, Spain: Centro de Investigacion y Technologia Agrarias.
- Seihhorst JW, Den Ouden H (1966) An improvement of bijloo's method for determining egg content of *Heterodera* cysts. *Nematologica*, 12, 170-171.
- Seinhorst JW (1965) The relation between nematode density and damage to plants. *Nematologica*, 11, 137-154.
- Shepherd AM (1986) Extraction and estimation of cyst nematodes. In: 'Laboratory methods for work with plant and soil nematodes.' (Ed. JF Southey). H.M.S.O. Books; Norwich, NR3 1PD, Norfolk, UK.
- Siddiqui ZA, Khan MW (1986) Nematodes causing damage to wheat crops in Libya. *International Nematology Network Newsletter*, 3, 23.
- Zancada C, Althofer MV (1994) Effect of *Heterodera avenae* on the yield of winter wheat. *Nematologica*, 40, 244-248.

CEREAL SOIL BORNE NEMATODES STATUS IN IRAN

ZAHRA TANHA MAAFI[1,*], ALI REZA AHMADI[2], NADER AZADBAKHT[3], MAZDOSHT GITTI[4], SEYED ABBAS HOSSEININEJAD[1], FARAHNAZ JAHANSHAHI AFSHAR[1], HADI KARIMIPOUR[5], MEHDI MINBASHI[1], MAJID PAKNIYAT[6], AMIR AHMADIAN YAZDI[7], AMIR RIVANDI[7], DARUSH SAFAEE[8], MOHAMMAD ALI AGHAJANI[9], JULIE M. NICOL[10], AND ABDELFATTAH A. DABABAT[11]

[1]Iranian Research Institute of Plant Protection, Agricultural Research Education and Extension Organization (AREEO), Tehran, Iran; [2-9]Plant Protection Research Department, Khuzestan, Lorestan, Hamadan, Kohgilouyeh va Boyer-Ahmad, Fars, Khorasan Razavi, Kermanshah, Golestan Agricultural and Natural Resources Research Center, AREEO, Iran; [10]CIMMYT Int, Consultant, Australia; [11]ICARDA-CIMMYT Wheat Improvement Program, P.K. 39 Emek, 06511, Ankara, Turkey. *Correspondence author email: tanhamaafi@iripp.ir

SUMMARY

Plant-parasitic nematodes affect agricultural crops worldwide resulting in heavy losses annually. The families Pratylenchidae and Heteroderidae are of great economic importance, as they cause substantial crop losses in many countries. Wheat is one of the most important strategic plant roles as a good host for the genera of *Pratylenchus* and *Heterodera* and is seriously affected in many wheat-producing areas particularly in rain-fed regions throughout the world. *Heterodera avenae* group species and root lesion nematode endoparasites of roots are widely distributed in cereal fields in most provinces in Iran. Surveys on identification, distribution and population density for both genera were conducted in several provinces. The results indicated *Heterodera filipjevi*

* Tanha Maafi Z, Ahmadi A, Azadbakht N, Gitti M, Hosseininejad SA, Afshar FJ, Karimipour H, Minbashi M, Pakniyat M, Yazdi AA, Rivandi A, Safaee D, Aghajani MA, Nicol JM, Dababat AA (2015) Cereal soil borne nematodes status in Iran. In 'Nematodes of Small Grain Cereals: current status and research.' (AA Dababat, H Muminjanov and RW Smiley) pp. 91-100. (FAO: Ankara, Turkey).

is the most abundant species of cereal cyst nematodes in cereal fields, followed by *H. latipons* and *H. avenae*, distributed in the west and southwest. Root lesion nematodes *P. neglectus* and *P. thornei* are the prevalent species which are accompanied by *Pratylenchoides ritteri* in the north east, and *Pr. ritteri* and *P. alkani* in the west of the country. The population densities in some cereal fields are higher than the economic damage threshold determined for these nematodes in other parts of the world. Assessment of the impact of cereal cyst and root lesion nematodes on a few wheat cultivars have been performed in some regions including Khuzestan Province (south west), Kermanshah and Hamadan provinces (west) and Khorasan Rezavi Province (east). The results indicated the common species of cereal cyst nematodes *i.e. H. filipjevi, H. latipons* and *H. avenae* as well as root lesion nematodes species viz *P. neglectus*, *P. thornei* and *Pr. alkani* are able to reduce the wheat grain yield significantly under field conditions. The reaction of some current wheat cultivars along with CIMMYT germplasms against two species of *P. neglectus* and *P. thornei*, revealed resistance in some current wheat cultivars, which needs to be confirmed under field conditions. Perspectives of future researches will be focused on management strategies through seeking resistance sources in Iranian wheat landraces and commercial cultivars by molecular markers as well as determining the economic importance of root lesion nematodes and cereal cyst nematodes in wheat production under drought stress conditions in different localities. Meanwhile, surveys of the distribution and frequencies of these groups of soil borne pathogens in other unstudied provinces should be emphasized.

INTRODUCTION

Nematodes affecting cereals are divided in two groups, parasites of underground and aboveground plant tissues. The underground parasitic nematodes of cereals are soil terrestrial nematodes which invade root systems of cereals and cause severe damage to plant growth parameters and crop yield. Cereal cyst nematodes (CCN) and root lesion nematodes (RLN) are categorised into this group and induce significant crop losses in wheat worldwide. *Heterodera avenae* is the most common species of CCN that has a global distribution and is found in most European countries, Australia, Canada, South Africa, North Africa, Japan, Saudi Arabia and Turkey (Dababat *et al.* 2015). *Heterodera avenae* is the most damaging species on temperate cereals (Nicol *et al.* 2003). *Heterodera filipjevi* is more confined to Asia and has been reported from Russia, Spain, Turkey, Central Asia, India, and was recently found in the USA (Nicol *et al.* 2003, Smiley *et al.* 2008, Dababat *et al.* 2015). The Mediterranean cyst

nematode, *H. latipons*, is mostly found in Mediterranean regions as well as in North Africa and some northern European countries (Nicol *et al.* 2003). Root lesion nematodes are migratory endoparasitic nematodes that have a global distribution and broad host ranges.

Out of more than 68 valid species of *Pratylenchus* Filipjevi, 1936 (Castillo and Vovlas 2007) eight species are found in cereals fields and, of these, *P. neglectus*, *P. thornei*, *P. penetrans* and *P. crenatus* have a worldwide distribution (Rivoal and Cook 1993). *Pratylenchus neglectus* and *P. thornei* are the most common species found in wheat fields in Iran. Moreover, among the group of root lesion nematodes, *Pratylenchoides* spp. were frequently found in cereal fields particularly in the west of Iran. *Pratylenchoides* belongs to Pratylenchidae family, however, recently it was moved to the subfamily Pratylenchoidinae, family Merliniidae (Sturhan 2012). Cereal cyst nematodes and root lesion nematodes have been frequently reported from cereal fields in different parts of Iran. The first evidence of the presence of CCN in Iran was revealed when Talatchian *et al.* (1976) surveyed sugar beet fields in eleven provinces to detect the sugar beet cyst nematode, *H. schachtii*. They found the "*H. avenae* group" as well as *H. latipons* and *H. schachtii*, but they did not directly identify *H. avenae*. The species *H. avenae* was then reported for the first time by Barooti and Loof (1990) from wheat fields in Marvast area, Yazd Province; however, it was then re-identified as *H. filipjevi* after precise morphological and molecular studies (Sturhan 1996, Rumpenhorst *et al.* 1996). Following identification of cyst forming nematodes in Iran, five species belonging to the "*H. avenae* group", namely *H. avenae* type B, *H. filipjevi*, *H. hordecalis*, *H. latipons* and *Heterodera* sp. 1, were reported from Iran, and *H. filipjevi* had the dominant distribution followed by *H. latipons* and *H. avenae* type B (Tanha Maafi *et al.* 2007).

The above-ground parasites, *i.e.*, the family Anguinidae Nicoll, 1935 (1926), that include *Anguina* Scopoli, 1777 and *Ditylenchus* Filipjev, 1936, attack upper parts of cereals. *Ditylenchus dipsaci* Filipjev, 1937 is a migratory endoparasite and invades the foliage and the base of stems of cereal plants. Oat and rye are the main hosts for *D. dipsaci*, and it is widespread throughout western and central Europe, the USA, Canada, Australia, Brazil, Argentina, and North and South Africa (Plowright *et al.* 2002). There are several reports of *D. dipsaci* occurring in Iran, however it has not been reported from wheat thus far.

Anguina tritici is the causal agent of wheat seed gall and is a migratory ectoparasite of leaves and growing points of plants. The juveniles penetrate the flower

primordium at flowering time and then turn into adults. As a result, ovules and other flowering parts of a plant are transformed into galls or 'cockles.' The gall seed nematode currently is not a serious problem in wheat producing areas where there is use of modern seed cleaning systems. However, farmers who are using their own seeds encounter problems from the early stages of wheat growth, even leading to death of new emerged plants. Twisted and waved leaves are the common symptoms of infection to *A. tritici*. Wheat is the main host for *A. tritici*. It is distributed throughout western Asia and North Africa, the Indian subcontinent, China, parts of Eastern Europe, Iraq and Pakistan (Mc Donald and Nicol 2005), and in Turkey (Tulek *et al.* 2015). The damage of *A. tritici* is remarkable occasionally in some wheat-producing areas in Iran where farm-saved seeds are grown by conventional small-scale growers.

HISTORY

Distribution and abundance of cereal nematodes were carried out by collecting soil and root samples during growing seasons 2008-2012 from wheat and barley fields in six provinces of cereal-producing areas in the center, east, north east, west and south east of Iran. The samples were analyzed according to current nematology techniques. The population density of cereal nematodes was determined for the collected samples. The identification of CCN was performed based on morphological and molecular data (Baldwin and Mundo-Ocampo 1991, Subbotin *et al.* 1999). The RLN species were distinguished on the basis of morphological and morphometric characters (Loof 1991) and molecular characteristics (Waeynberg *et al.* 2000). The distribution map of cereal nematodes was prepared for the surveyed provinces. The effect of *H. filipjevi* and *H. latipons* on wheat cultivar Sardari was evaluated with different levels of initial populations under microplot conditions (Hajihassani *et al.* 2010a, 2010b). The crop loss assessment of *H. filipjevi* on some cultivars of wheat and barley was performed under field conditions in Ramshir region during 2008-2009 in Khuzestan Province that were either untreated or treated with aldicarb nematicide to reduce nematode potential for damage. Meanwhile, the impact of *H. avenae* type B was evaluated in a field trial with four bread and durum wheat cultivars at Behbahan Agricultural Research Station (BARS) in south west of Iran during the growing season 2009-2010. Both experiments were arranged in a randomized complete block design. The biology of *H. avenae* type B was determined under BARS during growing season 2009-2010. Investigation on yield loss caused by root lesion nematodes was assessed on wheat and barley cultivars at three regions, viz Khorasan Razavi, Neyshabour Agricultural Re-

search Station (NARS) in the east, Hamadan, Ekbatan Agricultural Research Station (EARS) and Kermanshah, Islamabad-Gharb Agricultural Research Station (IARS) in the west under field conditions. Moreover some commercial wheat cultivars and CIMMYT germplasm were screened against *P. neglectus* and *P. thornei* under control conditions.

STATUS

The results showed that cereal nematodes are widely distributed in the surveyed regions. Three species of CCNs were identified, *H. filipjevi* had the highest frequency among the identified species, *H. latipons* was the second most abundant species followed by *H. avenae*. Root lesion nematodes were found to widespread in the surveyed wheat producing regions. Four species of RLN were identified; *P. neglectus*, *P. thornei*, *P. pseudopratensis*, *Pr. ritteri*. The most dominant species was *P. neglectus*, however *Pr. ritteri* had a high frequency in the north east and in Lorestan Province, which is located in the west of country, where it was ranked after *P. neglectus*. In crop loss trials of cereal cyst nematodes, *H. filipjevi* caused significant reduction of grain yield, biomass, shoot weight, shoot height and tillering by 52 (40-73), 40 (14-53), 38 (6-67), 15 (8-21) and 24 (10-39) percent, respectively, on wheat and barley cultivars in Ramshir region in Khuzestan Province where the average initial population was determined to be 10 eggs and second-stage juveniles (J2) per g of soil. The trial conducted at BARS was infested with *H. avenae* type B, which induced yield reduction by 11-21% for four wheat cultivars; the average initial population density was 62 eggs and J2 per g soil.

The root lesion nematodes caused significant impact of crop yield in three regions where the yield loss assessment trials were conducted. A two-year experiment conducted in IARS contained *Pr. alkani* and showed a reduction of grain yield for wheat cultivars Azar 2 and Sardari (22.7% and 20.5%, respectively) in comparison with the control treatment including nematicide application. Other treatments, i.e. barley cultivars Sararod 1 and Rizhaw, were damaged as well by 16.1 and 13% reduction of grain yield, respectively. The results of the trials done in NARS were infested by RLNs and indicated similar results in grain reduction for wheat cultivars, Sardari (8%), Sabalan (19%) and Pishtaz (6%) during a two-year experiment. The field trials in EARS showed 6.7% and 22.3% increase in grain of wheat due to nematicide application in year 2013 and 2014, respectively.

The evaluation of commercial wheat cultivars to *P. neglectus* and *P. thornei* showed that cvs. Dez, Shiraz, Akbari, Atrak, Parsi, Sivand, Alvand, Pishtaz and line S-80-18 were resistant to *P. thornei*. Cultivars Moghan3, Pishtaz, Dez, Darya, Chamran, Nicknejad, Gascojen, Bam, Bahar, and line CD-5509 had resistant reactions against *P. neglectus*. Two commercial wheat cultivars, Dez and Pishtaz, were resistant to both RLN species.

IMPACTS

Cereal nematode research in Iran was performed in terms of different aspects to indicate the important role of these groups of nematodes in cereal production in Iran. Cereal cyst and root lesion nematodes are widely distributed in cereal fields. *Pratylenchus neglectus* showed the highest frequency compared to other RLN species, however *Pr. ritteri* was the dominant species in the north east and in Lorestan and Kermanshah provinces located in the west of country, where it was ranked after *P. neglectus*. *Pratylenchoides* spp. were frequently isolated from roots of wheat, occasionally containing high population densities, hereupon it should be taken into consideration as a potential RLN in wheat fields where it occurs.

According to the literature, *P. thornei* is more capable than *P. neglectus* for causing yield loss (Castillo and Vovlas 2007, Vanstone *et al.* 1998, Nicol *et al.* 2003, Smiley *et al.* 2005a, 2005). Recent investigations showed even more damage from *P. neglectus*, with mean yield improvement due to nematicide application being greater for spring wheat infested by *P. neglectus* compared to *P. thornei*, 31% *vs* 8%, and for winter wheat it was 9 and 11% as a result of infestation to *P. neglectus* and *P. thornei*, respectively (Smiley 2009, Smiley and Machado 2009). The population density of RLN in some collected soil samples was more than two nematodes per gram soil, which is rather higher than those of the economic damage threshold reported for these nematodes in other parts of the world, *i.e.* for *P. thornei* in Mexico it was 420 nematodes/kg soil, in western Australia it was 1000/kg soil, and in Queensland it was 2500/kg soil (Riley and Kelly 2002, Nicol and Ortiz-Monasterio 2004, Thompson 1993).

The economic damage threshold levels are not available for *H. filipjevi*, *H. latipons* and *H. avenae* type B, nevertheless these species of CCNs showed high density in some of the regions, occasionally equal or above the levels reported for *H. avenae* (Dixon 1969, Gill and Swarup 1971, Nicol and Rivoal 2008), implying crop yield reduction is most likely anticipated in these regions.

Damage caused by cereal nematodes in addition to population density depends on several factors, *i.e.* availability of water and nutrition, genotypical factors, and tolerance and resistance of cultivars which needs to be determined under different environmental conditions.

PROSPECTS

The sources of genetic resistance to cereal cyst nematodes in Iranian landraces wheat are unknown. Wheat is originally from the Near East and has been cultivated in this region for a long time. At least some landraces are likely assumed to contain resistance genes, as genetic resistance of Iranian landrace wheat to root lesion nematode, *Pratylenchus thornei*, was determined in Australia (Sheedy & Thompson 2009). Hereupon, prospective future researches will be focused upon management strategies through seeking resistance sources in Iranian wheat landraces and commercial cultivars by molecular markers as well as phenotypic screening.

There is comparatively little information on the economic importance of *H. filipjevi*, *H. latipons*, *H. avenae* type B, and root lesion nematodes species. The wide distribution and the preliminary crop loss trials indicate that these species can be considered as the most important cereal nematodes in the region and need intensive researches. The economic importance of RLNs and CCNs in wheat-production systems under drought stress conditions in different localities needs additional research. Meanwhile, surveys on the distribution and frequencies of these groups of soil borne pathogens in other unstudied provinces should be emphasized.

ACKNOWLEDGEMENT

The authors would like to thank Iranian Research Institute of Plant Protection (IRIPP), for support, coordination and providing the research facilities.

REFERENCES

- Baldwin JG, Mundo-Ocampo M (1991) Heteroderinae, cyst- and non-cyst-forming nematodes. In 'Manual of Agricultural Nematology', (Ed. WR Nickle) pp. 275-362. (Marcel Dekker, Inc. New York).

- Barooti S, Loof PAA (1990). The cereal cyst nematode *Heterodera avenae* occurring on wheat in Marvast area of Iran. Abstracts of the Second International Nematology Congress. *University Wageningen. The Netherlands* 25, 376.
- Castillo P, Vovlas N (2007) 'Pratylenchus (Nematoda: Pratylenchidae): Diagnosis, Biology, Pathogenicity and Management'. *Nematology Monographs and Perspectives*. (Brill Leiden-Boston, The Netherlands).
- Dababat AA, Imren M, Gul EO, Ashrafi S, Yavuzaslanoglu E, Toktay H, R. Pariyar S, Elekcioglu H, Morgounov A, Mekete M (2015) The importance and management strategies of cereal cyst nematodes, *Heterodera* spp., in Turkey. *Euphytica* 202:173-188. DOI 10.1007/s10681-014-1269-z
- Dixon GM (1969) The effect of cereal cyst eelworm on spring sown cereals. *Plant Pathology*, 18, 109–112.
- Gill JS, Swarup G (1971) On the host range of cereal cyst nematode, *Heterodera avenae,* the casual organism of 'molya' disease of wheat and barley in Rajasthan, India. *Indian Journal of Nematology* (1), 63–67.
- Hajihasani A, Tanha Maafi Z, Nicol JM, Seraji A (2010a) Relationships between population densities of *Heterodera latipons* and yield losses of winter wheat in microplots. *Australian Plant Pathology* 39, 530-535.
- Hajihasani A, Tanha Maafi, Z, Nicol JM, Rezaee S (2010b) Effect of the cereal cyst nematode, *Heterodera filipjevi*, on wheat in microplot trials. *Nematology* 12, 357-363.
- Mc Donald AH Nicol MJ (2005) Nematode Parasites of Cereals. In: 'Plant Parasitic Nematodes in Subtropical and Tropical Agriculture.' (Ed. M Luc, RA Sikora, Bridge J) pp. 131-191 (CAB International 2nd Edition)
- Nicol JM, Ortiz-Monasterio I (2004) Effects of the root lesion nematode, *Pratylenchus thornei,* on wheat yields in Mexico. *Journal of Nematology, 6*, 485–493.
- Nicol JM, Rivoal R (2008) Global knowledge and its application for the integrated control and management of nematodes on wheat In: 'Integrated Management and Biocontrol of Vegetable and Grain Crops Nematodes'. (Ed. A Ciancio, KG Mukerji) pp. 251–294 (Springer).
- Nicol JM, Rivoal R, Taylor S (2003) Global importance of cyst (*Heterodera* spp.) and lesion nematodes (*Pratylenchus* spp.) on cereals: distribution, yield loss, use of host resistance and integration of molecular tools. *Nematology Monographs & Perspectives,* 2, 1-19.
- Plowright RA, Caubel G, Mizen KA (2002) *Ditylenchus* species. In: '*Plant Resistance to Parasitic Nematodes.*' (Ed. JL Starr, R Cook, J Bridge) pp. 107–139 (CAB International, Wallingford, UK).

- Riley IT, Kelly SJ (2002) Endoparasitic nematodes in cropping soils of Western Australia. *Australian Journal of Experimental Agriculture*, 42, 49–56.
- Rivoal R, Cook R (1993). nematode pests of cereals. In: 'Plant parasitic nematodes in temperate agriculture'. (Ed. K Evans, DL Trudgill JM Webster) pp. 259-303 (Wallingford, UK, CAB International).
- Rumpenhorst H J, Elekcioglu IH Sturhan D, Ozturk G, Enelli S (1996) The cereal cyst nematode *Heterodera filipjevi* (Madzhidov) in Turkey. *Nematologia mediterranea*, 24, 135-138.
- Safaee S, Hossaeininejad SA, Tanha Maafi Z, Nicol JM, Kafashi AK, Younesi H, Sheikhieslami M (2014) Evaluation of yield losses caused by root lesion nematode (*Pratylenchoides alkani*)under field condition in Kermanshah province. *21st Iranian Plant Protection Congress*, Urmieh, September, p. 293.
- Sheedy JG, Thompson JP (2009) Resistance to the root-lesion nematode *Pratylenchus thornei* of Iranian landrace wheat. *Australasian Plant Pathology*, 38, 478-489.
- Smiley RW (2009) Root-Lesion nematodes reduce yield of intolerant wheat and barley. *Agronomy Journal*, 101, 1322–1335.
- Smiley RW, Machado S (2009) *Pratylenchus neglectus* reduces yield of winter wheat in dryland cropping systems. *Plant Disease*, 93, 263–271.
- Smiley RW, Whittaker RG, Gourlie JA, Easley SA Ingham RE (2005) Plant-Parasitic nematodes associated with reduced wheat yield in Oregon: *Heterodera avenae. Journal of Nematology*, 37 (3), 297-307.
- Smiley RW, Yan GP, Handoo ZA (2008) First record of the cyst nematode *Heterodera filipjevi* on wheat in Oregon. *Plant Disease*, 92, 1136.
- Sturhan D (1996) Occurrence of *Heterodera filipjevi* (Madzhidov, 1981) Stelter, 1984 in Iran. *Pakistan Journal of Nematology,* 14, 89-93.
- Sturhan D (2012) Contribution to a revision of the family Merliniidae Ryss, 1998, with proposal of Pratylenchoidinae subfam. N., *Paramerlinius* gen. n., *Macrotylenchus* gen. n. and description of *M. hylophilus* sp. n. (Tylenchida). *Journal of Nemate Morphology and Systematic* 15(2): 127-147.
- Subbotin SA, Waeyenberge L, Molokanova IA, Moens M (1999) Identification of *Heterodera avenae* group species by morphometrics and rDNA-RFLP. *Nematology,* 1, 195-207.
- Talatchian P, Akhiani A, Grayeli Z, Shah-Mohammadi M, Teimouri F (1976) Survey on cyst forming nematodes in Iran in 1975 and their importance. *Iranian Journal of Plant Pathology*, 12, 42-43.
- Tanha Maafi Z, Sturhan D, Kheiri A, Geraert E (2007) Species of the *Heterodera avenae* group (Nematoda: Heteroderidae) from Iran. *Russian Journal of Nematology*, 15(1), 49-58.

- Thompson JP (1993) Assessing damage to wheat by *Pratylenchus thornei*. Proceedings of the *Pratylenchus* Workshop, *9th Biennial Australasian Plant Pathology Conference*, Hobart, Tasmania.
- Tulek A, Kepenekci i, Cifticigil TH, Ozturk I, Akin K, Seidi M, Yildirim M, Dababat AA (2015) Effects of seed-gall nematode (Anguina tritici) on bread wheat grain characteristics and yields in Turkey. *Nematology* (In press).
- Waeyenberge L, Ryss A, Moens M, Pinochet J, Vrain TC (2000) Molecular characterisation of 18 *Pratylenchus* species using rDNA restriction fragment length polymorphism. *Nematology* 2, 135-142.

EVALUATION OF WHEAT YIELD LOSSES CAUSED BY ROOT LESION NEMATODES IN HAMADAN PROVINCE, IRAN

MAZDOSHT GITTI[1,*], SEYED ABBAS HOSEININEJAD[2], AND ABDELFATTAH A. DABABAT[3]

[1]Plant Protection Research Department, Hamadan Agricultural and Natural Resources Research Center, AREEO, Hamadan, Iran. [2]Iranian Research Institute of Plant Protection, Agricultural Research Education and Extension Organization (AREEO), Tehran, Iran, [3]ICARDA-CIMMYT Wheat Improvement Program, P.K. 39 Emek, 06511, Ankara, Turkey. *Correspondence author email: Mazdyashtg@gmail.com

SUMMARY

Pratylenchus thornei and *P. neglectus* are the most common root lesion nematodes (RLN) on wheat with economically important damaging capabilities. On the basis of previous studies both species are prevalent in the province and are damaging crops especially in rainfed wheat fields. In order to evaluate yield losses caused by root lesion nematodes on wheat in Hamadan, a two-year field trial with four split-plot replications on a randomized completed block design were conducted in a naturally infested field during 2012- 2014. Two wheat cultivars (Sardari and Azar), two barley cultivars (Makuii and Abidar), and a triticale cultivar (Juanillo92) were sown in 2 × 5 m plots in 10 lines. Five lines of each plot were treated with aldicarb 10G, 0.8 g *a.i..s*/m^2 soil at sowing time. Soil samples were taken from each plot by auger one month after sowing and one month before harvest, and root lesion nematodes were extracted and counted. For both years the grain yield was recorded for each plot. Analysis of data with SAS statistical program showed there was a significant difference between means and nematicidal application which caused 84% and 92% de-

* Gitti M, Hoseininejad SA, Dababat AA (2015) Evaluation of wheat yield losses caused by root lesion nematodes in Hamadan Province, Iran. In 'Nematodes of Small Grain Cereals: current status and research.' (AA Dababat, H Muminjanov and RW Smiley) pp. 101-108. (FAO: Ankara, Turkey).

crease in RLN populations and 6.7% and 22.3% increase of grain of wheat in the first and second year, respectively. There was a significant linear relationship between RLN density and wheat grain yield of Sardari and Azar cultivars with the estimated regression equation; $Y= 0.1425 x^2 – 13.937 x + 1513$ and $R^2 = 0.0875$, where Y is the grain yield in gr/m^2 and x is the number of RLN per gram oven-dried soil. Sardari wheat cultivar was more susceptible than Azar cultivar whereas barley and triticale revealed less difference in grain yields with different root lesion nematode densities.

INTRODUCTION

Wheat (*Triticum aestivum* L.) as a main crop is planted each year on 303000 ha as rainfed with 1103 kg/ha yield in the low-precipitation region (150 to 300 mm) and 103000 ha with 3800 kg production per ha in irrigated fields in Hamadan Province. In most areas winter wheat is rotated with a 14-month fallow (crop-free) period and in some areas winter wheat without fallow (Anon 2014). However, wheat or barley (*Hordeum vulgare* L.) are sown in rainfed fields annually while irrigated fields have rotations that include broadleaf crops such potato, alfalfa, sugarbeet, chickpea or canola.

Root-lesion nematodes (*Pratylenchus neglectus* (Rensch, 1924) Filipjev Schuurmanns & Stekhoven, 1941 and *P. thornei* Sher & Allen 1953) are present in 82% of the fields in the province and effects of crop management practices on nematode populations have not been clearly defined (Tanha Maafi *et al.* 2009). Direct associations between *Pratylenchus* spp. population density and frequency of cereal cropping have been reported in other countries (Gair *et al.* 1969, Riley *et al.* 2002). A replicated 2-year experiment was established during 2012-2014 to examine the aspects of RLN damage on wheat at a low precipitation site known to be infested with *P. neglects* and *P. thornei*.

METHODS

The experiment was performed at the Ekbatan Agricultural Research Station (EARS) located 5 km northwest of Hamadan. A uniform crop of winter wheat was planted over the intended experimental area during 2012 in order to maintain the RLN population. The experimental area was divided into 24 plots of 2 × 5 m arranged as four randomized blocks of six plots representing six treatments. Plots had 10 lines, and five lines of each plot were treated with aldicarb 10G, 0.8 g *a.i..s*/m^2 soil at sowing time. The six treatments included biennial

Figure 1. The experimental site of evaluation of wheat and barley yield losses caused by root lesion nematodes in Hamadan Province, Iran, 2014

Sardari and Azar winter wheat, biennial Makuii and Abidar winter barley, and triticale cultivar Juanillo92. All crops were harvested in mid September. Grain yield was measured using a weigh wagon to determine yield per plot. Routine soil sampling for nematode extraction was collected each year to assess *Pratylenchus* populations in individual treatments when soil was moist. Soil samples were taken from each plot by an auger (2.5-cm diameter) one month after sowing and one month before harvest. Samples consisted of 20 cores composited for each of 24 plots. All soil-dwelling nematodes from 250-g subsamples of soil plus root fragments were extracted by the Whitehead tray method (Whitehead and Hemming 1965). Nematodes were washed into 100-ml cups and suspensions were stored at 5 °C before counting. One ml of suspension was placed on a counting slide, and all plant-parasitic nematode genera on the slide were counted, identified, and recorded as nematodes/kg of oven-dried soil.

Results were analyzed using one-way analysis of variance (ANOVA). Each analysis was performed using SAS Statistical Software. Associations of grain yields and nematode populations were evaluated by regression analysis.

RESULTS

Numbers of isolated RLN were greater than any other plant-parasitic species, which were detected infrequently and in low numbers. *Pratylenchus* populations did not significantly differ ($P < 0.05$) among treatments during the 2 years of the experiment. Aldicarb reduced ($P < 0.01$) *Pratylenchus* densities in soil and roots for two years in treated plots (Table 1). Applying aldicarb caused a 84% and 92% decrease in RLN populations and 6.7% and 22.3% increase in grain of wheat during the first and second year, respectively. The yield × aldi-

carb interaction was significant and when data for control plots (not treated with aldicarb) were analyzed separately, the varietal difference was significant at $P < 0.05$. Grain yields also were improved by aldicarb in each experiment ($P < 0.01$). There was a significant negative linear relationship between RLN density and wheat grain yield of Sardari and Azar cultivars with the estimated regression equation; $Y= 0.1425 x^2 – 13.937 x + 1513$ and $R^2 = 0.0875$, where Y is the grain yield in gr/m^2 and x is the number of RLN per gram oven-dried soil.

Table 1. Density of Pratylenchus in one gram of soil in aldicarb-treated and untreated plants at Ekbatan Agricultural Research Station during 2013 and 2014

Year	Cereals									
	Sardari (wheat)		Azar (wheat)		Abidar (barley)		Makuii (barley)		Triticale	
	Aldicarb	Check	Aldicarb	Check	Aldicarb	Check	Aldicarb	Check	Aldicarb	Check
2013	0.75	9.30	0.30	10.90	1.05	5.10	1.25	4.35	1.95	4.00
2014	0.65	20.65	0.60	23.15	0.80	6.60	0.85	3.25	1.50	3.00

Table 2. Grain yields of aldicarb-treated and untreated plants at Ekbatan Agricultural Research Station during 2013 and 2014

Year	Cereals									
	Sardari (wheat)		Azar (wheat)		Abidar (barley)		Makuii (barley)		Triticale	
	Aldicarb	Check	Aldicarb	Check	Aldicarb	Check	Aldicarb	Check	Aldicarb	Check
2013	1377	1242	1592	1540	1330	1380	1432	1425	1375	1727
2014	1747	1370	1359	1168	1406	1293	1900	1254	9995	9077

DISCUSSION

We report the first field-derived evidence that *Pratylenchus* spp. causes economic damage to wheat in the west regions of Iran. In soils with high populations of *Pratylenchus* spp., aldicarb application improved yield and grain weight. Observations of yield differences due to varietal tolerance and resistance to *Pratylenchus* spp. in the west of Iran are important because they indicate a potential for improving yields of locally-adapted varieties by incorporating parental lines with improved tolerance and resistance to *Pratylenchus* spp. into the breeding programs. However, Sardari and Azar are well adapted to production systems in the west of Iran.

Compared to non-treated controls, aldicarb treatments yields were higher by an average of 14.5%. Invasion of roots by *Pratylenchus* spp. generally does not

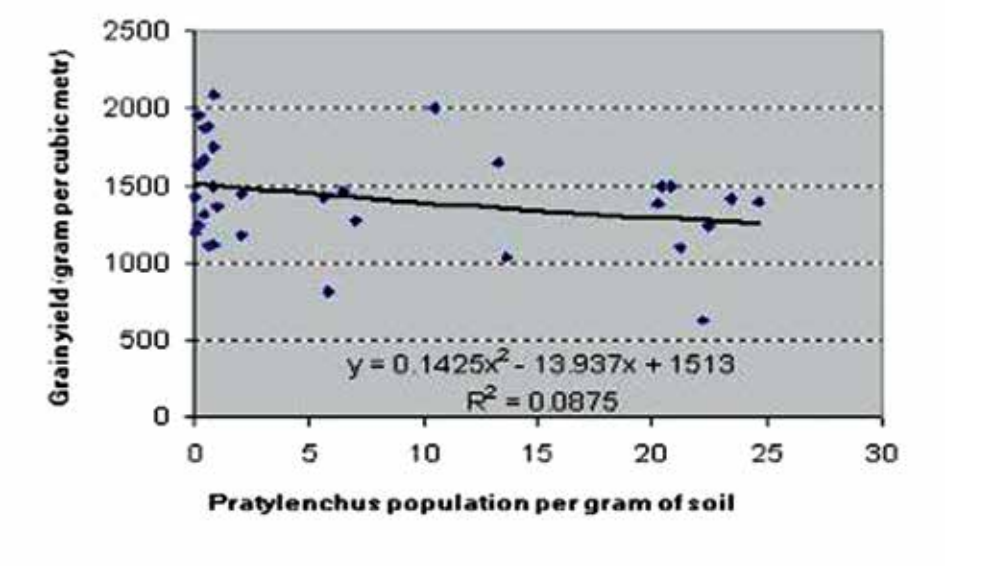


Figure 2. Relationship between numbers of Pratylenchus spp. and yield of Sardari and Azar winter wheat during two years at the Ekbatan Agricultural Research Station

cause diagnostic symptoms to occur in the foliar canopy. Plants with heavily damaged roots may exhibit stunting, poor vigor, reduced tillering, and premature wilt at the onset of moisture stress (Doyle *et al.* 1987, Orion *et al.* 1984, Van Gundy *et al.* 1974).

Although crop rotation is a preferred management practice, limiting damage by *Pratylenchus* spp. through rotation is a continuing challenge because RLN species infest a wide range of host plant species (Hollaway *et al.* 2000, Talavera and Vanstone 2001, Thompson *et al.* 1999, Vanstone and Russ 2001a 2001b). In general, most commercial wheat and chickpea varieties are considered good hosts for *P. thornei.* Poor hosts include many varieties of barley, durum wheat, canola, safflower, lupine, lentil, field pea, and flax. Of the listed crops, only barley is adapted to the Hamadan small grain-production region, where precipitation has a winter-dominant pattern and temperatures are very low, and summers are hot and dry. Assessment of barley as a potential break crop is required. Likewise, much progress and promise exists for breeding wheat varieties with sufficiently high tolerance and (or) resistance to stabilize yields and reduce the reproductive efficiency for *Pratylenchus.*spp. Development of tolerant, locally-adapted varieties is essential for limiting damage by *Pratylenchus* spp. *Pratylenchus* spp. population densities were strongly influenced by cropping systems in the low-precipitation region. These results are in agreement with previous observations (Gair *et al.* 1969, Riley *et al.* 2002). Although

populations of *Pratylenchus* spp. were not monitored in the volunteer cereals and weed grasses in this experiment, there was ample opportunity for multiplication of nematodes through the 10-month winter wheat growth cycle and also, when temperatures permitted, for as many as seven months of cycle. We conclude that management of *Pratylenchus* spp. populations in the winter wheat region of western Iran must include elimination of the bridging and multiplication potential by unwanted hosts during the fallow period, as has been shown to be essential for reducing risks posed by other pests and diseases of wheat roots and foliage.

ACKNOWLEDGMENTS

We would like to thank Dr. Zahra Tanha Maafi, Iranian Research Institute of Plant Protection, Tehran, Iran for her contribution and support.

REFERENCES

- Anon (2014) Agricultural Statistical Yearbook. Tehran, Iran, Ministry of Jihad-E- Agriculture, Statistical and Information Technology Unit, 271 pp.
- Doyle AD, McLeod RW, Wong PTW, Hetherington SE, Southwell RJ (1987) Evidence for the involvement of the root lesion nematode *Pratylenchus thornei* in wheat yield decline in northern New South Wales. *Australian Journal of Experimental Agriculture*, 27, 563–570.
- Gair R, Mathias PL, Harvey PN (1969) Studies of cereal nematode populations and cereal yields under continuous or intensive culture [*Heterodera avenae, Pratylenchus neglectus, Trichodorus primitivus*]. *Annals of Applied Biology*, 63, 503-512.
- Hollaway GJ, Taylor SP. Eastwood RF, Hunt CH (2000) Effect of field crops on density of *Pratylenchus* in southeastern Australia. Part 2: *P. thornei. Journal of Nematology*, 32, 600–608.
- Orion DJA, Kirkun J (1984) Field observations on *Pratylenchus thornei* and its effects on wheat under arid conditions. *Revue de Ne´matologie*, 7, 341–345.
- Riley IT, Kelly SJ (2002) Endoparasitic nematodes in cropping soils of Western Australia. *Australian Journal of Experimental Agriculture*, 42, 49-56.
- Smiley RW (2009) Root-lesion nematodes reduce yield of intolerant wheat and barley. *Agronomy Journal*, 101, 1322-1335.
- Talavera M, Vanstone VA (2001) Monitoring *Pratylenchus thornei* densities in soil and roots under resistant (*Triticum turgidum durum*) and susceptible (*Triticum aestivum*) wheat cultivars. *Phytoparasitica*, 29, 29–35.

- TanhaMaafi Z, Nicol JM, Kazemi H, Ebrahimi N, Gitti M, Ghalandar M, Mohammadi-pour M, Khoshkhabar ZH (2009) Cereal cyst nematodes, root rot pathogens and root lesion nematodes affecting cereal production in Iran. Proceeding of the first workshop of the international cereal cyst nematode initiative, Antalya, Turkey.
- Thompson JP, Brennan PS, Clewett TG, Sheedy JG, Seymour NP (1999) Progress in breeding wheat for tolerance and resistance to root-lesion nematode (*Pratylenchus thornei*). *Australasian Plant Pathology*, 28, 45-52.
- Van Gundy SD, Perez BJG, Stolzy LH, Thomason IJ (1974) A pest management approach to the control of *Pratylenchus thornei* on wheat in Mexico. *Journal of Nematology*, 6, 107–116.
- Vanstone VA, Russ MH (2001a) Ability of weeds to host the root lesion nematodes *Pratylenchus neglectus* and *P. thornei*. I. Grass weeds. *Australasian Plant Pathology*, 30, 245–250.
- Vanstone VA, Russ MH (2001b) Ability of weeds to host the root lesion nematodes *Pratylenchus neglectus* and *P. thornei*. II. Broadleaf weeds. *Australasian Plant Pathology*, 30, 251–258.
- Vanstone VA, Hollaway GJ, Stirling GR (2008) Managing nematode pests in the southern and western regions of the Australian cereal industry: Continuing progress in a challenging environment. *Australasian Plant Pathology*, 37, 220-234.
- Whitehead AG, Hemming JR (1965) A comparison of some quantitative methods of extracting small vermiform nematodes from soil. *Annals of Applied Biology*, 55, 25-38.

SURVEY ON ROOT-LESION NEMATODES IN CEREAL FIELDS IN SOUTHWESTERN OF IRAN

ALI REZA AHMADI[1,*], ZAHRA TANHA MAAFI[2] AND ABDELFATTAH A. DABABAT[3]

[1]Plant Protection Research Department, Khuzestan Agricultural and Natural Resources Research Center, AREEO, Ahvaz, Iran, [2]Iranian Research Institute of Plant Protection, Agricultural Research Education and Extension Organization (AREEO), Tehran, Iran, and [3]ICARDA-CIMMYT Wheat Improvement Program, P.K. 39 Emek, 06511, Ankara, Turkey. *Corresponding author email: alirahmadi2000@gmail.com

SUMMARY

Khuzestan Province has been shifted in first and second rank among the cereal producing provinces in Iran during recent years with an area of 700.000 ha and average annual yield production of 2.3 t / ha. The root lesion nematodes (RLNs), *Pratylenchus* spp., are distributed throughout the world and can significantly impact grain yield in cereal fields worldwide. In a survey to study the RLNs in cereal fields in Khuzestan Province, 200 soil and root samples were collected from wheat and barley fields in different districts during 2008-2011. The soil and root samples were processed by current nematology techniques and extracted RLNs were subject to identification and determination of population density. Two species of RLN, *P. thornei* and *P. neglectus*, were identified based on morphological and morphometric features. We found that *P. thornei* was the dominant species in both wheat and barley fields. The result showed that RLNs were found in 35 and 42% of wheat and barley fields, respectively. Population densities of RLNs in root samples taken from wheat fields ranged from 1 to 365 (mean 39) nematodes/g root. The range in soil samples was 0 to 1334 (mean 228) nematodes/250 cm^3. Soil and root samples collected from

* Ahmadi AR, Tanha Maafi Z, Dababat AA (2015) Survey on root-lesion nematodes in cereal field in southwestern of Iran. In 'Nematodes of Small Grain Cereals: current status and research.' (AA Dababat, H Muminjanov and RW Smiley) pp. 109-114. (FAO: Ankara, Turkey).

barley fields contained rather less population density compared to wheat samples, it ranged from 3 to 54 (mean 6) nematodes/g root and from 30 to 734 (mean 68) nematodes/250 cm^3 soil. Yield damage caused by root lesion nematodes depends on several parameters such as drought stress, wheat varieties, crop rotation, etc. Further studies are necessary to determine the threshold values for economic damage of root lesion nematodes in the region.

INTRODUCTION

Cereals are the most important food sources in the world and 58 percent of the annual global cultivation have been allocated to wheat, corn and rice. By 2030, world population will reach eight billion, so reliance on cereals will increase (Fischer *et al.* 2009). Wheat is cultivated in all parts of Iran over an area of 7 million ha with an annual production of 14 million tonnes, making it the 13th wheat producing country in the world during 2013 (FAO 2013). The area under wheat and barley cultivation in Khuzestan Province is 0.7 million ha with a total production of 1.6 million tonnes, making it the second most important cereal producing province, contributing 17% of the annual production in Iran in 2012-2013 (Anon 2014). The root lesion nematodes (RLNs) are a major pest of cereal throughout the world. Eight species of RLNs including *Pratylenchus brachyurus*, *P. coffeae*, *P. crenatus*, *P. neglectus*, *P. penetrans*, *P. pseudopratensis*, *P. thornei* and *P. zeae* were reported from maize, wheat, barley and rice (Pourjam *et al.* 1998, Ahmadi *et al.* 2010). *Pratylenchus neglectus*, *P. thornei*, *P. pseudopratensis* and *P. penetrans* were reported from wheat fields in Iran (Ghaderi *et al.* 2010). However, there is not enough information on the status of RLNs in cereal fields of Khuzestan Province. The aim of this study was to determine the occurrence, distribution and population density of RLNs in wheat and barley fields in southwest of Iran.

METHODS

The survey was performed in the cereal growing areas in southwest of Iran for three years (2008-2011). The survey included 169 wheat and 31 barley fields that were inspected and sampled in 22 regions during the grain filling period to harvest time, from mid-February to late May. Root tissue of 10 wheat and barley plants from each sample were examined under a stereomicroscope to observe disease symptoms. From each collected soil sample, 250 cm^3 soil was processed with the Whitehead tray method (Whitehead and Hemming 1965). The species of RLNs were identified based on morphological and morphomet-

ric features (Loof 1991). Distribution of RLNs were plotted on Khuzestan map with ArcGIS 9.3 software.

RESULTS

Root lesion nematodes occurred in 64 (39%) and 13 (20%) irrigated and rain-fed wheat and barley fields respectively (Table 1). The results showed that out of 200 soil and root samples, 77 samples (38.5%) were infested with average populations of 32 nematode/g of root and 201 nematodes/250 cm^3 of soil. Root lesion nematodes species were identified as *Pratylenchus neglectus* and *P. thornei*, which were widely spread in important cereal growing areas in the provinces, i.e., Ahvaz, Andika, Andimeshk, Baghmalek, Behbahan, Dezful, Gotvand, Hoveize, Izeh, Lali, Masjedsoleiman, Omidiyeh, Ramhormoz, Ramshir, Shadegan, Shushtar and Susa.

Population density of RLNs in soil samples of wheat fields ranged from 0 to 1334 nematodes (mean 228)/250 cm^3 soil and 1 to 365 (mean 39) nematodes/g root. The highest incidence in wheat fields was found in Lali district with 145 (48-242) nematodes/g root and 524 (235-813) nematodes/250 cm^3 soil. The lowest incidence was found in district Ramshir 4 (2-8) nematodes/g root and 135 (55-250) nematodes/250 cm^3 soil. Only in districts Haftghel, Hendijan and Korramshar were no RLNs found. Population density of RLNs in soil samples of barley fields ranged 30-734 nematodes (mean 68)/250 cm^3 soil and 3-54 (mean 6) nematodes/g root. The highest and lowest incidences were found in districts Lali and Ramhormoz, with 100 and 33.3 percent, respectively.

DISCUSSION

The aim of the present study was to determine the current occurrence, distribution and population density of RLNs in wheat and barley growing areas of Khuzestan province. Disease incidence in the irrigated fields, was more than the rain-fed fields, that confirms soil moisture and nutrients have a positive effect on the nematode population. Population density of nematode in wheat fields was higher than barley fields confirming that barley is rather a poor host for RLN (Vanstone *et al.* 2008). The economic damage thresholds for *P. thornei* and *P. neglectus* on wheat and barley vary greatly, 420 and 2500 *P. thornei*/kg of soil have been reported from different parts the world. *Pratylenchus thornei* and *P. neglectus* and mixture of two species were observed in 70, 23 and 13% of the Khuzestan surveyed fields respectively. *Pratylenchus thornei* was dominant species in

Table 1. Occurrence and incidence of root lesion nematodes in wheat and barley fields of Khuzestan Province, Iran during 2008-2011

District	No. of total samples		No. of infested samples		Mean and range of nematodes/g root	Mean and range of nematodes/250 cm^3 soil
	I[1]	R[2]	I	R		
Ahvaz	13	0	5	0	14 (5-30)	134 (60-240)
Andika	0	5	0	4	65 (14-167)	228 (132-398)
Andimeshk	7	0	2	0	46 (1-91)	242 (35-450)
Baghmalek	7	9	3	5	15 (1-46)	313 (30-500)
Behbahan	12	0	5	0	123 (19-365)	260 (0-534)
Dashteazadegan	14	0	1	0	14	210
Dezful	10	0	3	0	7(6-8)	318 (145-470)
Gotvand	8	0	6	0	19 (1-71)	213 (111-329)
Haftgel	0	5	0	0	0	0
Hendijan	5	0	0	0	0	0
Hoyeyzeh	8	0	2	0	37 (22-52)	326 (221-432)
Izeh	0	7	0	1	61	434
Korramshahr	7	0	0	0	0	0
Lali	1	3	1	2	85 (25-242)	334 (144-813)
Mahshar	5	0	3	0	6 (1-18)	114 (65-172)
Masjedsolaiman	0	2	0	1	77	250
Omidiyeh	5	0	3	0	29 (11-42)	155 (78-253)
Ramhormoz	14	0	8	0	41 (14-130)	256 (89-734)
Ramshir	7	0	4	0	4 (2-8)	135 (55-250)
Shadegan	6	0	1	0	2	100
Shushtar	18	5	7	0	36 (1-351)	175 (0- 904)
Susa	17	0	10	0	20 (0-199)	245(98-1334)
Overall & Mean	164	36	64	13	32 (0-365)	201 (0-1334)

[1]Irrigated, [2]Rainfed

both wheat and barley fields. *P. thornei* is more damaging than *P. neglectus* in the world (Smiley 2009). The use of resistant and tolerant varieties of wheat to RLNs is the most important management practices. This study suggests the need for more research on the reaction of the bread and durum wheat varieties to RLNs.

ACKNOWLEDGMENTS

We would like to thank Engs. N. Pashemforush and F. Hadadi (Plant Protection Research Department, Agricultural Research and Natural Resources Center of Khuzestan) for technical assistance.

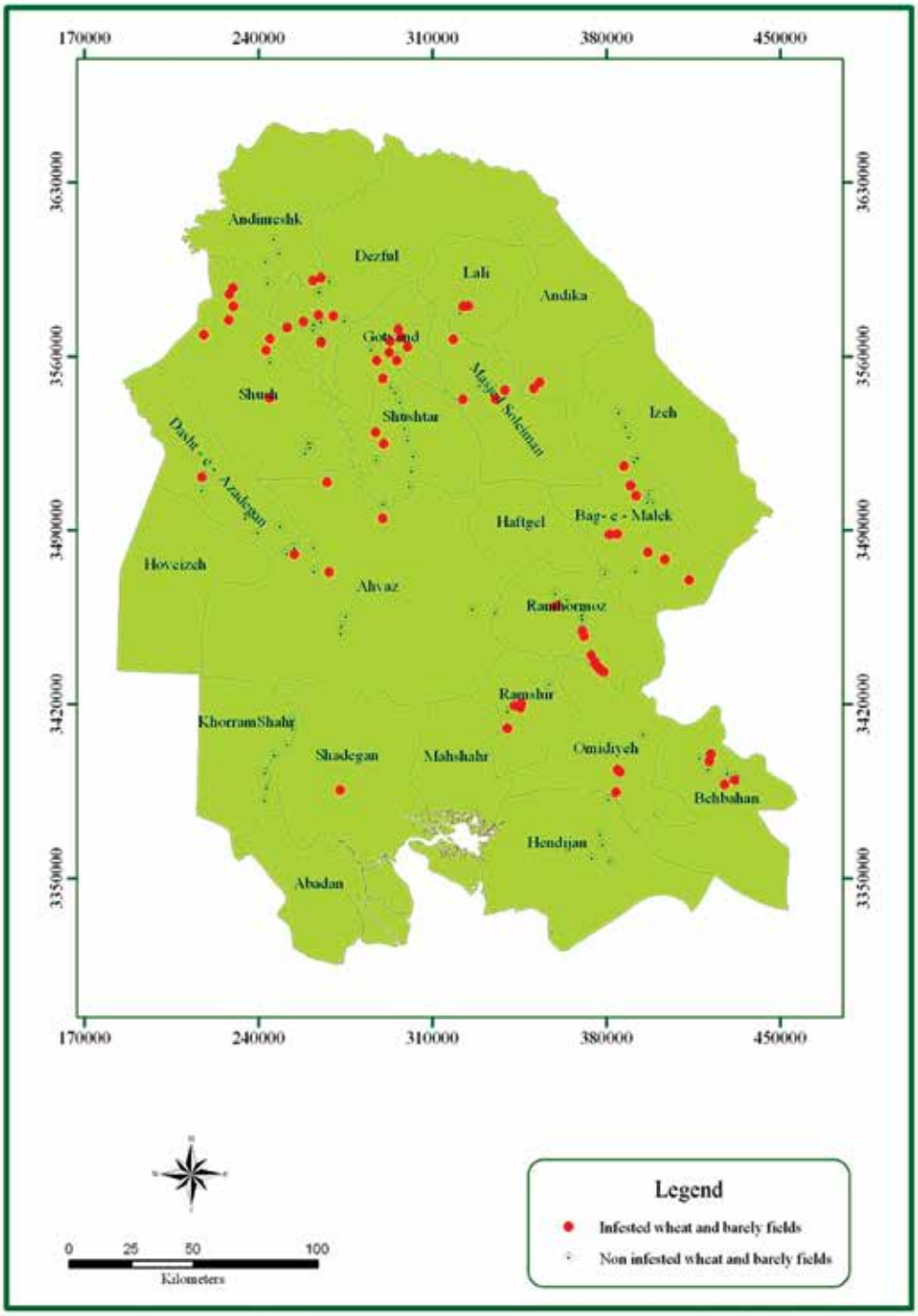


Figure 1. GIS map distribution of root lesion nematodes in the wheat and barley fields of Khuzeatan Province during 2008-2011.

REFERENCES

- Ahmadi, AR, Tanha Maafi Z, Fayazi F (2010) Identification and population density of cyst nematodes and root-lesion nematodes in cereals fields of Khuzestan province. Proceedings of the 19th Iranian plant protection congress, volum II plant diseases, P: 220.(In Farsi with English summary).
- Anon (2014) Agricultural Statistical Yearbook. Tehran, Iran, Ministry of Jihad-E- Agriculture, Statistical and Information Technology Unit.
- Anon (2013) FAO Statistical Yearbook. Rome, Italy.
- Fischer RA, Byerlee D, Edmeades GO (2009) Can technology deliver on the yield challenge to 2050? FAO expert meeting on how to feed the world in 2050.
- Ghaderi R, Karegar A, Banihashemi Z, Taghavi M (2010) Distribution and population fluctuation of root- lesion nematodes, *Pratylenchus* spp., in irrigated wheat and corn fields in Marvdasht, Fars province. *Iranian Journal of Plant Pathology*, 45, 95-98.
- Loof PAA (1991) The family *Pratylenchidae* Thorne, 1949. In:'Manual of Agricultural Nematology.'(Ed. WR Nickle) pp. 363-421. (Marcel Dekker Inc.: New York,USA).
- Purjam E, Kheiri A, Geraert E, Alizadeh A (1998) Occurrence of some *Pratylenchus* species in Iran. Proc. 13th Iranian Plant Protection Congress, Volum II: *Plant Diseases*, p 318. (In Farsi with English summary).
- Vanstone VA, Hollaway GJ, Stirling GR (2008) Managing nematode pests in the southern and western regions of the Australian cereal industry: Continuing progress in a challenging environment. *Australian Plant Pathology*, 37, 220-234.

ASSESSMENT OF GRAIN YIELD REDUCTION CAUSED BY *HETERODERA FILIPJEVI* IN WHEAT CULTIVARS UNDER NORMAL IRRIGATION AND DROUGHT STRESS IN NATURAL FIELD CONDITIONS

HADI KARIMIPOUR FARD[1,*], EBRAHIM POURJAM[2] AND ZAHRA TANHA MAAFI[3]

[1]Plant Protection Research Department, Kohgilouyeh va Boyer-Ahmad Agricultural and Natural Resources Research Center, AREEO, Yasuj, Iran, [2]Department of Plant Pathology, College of Agriculture, Tarbiat Modares University, Tehran, Iran, and [3]Iranian Research Institute of Plant Protection, Agricultural Research Education and Extension Organization (AREEO), Tehran, Iran. *Corresponding author email: pourjame@modares.ac.ir

SUMMARY

In order to assess the grain yield loss caused by *Heterodera filipjevi* on wheat cultivars an experiment was conducted in a wheat field infested with *H. filipjevi* during 2013-2014 at Kabotarabad Agricultural Research Station, Isfahan Province. The experiment was carried out in a split-plot factorial design with six treatments including three wheat cultivars (viz, Back-cross Rowshan, Pishtaz and Parsi) with and without applying nematicide (Aldicarb 10G). Each treatment consisted of a 6 m^2 plot which was replicated five times. The trials were performed in two different conditions under normal irrigation and under drought stress conditions. The initial and final population of each plot was determined before sowing the seeds and after harvesting. Plant growth parameters (i.e., yield, yield components, and growth characteristics) were

* Karimipour Fard H, Pourjam E, Tanha Maafi Z (2015) Assessment of grain yield reduction caused by *Heterodera filipjevi* in wheat cultivars under normal irrigation and drought stress in natural field conditions. In 'Nematodes of Small Grain Cereals: current status and research.' (AA Dababat, H Muminjanov and RW Smiley) pp. 115-120. (FAO: Ankara, Turkey).

measured and the nematode reproduction factor was calculated. The mean *Pi* was defined as 22.4 eggs + J2/g of soil. The results showed cereal cyst nematode, *H. filipjevi*, significantly affected grain yield in all three wheat cultivars in both irrigation conditions. In drought stress conditions, the cultivars Parsi, Back-cross Rowshan and Pishtaz showed 28.16%, 26.85% and 23.69% yield reduction, respectively, in comparison to the same cultivars that were treated with nematicide. The results in the normal irrigation system indicated a significant reduction of grain yield by 22.83%, 21.8% and 19.5% in Back-cross Rowshan, Pishtaz and Parsi, respectively, compared to these varieties treated by nematicide. The nematode reproduction factor ranged from 1.88 to 0.29 in plots minus and plus nematicide.

INTRODUCTION

Cereal cyst nematode (CCN) is globally acknowledged to be an economically important biotic constraint in predominantly rain-fed wheat production system in many wheat growing regions (Nicol and Rivoal 2008). The studies carried out on distribution and losses of *Heterodera filipjevi* in Turkey showed that *H. filipjevi* is widely distributed in Turkey and the average yield loss was 40% on wheat which might be even higher particularly in drought conditions (Rivoal and Nicol 2009). The economic importance of *H. filipjevi* was investigated at Cifteler and Haymana regions in Turkey, with results showing average yield losses of 20% and 36% for these two regions respectively (Elekcioğlu *et al.* 2009). *H. filipjevi* is the dominant species of cereal cyst nematode in most cereal growing areas of Iran and is widespread in different areas (Damadzahed and Ansaripour 2001, Tanha Maafi *et al.* 2007). Preliminary studies of distribution and population density of *H. filipjevi* in wheat fields of Isfahan province in Iran showed 51.7% of soil samples taken from different wheat fields were infested with *H. filipjevi*, with the mean population of 1658 eggs plus J2/g of soil (Karimipour Fard and Tanha Maafi 2010). The effect of different initial population levels of *H. filipjevi* on wheat cultivar Sardari was investigated in microplot conditions, the results indicated the grain yield loss was demonstrated even at the lowest population density (2.5 eggs and J2/g of soil) and reached a maximum loss of 48% with an initial population density of 20 eggs and J2/g of soil (Hajihasani *et al.* 2010). There is no information about the impact of *H. filipjevi* on wheat cultivars under field conditions in Isfahan Province. This study was conducted to assess the grain yield reduction caused by *H. filipjevi* on three wheat cultivars under normal irrigation and drought stress in natural field conditions.

METHODS

The experiment was conducted in an infested field in Kabotarabad Agricultural Research Station, 30 km east of Isfahan during the 2013-2014 growing season. The experiment was carried out in a split-plot factorial design with six treatments including three wheat cultivars (viz, Back-cross Rowshan, Pishtaz and Parsi) with and without applying nematicide (Aldicarb 10G). Each treatment consisted of 6 m^2 plots which were replicated five times, for a total of 60 plots. The trials were performed under normal irrigation conditions and also under drought stress. Seven irrigations were done for all plots, whilst the plots under drought stress conditions received the last four irrigation with 10 days delay. The initial and final population of each plot was determined before sowing the seeds and after harvesting. Plant growth parameters (i.e., yield, yield components, and growth characteristics) were measured and the nematode reproduction factor was calculated. Data were analyzed and the percentage of yield reduction was calculated for three wheat cultivars under normal irrigation and drought stress.

RESULTS

The average initial population density of *H. filipjevi* in experimental plots was determined to be 23.4 eggs and J2/g of soil. The results showed significant differences between plant growth parameters and nematodes indices in treated and untreated plots. Cereal cyst nematode, *H. filipjevi*, significantly affected grain yield in all three wheat cultivars in both irrigation conditions. In drought stress conditions, the cultivars Parsi, Back-cross Rowshan and Pishtaz showed 28.16%, 26.85% and 23.69% yield reduction, respectively, in comparison to the same cultivars treated with nematicide (Figure 1). The results in normal irrigation system indicated significant reduction of grain yield by 22.83%, 21.8% and 19.5% in Back-cross Rowshan, Pishtaz and Parsi, respectively, in plots treated by nematicide (Figure 1). Back-cross Rowshan cultivar treated with nematicide produced the most grain yield (4731.56 kg/ha) in the normal irrigation system. The nematode reproduction factor ranged from 1.88 to 0.29 in plots minus and plus nematicide, respectively (Figures 2 and 3).

DISCUSSION

Based on the results in this study, *H. filipjevi* caused grain yield reduction in the three surveyed cultivars. The results confirmed that cereal cyst nematode,

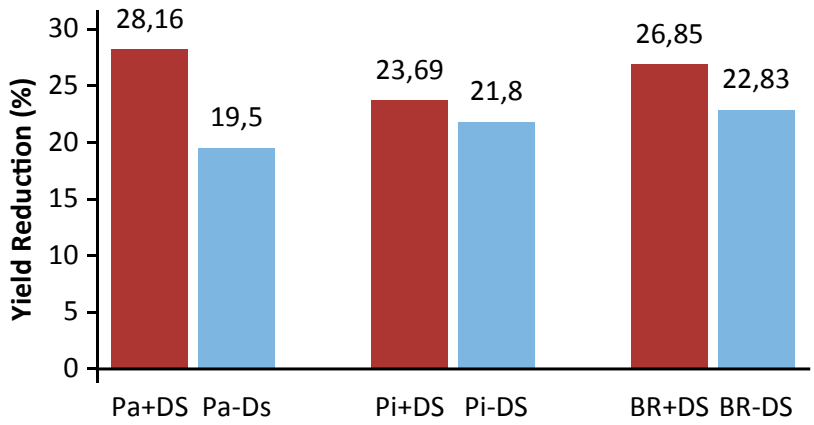


Figure 1. Percentage grain yield reduction caused by H. filipjevi in three cultivars; Parsi (Pa), Pishtaz (Pi) and Back-cross Rowshan (BR) under drought stress condition (+DS) and normal irrigation (-DS).

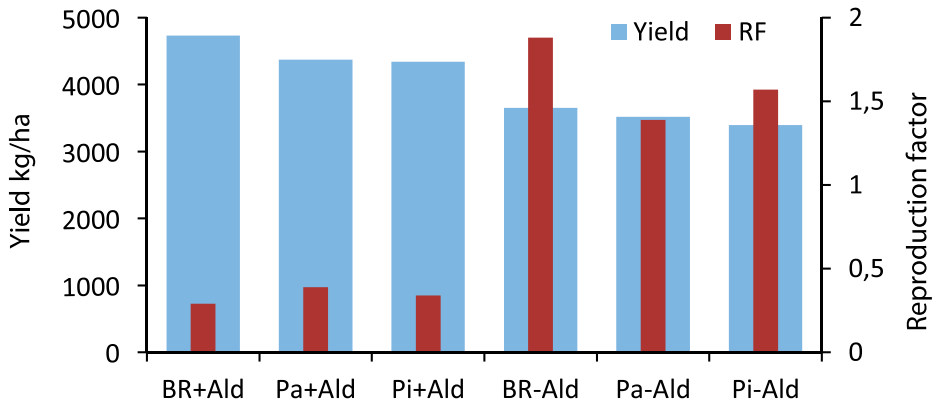


Figure 2. Yield of different treatments; Parsi (Pa), Pishtaz (Pi) and Back-cross Rowshan (BR) cultivars plus (+) or minus (-) Aldicarb 10G (Ald), and the nematode multiplication rate in the normal irrigation system.

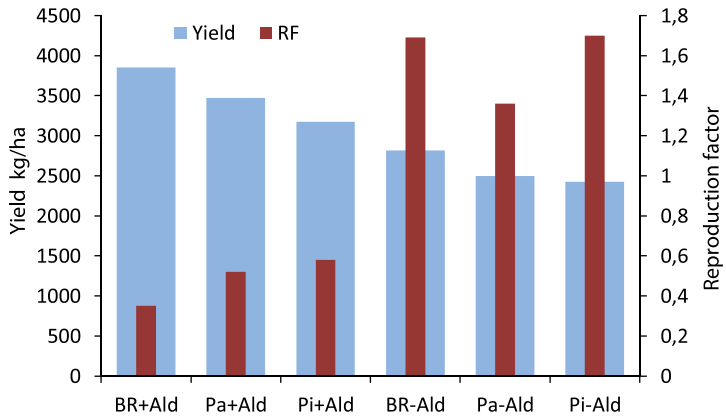


Figure 3. Yield of different treatments; Parsi (Pa), Pishtaz (Pi) and Back-cross Rowshan (BR) cultivars plus (+) or minus (-) Aldicarb 10 G (Ald) and the nematode multiplication rate under drought stress conditions.

H. filipjevi, is an efficient causal agent of reduced wheat production under Isfahan conditions, and that this nematode causes severe damage to wheat yield. Data on damage of cereal cyst nematodes are mainly concentrated on *H. avenae*, the most prevalent species of cereal cyst nematodes in Europe and temperate zones, whilst the impact of *H. filipjevi*, which is widespread in relatively warmer regions such as west Asia and the Middle East, has not been adequately evaluated on wheat. The economic importance of *H. filipjevi* as a member of the '*H. avenae* group' on wheat was proved in this current study, which validated the findings of previous investigations (Nicol *et al.* 2006, Elekcioğlu *et al.* 2009). Grain yield reduction percentage under drought stress conditions was significantly higher than under normal irrigation conditions, indicating that water stress enhances the severity of injuries in infected plants. This finding corresponds to those provided in previous studies about more losses from *H. filipjevi* occurring under drought conditions in Turkey (Rivoal and Nicole 2009). The means of the reproduction factors were inversely related to means of yield in the different treatments, and plots minus and plus nematicide contained the least and highest multiplication rates, respectively.

ACKNOWLEDGMENTS

We would like to thank Dr M. Nasr Esfahani, Eng. H. Almasi (Plant Protection Research Department, Agricultural and Natural Resources Research Center of Isfahan) and Eng. Homayoun Darkhal (Kabotarabad Agricultural Research Station, Agricultural Research and Natural Resources Center of Isfahan).

REFERENCES

- Damadzadeh M, Ansaripour B (2001) Identification and distribution of *Heterodera filipjevi* in the Esfahan province of Iran. *Russian Journal of Nematology*, 9, 57-58.
- Elekcioglu IH, Nicol JM, Bolat N, Sahin E, Yorgancılar A, Braun HJ, Yorgancılar Ö, Yıldırım AF, Kılınc AT, Toktay H, Calışkan M (2009) Longterm studies on the cereal cyst nematode *Heterodera filipjevi* in Turkey: international collaboration with regional implications. In: 'Cereal Cyst Nematodes: Status, Research and Outlook.' (Eds. IT Riley, JM Nicol, AA Dababat) pp. 11-16. (CIMMYT: Ankara , Turkey).
- Hajihasani A, Tanha Maafi Z, Nicole JM, Rezaee S (2010) Effect of the cereal cyst nematode, *Heterodera filipjevi*, on wheat in microplot trials, *Nematology*, 12(3), 357-363.

- Karimipour Fard H, Tanha Maafi Z (2010) Three years studies on distribution and population density of *Heterodera filipjevi* in cereal fields of Isfahan province, Iran. *Proceedings 30th International Symposium of the European Society of Nematologists, Vienna, Austria.*
- Nicol JM, Bolat N, Sahin E, Tulek A, Yildirim AF, Yorgancilar A, Kaplan A, Braun HJ (2006) The cereal cyst nematode is causing economic damage on rainfed wheat production systems of Turkey. *Phytopathology*, 96, S169. [abstract]
- Nicol JM, Rivoal R (2008) Global knowledge and its application for the integrated control and management of nematodes on wheat. In: 'Integrated management and biocontrol of vegetable and grain crop nematodes.' (Eds. A Ciancio, KG Mukerji) pp. 243-287. (Springer Academic Publishing: Dordrecht, The Netherlands).
- Rivoal R, Nicol JM (2009) Past research on the cereal cyst nematode complex and future needs. In: 'Cereal Cyst Nematodes: Status, Research and Outlook.' (Eds. IT Riley, JM Nicol, AA Dababat) pp.3-10 (CIMMYT: Ankara , Turkey).
- Tanha Maafi Z, Sturhan D, Kheiri A, Geraert E (2007) Species of the *Heterodera avenae* group (Nematoda: Heteroderidae) from Iran. *Russian Journal of Nematology*, 15(1), 49-58.

WHEAT PERCEPTIVENESS OF STUDY OF SPRING WHEAT NEMATODES IN KAZAKHSTAN

DUTBAYEV Y.[1,*], SULEYMANOVA G.[1], LESOVA ZH.[2], SULTANOVA N.[3], AND ABDELFATTAH A. DABABAT[4]

[1]Kazakh National Agrarian University, Abay, 8 Avenue, Almaty, Kazakhstan, [2]Almaty Technological University, Almaty, Kazakhstan, [3]Kazakh Scientific Research Institute of Plant Protection and Quarantine, KazybekBii, 1, Almaty, Kazakhstan, [4]CIMMYT Global Wheat Program, Ankara-Turkey. *Corresponding author email: edutbaev@mail.ru

SUMMARY

With the aim of increasing global wheat production by 2030, the potential wheat yield must be increased up to 30-40%. Each year the crop productivity must be increased between 1,6-1,8%, including 1% coming from improving the genetic and breeding methods. To achieve this aim, we must use the wild relatives of wheat to breed for specific traits, especially diseases. An important direction for improving the genetic potential of wheat is to involve and to pyramid genes for resistance to biotic and abiotic stresses. At this study, a synthetic hexaploid wheat was tested at the experimental base of Kazakh Scientific Research Institute for Plant Growing and Farming, and at CIMMYT-Turkey. Five lines of synthetic wheat were selected which have yield productivity on a level equal to local standards. In Turkey, the lines were tested under both field and greenhouse conditions under artificial inoculation for leaf rust, stripe rust, stem rust, common bunt and soil borne nematodes and root rot. Eight out of 49 lines were resistant to crown rot while 13 lines were resistant to nematodes. On lines LANGDON/KU-2144 and LANGDON/KU-2124 in field conditions nematodes were absent and under greenhouse conditions there were 2-4 nematodes/plant.

* Dutbayev Y, Suleymanova G, Lesova Zh, Sultanova N, and Dababat AA (2015) Wheat perceptiveness of study of spring wheat nematodes in Kazakhstan. In 'Nematodes of Small Grain Cereals: current status and research.' (AA Dababat, H Muminjanov and RW Smiley) pp. 121-130. (FAO: Ankara, Turkey).

INTRODUCTION

The FAO 2050 predicts that the world population will be increased to 9 billion (FAO Statistical Yearbook 2013). Therefore, to satisfy the needs of the global population by 2030 it is necessary to increase the potential wheat yield by 30-40%. For this purpose, it is necessary to increase the annual yield by an average of 1.6-1.8%, including a 1% gain by improving breeding and genetic methods. This can be achieved by using new genetic sources for resistance to diseases especially the soil borne diseases. An increase in spring wheat productivity in Central and Northern Kazakhstan is one of the main goals in the next years. Also a holistic approach to improve and to increase yield will include reduce diseases, pests and weeds in wheat. In these regions the most important diseases of wheat are Septoria blotches, Helminthosporium blotches, and common root rot. The yield losses can reach 30% and more (Koyshibayev 2002). However, effects of nematodes on wheat is still not well investigated. The average yield in Kazakhstan is still very low especially due to the short season. Attempts to study nematode dynamics in the Kazakhstan wheat growing areas will be of significant importance for increasing the yield. If those diseases are reported we will need to start breeding to develop resistant lines.

It is necessary to improve the phytosanitary situation to understand the biology of soil borne pathogens. These agents were previously reported as facultative parasites. The best methods to control the soil borne pathogens is by the use of resistant varieties, which is easy to handle, cheap and safest. In a study carried out in the 1980s and 1990s by scientists of Kazakh Research Institute of Plant Protection and the Kazakh Research Institute of Grain Farming, investigations were conducted on common root rot of spring wheat (Koyshibayev 2002, Gorodilova and Shevtsov 1972, Tupenevich 1970, Djiembayev 1971, Bedina 1974, Alzhanov 1977, Kotova 1977). The main agent of common root rot is the fungus *Bipolaris sorokiniana* Sacc. (*Helminthosporium sativum*P.K. et B.). This fungus and *Fusarium spp.* were determined to be the soil borne agents of diseases in previous crops. Studies included black grain fallow, grain grasses, and green manure crops, as well as elements of technologies for wheat cultivation, such as rates and dates for sowing seeds, rates of application of organic and mineral fertilizers, and systems of fallow tillage.

In 2004-2008, Dutbayev studied *B. sorokiniana* in Almaty using artificial inoculations and in naturally-infected fields in North Kazakhstan and Aktobe oblasts. Studies included evaluations of CIMMYT's nursery of wheat (103

samples) with sources of resistance to root rot (Dutbayev and Tsigankov 2007). During wheat maturity 22 lines were resistant (frequency of root rot was up to 5%). A local cultivar Saratovskaya had 42% root rot, and the average root rot for the 22 lines was up to 12-20%.

In the present days soil biology is changing; pathogens, chemical and biological contents of soil are changing, and methods of agent identification are improving. Cereal cyst nematodes (CCNs) are plant parasites that significantly limit global cereal production. The most frequently reported pathogenic species are *Heterodera avenae, H. filipjevi*, and *H. latipons.* So far, one of the most cost-effective, environmentally friendly, and easily adopted control measures to control the cereal cyst nematodes is the use of genetic host resistance, which maintains nematode populations below the economic damage threshold level. Many effective sources of resistance to CCNs have been identified in cereals, however, their effectiveness and usefulness is dependent on the interaction of the specific putative resistant accession and the CCN pathotype found in a specific region. At CIMMYT, in a study conducted by Dababat *et al.* (2014), 719 wheat lines from the Facultative and Winter Wheat Observation Nurseries, representing a broad geographical spectrum of breeding lines and varieties from Europe, Central Asia, and the International Winter Wheat Improvement Program, were screened against *H. filipjevi* under controlled conditions. The results indicated that 114 and 90 genotypes were ranked resistant and moderately resistant, respectively, representing 15.8% and 12.5% of the screened genotypes. The frequency of resistant genotypes observed in the germplasm varied significantly among the original countries of origin, and was highest for genotypes that originated from Bulgaria (59.3%). From those phenotyped germplasms, a set of 289 lines was genotyped to understand if resistance sources are located at the same site or originate from different locations in the genome. Twelve lines from Kazakhstan included one that was highly resistant, three that were resistant, and seven that were susceptible. Luckily, the International Winter Wheat Improvement Program (IWWIP) at CIMMYT-Turkey is distributing international nurseries to more than 150 collaborators representing more than 50 countries around the world, and most of the germplasm was screened for soil borne diseases in Turkey.

That's why we need to identify resistance in spring wheat to increase grain production in the main grain regions of Kazakhstan to reduce yield losses associated with soil borne pathogens, including plant-parasitic nematodes and root rotting fungi.

MATERIALS & METHODS

Cereal Cyst Nematodes: The Soil Borne Pathogens Program at CIMMYT-Turkey annually receives 1000 lines of winter wheat lines to be screened for the CCNs (*Heterodera avenae*, *H. filipjevi*, and *H. latipons*), with a main focus on *H. filipjevi*. In Turkey, in 2014, the nursery of synthetic wheat 13JAP-SYNT (49 samples) was screened at the Soil Borne Pathogens Program under greenhouse conditions for *Heterodera filipjevi* using multiple replications. Each plant was inoculated with 400 second stage juveniles (J2) one week after sowing. Based on one year of screening under growth room and greenhouse conditions, lines were rescreened in the field using both naturally-infested and artificially-inoculated plots.

At Almaty Technological University, we are going to survey wheat growing regions to identify nematodes to the species level and also determine the predominant causal agents of root rots in spring wheat in northern and central Kazakhstan. The biology of the nematode population dynamics will be researched. This will require a comprehensive survey of spring wheat production areas in central and northern Kazakhstan to determine the distribution of spring wheat nematodes and root rots. The main target is to train three scientists at CIMMYT-Turkey on pathological methods of working with nematodes and, in general, with soil borne pathogens. In Kazakhstan, we are lacking the expertise to work on soil borne pathogens, and especially nematodes. The next step will be to evaluate Kazakh-released varieties, breeding lines and introduced germplasm for resistance to nematodes and root rots under greenhouse and field conditions. For artificial inoculation, the nematodes will be extracted from infested fields and used as a source for inoculum in the screenings. In laboratory and greenhouse conditions, we will study the fungicidal influence of nanoparticles of AgNPs against diseases of wheat.

The same methods will be followed at the Soil Borne Pathogens Program at CIMMYT-Turkey. Soil samples will be collected from wheat growing fields and cysts will be extracted by using the Fenwick-Can technique (Fenwick 1940). Cysts will be collected by hand picking and will be surface sterilized with 0.5% NaOCl for 10 min and rinsed several times in autoclaved distilled water. Cysts will be stored at 4°C in distilled water on a special sieve to enhance hatching. Juveniles will be obtained by placing the cysts on Baermann sieves over tap water at room

temperature for 7 days to stimulate hatch. After seed germination, 400 J2 of *H. filipjevi* in one ml of distilled water will be inoculated into three 2-cm-deep holes made into the soil around the base of the seedling. Plants will be incubated in a growth chamber at 25°C with a 16 h of photoperiod of light and with a relative humidity of 70%. Plants will be top watered whenever needed, and fertilised with NitrophoskaSolub/Hakaphos (N: P: K, 8:12:24). The plants will be harvested nine weeks after nematode inoculation. Two weeks before harvest, irrigation will be stopped to allow the soil to dry for better extraction of cysts. Cysts will be extracted by a modified floatation method (Coyne *et al.* 2007). Roots will be first washed carefully with a stream of water to remove surrounding soil. The roots will be then washed thoroughly with a strong stream of water to separate white females and/or mature cysts from the roots. These will be collected in a small container. The soil in the bucket will be then stirred for 10 s and left for about 30 s to allow the heavy sand and soil debris to settle and then poured through sieves of 850 µm and 250 µm aperture for cyst collection. The process will be repeated three times to ensure that all cysts are extracted. Cysts will be collected from the 250µm sieve. The total number of cysts will be counted under a binocular microscope and recorded. Wheat genotypes will be recorded with mean cyst number and classified into five groups as per Dababat *et al.* (2014), according to the check lines used. The groups are: resistant (R = equal or fewer cysts than in a known resistant check), moderately resistant (MR = slightly more cysts than in a resistant check); moderately susceptible (MS = significantly more cysts than in a resistant check but not as many as in the susceptible check, susceptible (S = as many cysts as in the susceptible check and number of cysts per root system considered damaging, and highly susceptible (HS = more cysts than in the susceptible check).

Common Root Rot: The ratings of common root rot have been conducted during wheat maturity. Development of diseases were conducted by using the M. Koishibayev technique (2004), using artificial infection to screen for one year under growth room and greenhouse conditions, and planting in the field under both naturally-infested and artificially-inoculated conditions. Diseased plant samples were collected from Ankara. *B. sorokiniana* conidia were extracted by using the Ledingham and Chinn technique (1955).

RESULTS

CIMMYT and Kyoto University developed lines of synthetic hexaploid wheats (SHWs) using diverse accessions of the D genome donor *Aegilops tauschii*.

Studies of synthetic hexaploid wheat lines were conducted since 2014 at the Kazakh Scientific Research Institute for Plant Growing and Farming and at several research institutes in Turkey. In Kazakhstan, the nursery 13JAP-SYNT was sown in autumn 2013. In spring 2014 on naturally-infected areas of winter wheat, diseases of local winter wheat cultivars Zhetisu, Azharly and Farabi developed levels of stripe rust up to 10-20%, leaf rust up to 5-10%, and leaf spot blotches up to 5-20%. Stem rust was absent. Forty one of 50 lines showed no rust at heading. We selected 5 lines with yield levels similar to, or above, local standard cultivars. Field and greenhouse tests in Turkey identified 43 of 49 lines (88%) resistant to leaf rust, 24 lines (49%) resistant to stem rust, four lines resistant to stripe rust, 23 lines resistant to common bunt, eight lines resistant to root rot, and 13 lines resistant to soil nematodes. In Turkey, in field and in greenhouse conditions, under artificial inoculation by soil borne nematodes and root rot, we conducted evaluations of wheat diseases. Resistance showed in 43 of 49 lines (88%) for leaf rust, in 24 lines of 49 (49%) for stem rust, four lines for stripe rust, 23 lines for common bunt, eight lines for root rot, and 13 lines for nematodes. On lines LANGDON/KU-2144 and LANGDON/KU-2124 nematodes were absent under field conditions, and in the greenhouse there were 2-4 nematodes/plant. In Kazakhstan up to present time soil borne diseases including nematodes on wheat have never been studied before (Table 2).

Table 1. Promising lines from the International Winter Wheat Improvement Program (IWWIP) nursery synthetic wheat 13JAP-SYNT (49 samples), Kazakhstan-Turkey during 2014

Entry	Cross name	Weight of grain/ spike(g)	Length of spike (cm)	Number of ears	Density of spike	Weight of 1000 grain (g)
20	GEREK	0.91	11.18	14.17	1.51	20.80
7	LANGDON/KU-20-8	1.12	10.21	14.25	1.40	24.20
41	LANGDON/KU-2109	1.07	12.52	13.25	1.07	42.16
42	LANGDON/KU-2132	1.02	11.94	17.17	1.44	26.64
26	LANGDON/AT 55	1.02	10.01	16.00	1.62	36.61
16	LANGDON/KU-2098	0.97	13.02	13.58	1.04	32.35
33	LANGDON/KU-2078	0.94	11.44	15.67	1.37	42.68
17	LANGDON/KU-2100	0.93	10.18	13.50	1.35	28.49
38	LANGDON/KU-2093	0.88	10.78	14.62	1.36	26.59
47	LANGDON/KU-2816	0.86	10.87	13.67	1.44	21.56
24	LANGDON/KU-2829A	0.82	10.51	15.33	1.47	19.32

Table 2. Evaluation of samples of synthetic wheat of nursery 14JAP-SYNT (CIMMYT-Turkey, 2014)

No. of samples	Cultivar, line	Development of wheat diseases						
		Rusts			Common bunt	Common root rot	Nematodes/plant	
		leaf	stripe	stem			field	Green-house
1	Bezostaya (local)	10MS	20S	40MS	51,0	3,7	12	20
2	Gerek	5MS	80S	50MS	11,0	4,3	14	10
40	Karahan	20MS	40S	80S	21,0	3,7	9	14
6	LANGDON/IG 131606	5MS	80S	10MS	11,3	4,0	2	4
37	LANGDON/KU-2091	5MS	90S	10MR	0,0	2,7	4	6
38	LANGDON/KU-2093	5MS	90S	10MR	16,5	1,7	10	11
39	LANGDON/KU-2103	5MS	90S	60S	16,9	4,3	4	7
14	LANGDON/KU-2096	5MS	100S	R	18,0	2,0	8	6
23	LANGDON/KU-2159	R	90S	60S	9,6	3,0	6	6
4	LANGDON/IG 48042	10MS	80S	R	11,0	1,7	10	8
44	LANGDON/KU-2155	10MS	70S	50MS	0,0	4,0	2	5
46	LANGDON/KU-2158	TMS	90S	40MS	20,0	3,0	6	9
12	LANGDON/KU-2088	5MS	100S	0	0,0	3,7	3	8
48	LANGDON/PI 499262	10MS	0	80S	3,2	2,0	20	26
49	LANGDON/PI 508262	R	20MR	20MR	48,5	2,0	26	24
22	LANGDON/KU-2144	R	10MR	R	0,0	3,3	0	5
21	LANGDON/KU-2124	R	10MS	R	0,0	0,0	0	2
32	LANGDON/KU-2076	R	90S	20MS	0,0	3,0	5	6

DISCUSSION

We are aiming to improve our capacity building by sending students from Kazakhstan to at CIMMYT-Turkey to be trained to study soil borne pathogens. So far, Kazakhstan lacks the expertise to work either on cereal nematodes or on root rot diseases caused by fungal pathogens. Upon their return to Kazakhstan, the students will survey spring wheat production areas in central and northern Kazakhstan to determine the distribution and species identities of the nematodes and root rots. Kazakh-released varieties, breeding lines and introduced germplasm will be evaluated for resistance to nematodes and root rots under greenhouse and field conditions. We aim to identify a new source(s) of resistance to control soil-borne pathogens on spring wheat in Kazakhstan.

Due to the short season for wheat production in Kazakhstan, the yield is less than 1 ton/ha. Finding resistant lines in the cultivated cultivars will be of great values to increase yields to higher levels.

ACKNOWLEDGEMENTS

Funding was from the Grant of Ministry and Education of the Republic of Kazakhstan, of State registration of the project #01120PK00443 "Increasing of productivity of winter wheat based on resistant to bunt diseases lines and optimization of utilizing of seed treatments and micro fertilizers in 2012-2014", and for the teaching of PhD students in the specialty "Plant Protection and Quarantine" at the Kazakh National Agrarian University. We thank Dr. Alexei Morgounov for wheat nurseries and for the possibility to train a PhD student from KazNAU at CIMMYT-Turkey in 2014.

REFERENCES

- Alzhanov ZhSh (1977) Root rot of wheat in dry areas of northern Kazakhstan. *News of Agricultural Science of* Kazakhstan, 3, 51. (In Russian)
- Bedina SF (1974) Root rot of spring wheat in Kazakhstan. *News of Agricultural Science of Kazakhstan*, 9, 40-43. (In Russian)
- Coyne DL, Nicol JM, Claudius-Cole B (2007) Practical Plant Nematology: A Field and Laboratory Guide. pp. 41-47. International Institute of Tropical Agriculture, Cotonou, Benin.
- Dababat AA, Erginbas-Orakçı G, Toktay H, Imren M, Akin B, Braun HJ, Dreisigacker S, Elekcioğlu IH, Morgounov A (2014) Resistance of winter wheat to *Heterodera filipjevi* in Turkey. *Turkish Journal of Agriculture and Forestry*, 38,180-186.
- Djiembayev ZhT (1970) Root Rots of Cereal Crops.-Almaty, 54. (In Russian)
- Dutbayev Y, Tsigankov VI (2007) Evaluation of RGCB from Turkey for resistance Helminthosporium root rot. 3rd International Conference of Young Scientists and Graduate Students: Actual problems of farming and plant growing. Almalybak, pp. 50-52.
- FAO Statistical Yearbook (2013) World Food in Agriculture. Rome, Italy.
- Fenwick DW (1940) Methods for the recovery and counting of cysts of *Heterodera schachtii* from soil. *Journal of Helminthology,* 18, 155–172.
- Gorodilova LM, Shevtsov SI (1972) Root rot of wheat in condition of soil-protective system of Kazakhstan. Tselinograd, 44. (In Russian)
- Ledingham RL, Chinn SHF (1955) A flotation method for obtaining spores of *Helminthosporium sativum* from soil. *Canadian Journal of Botany*, 33, 298-303.
- Kotova VV (1977) Root rot and seed quality of spring wheat. *All Union Research Institute of Plant Protection*. pp. 82-84. (In Russian).

- Koyshibayev M (2002) Diseases of Cereal Crops. Almaty, 367. (In Russian)
- Tupenevich SM (1970) Root rot of spring wheat in dry areas of northern Kazakhstan and western Siberia - root rots of cereal crops and methods of its control. Moscow, 3-8. (In Russian).

THE PLANT-PARASITIC NEMATODES ASSOCIATED WITH CEREAL CROPS IN BOLU, TURKEY

MUSTAFA IMREN[1,*], ŞENOL YILDIZ[2], ECE KASAPOĞLU[3], HALIL TOKTAY[4], HALIL KÜTÜK[1], AND ABDELFATTAH A. DABABAT[5]

[1]University of Abant Izzet Baysal, Faculty of Agri. and Natural Sciences, Dept of Plant Protection, Bolu, Turkey; [2]University of Abant Izzet Baysal, Faculty of Agri. and Natural Sciences, Dept of Wildlife & Ecology, Bolu, Turkey; [3]Cukurova University, Faculty of Agriculture, Department of Plant Protection, Adana, Turkey; [4] University of Niğde, Faculty of Agri. Sciences and Technologies, Dept. of Plant Product. and Techn., Niğde, Turkey; [5]CIMMYT Global Wheat Program, Ankara-Turkey; *Corresponding author email: mustafaimren@ibu.edu.tr

SUMMARY

Plant-parasitic nematodes, especially *Heterodera* and *Pratylenchus* species, cause significant yield losses for wheat growing areas worldwide. This study was conducted during the 2014-2015 growing season to determine the genera of plant-parasitic nematodes associated with barley and wheat in the Bolu Province of Turkey. Population densities of plant-parasitic nematodes were determined in 100 soil and root samples collected from 75 cereal growing districts. Thirteen genera of plant-parasitic nematodes and free living nematodes were found associated with cereals. Among the plant-parasitic nematodes *Heterodera*, *Pratylenchus*, *Pratylenchoides*, *Paratylenchus*, *Merlinius*, *Helicotylenchus* and *Tylenchorhynchus* were the main nematodes found to be associated with cereal crops. The most important and damaging plant-parasitic nematodes were found to be *Heterodera* (82.6%) and *Pratylenchus* (73.3%). Results

* Imren M, Yildiz Ş, Kasapoğlu E, Toktay H, Kütük H, Dababat AA (2015) The plant-parasitic nematodes associated with cereal crops in Bolu, Turkey. In 'Nematodes of Small Grain Cereals: current status and research.' (AA Dababat, H Muminjanov and RW Smiley) pp. 131-140. (FAO: Ankara, Turkey).

also indicated that the genus *Pratylenchoides* (66.6%), *Paratylenchus* (26.6%), *Merlinius* (78.6%), *Helicotylenchus* (38.8%) and *Tylenchorhynchus* (37.3%) were widely distributed. In addition, all the genera are reported comprehensively for the first time in cereal areas in Bolu Province. This study highlights the distribution of the plant-parasitic nematodes associated with cereals in cold regions of the Bolu. Therefore, further comprehensive surveys are indispensable to define the distribution, frequency and identification of plant-parasitic nematodes species more accurately, and define particularly the *Heterodera* and *Pratylenchus* species in Bolu.

Keywords: Wheat, cereal nematodes, occurrence, frequency

INTRODUCTION

Turkey is one of the ten largest wheat producers in the world, but has low average yields of around 2.3 t/ha (Anonymous 2014). The Turkish Central Anatolian Plateau (CAP) is the main wheat production area of Turkey, with over five million hectares of cereals being under rain-fed and limited irrigation. Bolu is the important wheat production province of CAP with over 1 million hectares of wheat that produces around 120 thousand tons of grain (Anonymous 2014).

Bolu Province, having 1.015% of the area of Turkey [8,276 km^2 (827,600 ha)], is located in the western Black Sea region. Approximately 18% of the Province is comprised of agricultural land. Cereal monoculture prevails, as practiced under an annual fallow system. Plants are often under water stress as they depend on the long-term average annual rainfall between 400-450 mm and limited supplementary irrigation in Bolu Province (Anonymous 2014). Under these constrained growing conditions, the combined effects of abiotic and biotic stresses are known to affect cereal productivity (Nicol and Ortiz-Monasterio 2004). Disease caused by the sedentary cereal cyst nematodes (*Heterodera* spp.) and the migratory root lesion nematodes (*Pratylenchus* spp.) are globally important plant-parasitic nematodes on small grain cereals (Nicol *et al.* 2003).

The genus *Heterodera* contains as many as 70 species, including a complex of 12 species known as the *Heterodera avenae* group. Species in this group invade and reproduce only in living roots of cereals and grasses (Rivoal and Cook 1993, McDonald and Nicol 2005). They do not reproduce on any broadleaf plant. In Turkey, the most economically important species on cereals are *H. avenae* Wollenweber, *H. filipjevi* (Madzhidov) Shelter, and *H. latipons* Franklin

(Sahin *et al.* 2009, Imren *et al.* 2012, 2015, Dababat *et al.* 2015). Recent studies have estimated that up to 25% and 50% yield loss caused by *H. avenae* and *H. filipjevi* to commonly cultivated spring and winter wheat varieties in the Eastern Mediterranean Region and CAP, respectively (Nicol and Ortiz-Monasterio 2004, Imren and Elekcioğlu 2014, Dababat *et al.* 2015).

At least eight species of *Pratylenchus* are parasitic on small grain cereals (De Waele and Elsen 2002, Nicol 2002, Nicol *et al.* 2003, McDonald and Nicol 2005, Castillo and Vovlas 2007). Four species (*P. crenatus*, *P. neglectus*, *P. penetrans* and *P. thornei*) occur throughout the world in temperate cereal-producing regions and are the most economically important (McDonald and Nicol 2005). Also, *P. fallax, P. crenatus*, *P. neglectus*, *P. thornei* and *P. penetrans* are found in wheat and barley growing regions including the Eastern Mediterranean, South East Anatolian and CAP (Imren and Elekcioğlu 2008, Sahin *et al.* 2009, Kılıc *et al.* 2012, Öcal 2012).

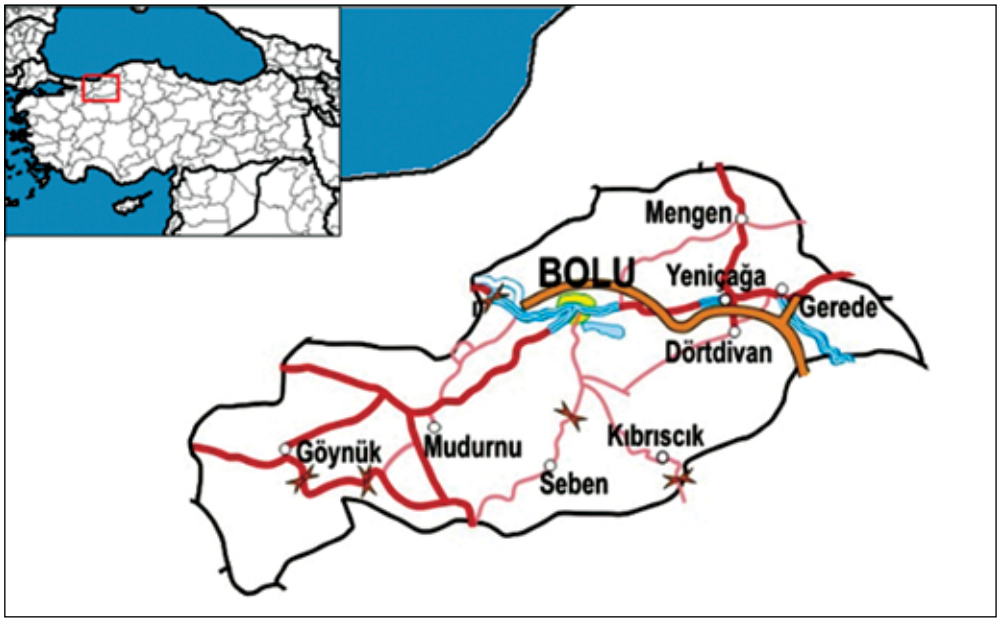
Nonetheless, little information is available about plant-parasitic nematodes especially cereal nematodes in barley and wheat fields of the CAP as well as Bolu Province; a survey of plant-parasitic nematodes was carried out to (i) identify the plant-parasitic nematodes associated with cereal-producing fields of the Province, and (ii) determine the frequency and population density of plant-parasitic nematodes in Bolu Province.

MATERIALS & METHODS

Sampling locations: A survey was conducted in Bolu Province to determine the frequency and abundance of plant-parasitic nematodes associated with cereals. Barley and wheat fields in Gerede, Yeniçağ, Dörtdivan, Mudurnu and central Bolu were surveyed in May and August of the 2014-2015 growing season. More than 75 soil samples were collected in the five locations (Figure 1).

Each sample consisted of a composite of 20 soil cores (2.5-cm-diam. × 20-cm-deep) taken in an evenly spaced systematic zigzag sampling pattern from a 10-ha section of each field (Lawrence and McLean 1999). Composite soil samples were sealed in plastic bags and stored less than 4 hours in a cooled iced chest for transport from the field and then transferred to a 5°C refrigerator. Each sample was thoroughly mixed, and a 100-cm^3 subsample was collected for nematode extraction. All samples were processed within 7 days of collection.

Figure 1. Sampling locations in Bolu Province, Turkey



Nematode extraction and identification: Migratory nematodes were extracted from 100 cm^3 of soil by using a modified "Petri-dish" Baermann funnel method. Collected nematodes were transferred to graduated cylinders, allowed to settle for 8 hours and then placed in 15 ml tubes. Nematodes were counted under a light microscope at 100× magnification and identified to the genus level. Their incidence and abundance were expressed as a percentage of their occurrence and as the number of nematodes/100 cm^3 of soil, respectively. After recording, nematodes were heat killed at 70-75 °C for 45 seconds and fixed in TAF (Hooper 1986). Preserved specimens were embedded in anhydrous glycerin and mounted on wax-circled glass slides.

Sedentary nematodes were extracted using the modified Fenwick can method (Fenwick 1940) from a 250-g soil sample. The cysts and soil residues were collected on a 250 µm pore sieve, transferred to cones of filter paper and stored dry at laboratory temperature. Cysts and soil residues were later floated on water in a vessel two-thirds filled with tap water and covered on its vertical interior sides with a band of filter paper. To help the cysts and soil debris move to the periphery of the vessel, a drop of liquid soap was added to the water surface. The filter paper band was then gently removed along with the attached cysts and soil debris and laid on a plastic sheet for examination under a stereomicroscope. The cysts were separated from other residues with a brush, and

transferred to a moist filter paper in a Petri dish. All nematodes were identified to genus level based on key morphological features (Siddiqi 2000, Handoo 2000, Handoo *et al.* 2007).

RESULTS

The total survey area represented 6.3% of the barley and wheat hectarage in Bolu Province. Plant-parasitic nematodes were present in 85% of soil samples. Thirteen genera of plant-parasitic nematodes were identified, including *Heterodera*, *Merlinus*, *Pratylenchus*, *Pratylenchoides*, *Amplimerlinus*, *Helicotylenchus*, *Paratylenchus* and *Tylenchorhynchus* (Table 1). Additionally, free-living nematodes were detected in all soil and root samples.

Table 1. Frequency and density of plant-parasitic nematodes associated with cereals in Bolu Province, Turkey

Genus	Density		Frequency (%)
	Average	Range	
Heterodera (cyts)	13	1-87	82.6
Pratylenchus spp.	140	20-840	73.3
Merlinus spp.	120	20-740	78.6
Amplimerlinus spp.	80	20-380	64.5
Pratylenchoides spp.	70	20-360	66.6
Helicotylenchus spp.	80	20-360	38.8
Paratylenchus spp.	60	20-220	26.6
Tylenchorhynchus spp.	70	20-280	37.3
Ditylenchus spp.	200	40-920	97.5
Tylenchus spp.	90	20-320	77.3
Aphelenchoides spp.	80	20-560	74.4
Filenchus spp.	40	20-160	70.6
Aphelenchus spp.	30	20-80	24.6

Among the plant-parasitic nematodes, *Heterodera* and *Pratylenchus* species had a very high occurrence, being found in 82.6% and 73.3% of the samples, respectively. Additionally, cyst and root lesion nematodes were found together in the same location in 68.8% of the sampling areas. Frequency of the other important genera, *Merlinus*, *Pratylenchoides*, *Amplimerlinus*, *Helicotylenchus, Tylenchorhynchus* and *Paratylenchus*, were 78.6, 66.6, 64.5, 38.8, 37.3 and 26.6%, respectively.

For *Heterodera*, we found an average of 13 cysts (range of 1-87) in 250 cm^3 of soil. Also the average eggs/cyst conservatively would be 180, and the range of

number of eggs or juveniles of *Heterodera* would be 1-62 per g of soil. *Pratylenchus* was also widely distributed, with a mean density of 140 juveniles and adults (range of 20–840) per 100 cm^3 of soil.

DISCUSSION

This survey provides information on the occurrence and density of plant-parasitic nematodes associated with cereal cultivation areas in Bolu Province. It is clear that cereal cyst nematodes and root lesion nematodes have widespread distributions in Bolu barley and wheat growing areas. Also, cyst and root lesion nematodes were found as mixed populations with a high density in many locations. Hence it is paramount to study their incidence, effect and control together. The complexity of these nematodes and the conditions in which they occur makes it essential to seek a realistic approach for their management. Such integrated management approaches include breeding for durable nematode resistance, production and distribution of healthy seed, developing appropriate crop management practices, monitoring nematode diversity, and applying recent advances in biotechnology to overcome disease losses. In addition, significant efforts are needed to better understand the influence of cropping systems and their interactions on the spread of nematodes.

In Turkey, the *H. avenae* group causes important economic losses in small grain crops. *Heterodera avenae*, *H. filipjevi* and *H. latipons* are the three economically important species of this group (Sahin *et al.* 2009, Imren *et al.* 2012, 2015, Dababat *et al.* 2015). *Heterodera filipjevi* is the most widespread species and generally occurs alone, but mixtures with *H. latipons* have been found in CAP (Abidou *et al.* 2005, Sahin *et al.* 2009). Additionally, Abidou *et al.* (2005) and Sahin *et al.* (2009) reported the occurrence of *H. filipjevi* in 85% and 78% of samples, respectively, in CAP. The frequency of recovery of this genus (82.6%) in our study was similar across the five growing areas in Bolu Province.

Root lesion nematodes, especially *P. thornei*, *P. neglectus* and *P. penetrans*, also appear to be of importance in world wheat cropping areas (Mc Donald and Nicol 2005). *Pratylenchus thornei* and/or *P. neglectus* was found in CAP in Turkey (Abidou *et al.* 2005, Sahin *et al.* 2009). The results from this survey indicate that the genus *Pratylenchus* is likely to be of economic concern wherever cereals are grown in these five locations and may be involved with suppression of cereal yields.

Moreover, *Pratylenchoides*, *Merlinus*, *Amplimerlinus*, *Paratrophurus* and the other tylenchid nematodes were the most predominant genera of plant-parasitic nematodes in some wheat producing areas. *Pratylenchoides sheri*, *Merlinius brevidens*, *Amplimerlinius vicia*, *Paratrophurus striatus* and *P. acristylus* were determined to be in wheat growing areas of the Southeast Anatolian region of Turkey (Imren and Elekcioğlu 2008). Therefore, the reported genera are probably of great economic importance to cereals in Bolu Province.

Andersen (1961) reported that damage thresholds for *H. avenae* in temperate regions were 0.2, 1 and 5 eggs + juveniles/g of soil for oats, wheat and barley, respectively. The frequency of recovery of this genus was high across the five some growing areas. Also, soil populations of *P. thornei* of more than 2.5 nematodes per g soil are considered to be damaging population densities in Australia (Vanstone *et al.* 1998). In some parts of Bolu, the genus *Pratylenchus* would be higher than that economic threshold. The damaging threshold for cereal cyst and root lesion nematodes are unknown in Bolu Province.

This survey provided important background information for planning and administering nematode management strategies in cereal fields of Bolu Province. The population density encountered in this survey may present a potential risk to cereal crops. Further research is required (i) to determine the significance of plant-parasitic nematodes and especially the cyst and the root lesion nematodes species on cereals, and (ii) to determine their interaction with different barley and wheat varieties. Accurate identification of the genus *Heterodera* and *Pratylenchus* to the species level is an essential prerequisite for using a control tactic that is based on selection of a resistant variety. Because identification with a microscope is difficult and time consuming, diagnostic labs typically identify cereal cyst nematodes only to the genus level. Molecular procedures using nematode DNA are now available to precisely differentiate individual species. Molecular procedures using DNA extracted from soil have been developed to simultaneously identify and quantify species.

Global complementation among regional or national research programs has proven to be highly beneficial for identifying and deploying germplasm with higher levels of resistance and tolerance to cereal cyst and root lesion nematodes. However, these global efforts currently lack effective funding and coordination, limiting the ability to realize the benefits already known to exist. Greater collaboration is therefore needed between advanced research institutions, international organizations such as CIMMYT and ICARDA, and sci-

entists in countries where these nematodes are known to be a problem. These collaborative efforts will provide greater understanding of the complexity, economic importance, and control of *Heterodera* and *Pratylenchus* populations, and of pathotype evolution or selection for *Heterodera* species.

ACKNOWLEDGMENTS

This study was funded by the number of 2014.10.06.725 of BAP project (Abant Izzet Baysal University Research Projects Coordination Unit).

REFERENCES

- Abidou H, El-Ahmed A, Nicol JM, Bolat N, Rivoal R, Yahyaoui A (2005) Occurrence and distribution of species of the *Heterodera avenae* group in Syria and Turkey. *Nematologia Mediterranea*, 33, 195–201.
- Andersen S (1961) Resistens mod Harveal *Heterodera avenae*. Meddelelse Nr. 68 fra den KG1. Veterinaer-og Landbohojskoles, Copenhagen, Denmark, 179 pp.
- Anonymous (2014) Turkish Statistical Institute. www.tuik.gov.tr
- Castillo P, Vovlas N (2007) *Pratylenchus*, Nematoda, Pratylenchidae: Diagnosis, biology, pathogenicity and management. *Nematology Monographs and Perspectives*, 6, 1-530.
- Dababat AA, Imren M, Erginbas-Orakci G, Ashrafi S, Yavuzaslanoglu E, Toktay H, Pariyar SR, Elekcioglu H, Morgounov A, Mekete M (2015) The importance and management strategies of cereal cyst nematodes, *Heterodera* spp., in Turkey. *Euphytica*, 202, 173-188.
- De Waele D, Elsen A (2002) Migratory endoparasites: *Pratylenchus* and *Radopholus* species. In: 'Plant Resistance to Parasitic Nematodes.' (Eds. JL Starr, R Cook, J Bridge) pp. 175-206. (CAB International, Wallingford, UK).
- Fenwick DW (1940) Methods for the recovery and counting of cysts of *Heterodera schachtii* from soil. *Journal of helminthology,* 18, 155–172.
- Handoo ZA, Khan A, Islam S (2007) A key and diagnostic compendium to the species of the genus *Merlinius* Siddiqi, 1970 (Nematoda: Tylenchida) with description of *Merlinius khuzdarensis* n. sp. associated with date palm. *Nematology*, 9, 251-260.
- Handoo ZA (2000) A key and diagnostic compendium to the species of the genus *Tylenchorhynchus* Cobb, 1913 (Nematoda: Belonolaimidae). *Journal of Nematology*, 32, 20-34.

- Hooper DJ (1986) Preserving and staining nematodes in plant tissues. In: 'Laboratory Methods for Work with Plant and Soil Nematodes - 6th ed.' (Ed. JF Southey) Reference Book 402, pp. 81-85. (Her Majesty's Stationery Office, London, UK).
- Imren M, Elekcioğlu İH (2008) Determination of Plant Parasitic Nematodes in Vegetable, Wheat and Grapevine Fields in Diyarbakır Province. *University of Cukurova Journal of Science and Engineering*, 17 (2), 116-121.
- Imren M, Waeyenberge L, Viaene N, Elekcioğlu İH, Dababat A (2015) Morphological and molecular identification of cereal cyst nematodes from the eastern Mediterranean region of Turkey. *Turkish Journal of Agriculture & Forestry*, 39 (1), 91-98.
- Imren M, Elekcioğlu İH (2014) Effect of Cereal Cyst Nematode *Heterodera avenae* (Tylenchida: Heteroderidae) on yield of some spring wheat varieties in Adana Province, Turkey. *Turkish Journal of Agriculture & Forestry*, 38 (6), 820-823
- Imren M, Waeyenberge L, Viaene N, Toktay H, Dababat A, Elekcioğlu IH (2012) Molecular characterization of cereal cyst nematodes from South Anatolian Region in Turkey using ITS-rDNA sequences. *Turkish Journal of Entomology*, 36, 491–499.
- Kilic M, Cikman E, Elekcioglu IH (2012) Taxonomic studies on the plant -parasitic nematode species in wheat cultivated areas in Mardin province. The 31st International Symposium of the European Society of Nematologists, Adana, Turkey, pp 117
- Lawrence GW, McLean KS (1999) Plant parasitic nematode pests of soybean. In: 'Soybean Production in the Midsouth.' (Eds. LG Heatherly, HF Hodges) pp. 291–309. (CRC Press, Boca Raton, Florida, USA).
- McDonald AH, Nicol JM (2005) Nematode parasites of cereals. In: 'Plant Parasitic Nematodes in Subtropical and Tropical Agriculture.' (Eds. M Luc, RA Sikora, J Bridge) pp. 131-191. (CAB International, Wallingford, UK).
- Nicol J, Rivoal R, Taylor S, Zaharieva M (2003) Global importance of cyst (*Heterodera* spp.) and lesion nematodes (*Pratylenchus* spp.) on cereals: Distribution, yield loss, use of host resistance and integration of molecular tools. *Nematology Monographs and Perspectives*, 2, 1-19.
- Nicol JM (2002) Important nematode pests of cereals. In: 'Bread Wheat: Improvement and Production. (Eds. BC Curtis, S Rajaram, G Macpherson) pp. 346-366. *FAO Plant Production and Protection Series*, No. 30. FAO, Rome, Italy.
- Nicol JM, Ortiz-Monasterio I (2004) Effects of the root-lesion nematode, *Pratylenchus thornei*, on wheat yields in Mexico. *Nematology*, 6, 485-493.

- Öcal A (2012) Determination of Distribution of Important Plant Parasitic Nematode Species on Important Culture Plants in Adıyaman Province, Dissertation, University of Cukurova.
- Rivoal R, Cook R (1993) Nematode pests of cereals. In: 'Plant Parasitic Nematodes in Temperate Agriculture.' (Eds. K Evans, DL Trudgill, JM Webster) pp. 259-303. (CAB International, Wallingford, UK).
- Sahin E, Nicol JM, Elekçioglu IH, Yorgancılar Ö, Yıldırım AF, Tülek A, Hekimhan H, Yorgancılar A, Kılınç AT, Bolat N, Erginbaş-Orakcı, G (2009) Frequency and diversity of cereal nematodes on the Central Anatolian Plateau of Turkey. In: 'Cereal Cyst Nematodes: Status, Research and Outlook.' (Eds IT Riley, JM Nicol, AA Dababat) pp. 100-105. (CIMMYT, Ankara, Turkey).
- Siddiqi MR (2000) Tylenchida: Parasites of Plants and Insects, 2nd ed. CAB International, Wallingford, UK.
- Vanstone VA, Rathjen AJ, Ware AH, Wheeler RD (1998) Relationship between root lesion nematodes (*Pratylenchus neglectus* and *P. thornei*) and performance of wheat varieties. *Australian Journal of Experimental Agriculture*, 38, 181-188.

OCCURRENCE AND DISTRIBUTION OF CEREAL NEMATODES IN EAST ANATOLIAN REGION OF TURKEY

HALIL TOKTAY[1,*], MUSTAFA İMREN[2], ATILLA ÖCAL[3], REFIK BOZBUĞA[4], YAKUP SALGUT[1], HANİFE DEMİRBAŞ[1], İBRAHIM HALIL ELEKÇIOĞLU[5], AND ABDELFATTAH A. DABABAT[6]

[1]Niğde University, Ayhan Sahenk Faculty of Agricultural Sciences and Technologies, Niğde, Turkey, [2]Abant İzzet Baysal University, Faculty of Agriculture and Natural Science, Department of Plant Protection, Gölköy Kampüs, 14280 Bolu, Turkey, [3]Batı Akdeniz Agricultural Research Institute, Muratpaşa, Antalya, Turkey, [4]Biological Research Institute, Adana, Turkey, [5]University of Cukurova, Faculty of Agriculture, Department of Plant Protection 01330-Sarıcam, Adana, Turkey, [6]CIMMYT -Turkey, P.K. 39 Emek 06511, Ankara, Turkey.
*Corresponding author email: haliltoktay@gmail.com

SUMMARY

Plant-parasitic nematodes are one of the important major biotic agents that cause economical global yield loss in wheat growing regions. Nematode damage is predominantly found in rainfed growing areas where water stress exists. Cereal cyst nematode is identified by cysts forming on root systems. Root lesion nematodes cause lesions on the root system and those injuries help in the penetration of other root pathogens. These two nematodes are the most important pests of wheat all over the world. Recent studies showed that cereal cyst nematodes cause yield losses up to 5-50% and the root lesion nematodes cause yield losses up to 19-70% in Turkey. East Anatolian Region is one of the most intensive winter wheat cultivation areas of Turkey. During 2013 and 2014, a survey on specific composition, frequency and geographical distribution of

* Toktay H, İmren M, Öcal A, Bozbuğa R, Salgut Y, Demirbaş H, Elekçioğlu İH, Dababat AA (2015) Occurrence and distribution of cereal nematodes in East Anatolian Region of Turkey. In 'Nematodes of Small Grain Cereals: current status and research.' (AA Dababat, H Muminjanov and RW Smiley) pp. 141-148. (FAO: Ankara, Turkey).

wheat nematodes, especially cereal cyst nematodes and root-lesion nematodes, attaching and associated with cereals was conducted in East Anatolia, Turkey. *Heterodera filipjevi*, *Pratylenchus neglectus* and *P. thornei* appeared to be the most common species in wheat fields of Elazig, Malatya, Sivas, Erzurum, Erzincan, Igdir, and Kars provinces. Among the cyst nematodes, *H. filipjevi* is more frequently distributed than *H. latipons*. It was observed that *H. latipons* was less widespread in a few fields of Erzincan, Elazığ and Malatya, whereas *H. filipjevi* infestation was highest in Elazig Province with 60%. Highest infestation rate in root lesion nematodes was 42.5% in Erzurum Province while the lowest 17.14% was in Sivas Province. Now we are screening some commercial Turkish bread wheat varieties and determining pathotype of cereal cyst nematode with an East Anatolian wheat nematode project (Tubitak, 112O565).

Key words: Cereal cyst nematodes, root-lesion nematodes, identification, East Anatolia

INTRODUCTION

Wheat is a strategic crop due to its very important global food value in human diets. Plant-parasitic nematodes damage plants mechanically and chemically by reducing plant vigor, inducing lesions, rots and deformations, and predisposing plants to infection by root-infecting fungi. The most important plant-parasitic species affecting wheat are in the genera *Heterodera* (cyst), *Pratylenchus* (root-lesion), *Meloidogyne* (root knot), *Ditylenchus* (stem), *Tylenchorhynchus* and *Merlinius* (stunt), *Paratrichodorus* (stubby-root), and *Anguina* (seed-gall) (Rivoal and Cook 1993, Nicol 2002, McDonald and Nicol 2005). Three species of cereal cyst nematodes (CCN; *H. filipjevi, H. avenae* and *H. latipons*) and two species of root-lesion nematodes (RLN), occurring either individually or in a complex, have been found to be associated in wheat growing areas in Turkey (Rumpenhorst *et al.* 1996, Subbotin *et al.* 2010, Nicol 2002, Şahin *et al.* 2009, İmren *et al.* 2012, Toktay *et al.* 2015, Dababat *et al.* 2015). Effects of cereal cyst (*Heterodera* spp.) and root-lesion (*Pratylenchus* spp.) nematodes on wheat are difficult to identify and control.

This study is aimed to determine the distribution and population density and to characterize cereal nematodes by morphological and molecular tools in the East Anatolian Region during survey in 2013 and 2014.

METHODS

Surveys were carried out in Eastern Anatolian in Sivas, Erzurum, Erzincan, Elazığ, Malatya, Iğdır, Ağrı, and Kars provinces in 2013-2014 where cereals are cultivated. Samples were taken two months before wheat harvesting time and with the help of spade at 15-20 cm depth and a subtotal of 2 kg of soil taken from capillary root and rhizosphere of wheat plant.

Each sample was then labelled with the following information: receiving date, area, the crop rotation in the previous year, type of plant, the phenological stage of plant, etc., and directly brought to the laboratory. Table 1 shows the provinces where the samples were taken and how many samples were collected from each province in the Eastern Anatolian Region of Turkey.

The morphologically and morphometric-allometric measurement values were used for diagnosis of second stage juveniles of CCN and adult males and females of RLN. For this purpose, the nematodes were killed in the second larval stage according to the procedure described by Hooper (1986).

The following parameters were used for morphological identification of adult CCN females: type of fenestration, fenestral length, semi-fenestral width, bridge width of the vulva, underbridge present or not and vulva slit length (Hooper 1986). Identification of CCN and RLN species were based on diagnoses according to Siddiqi (2000) and Handoo (2002).

For molecular identification of RLN and CCN, only one cyst or a single nematode of RLN was transferred into 45 µl of double distilled water (ddH_2O) in an Eppendorf tube and crushed using a micro homogeniser. After centrifugation of the crushed nematode content, 40 µl of the mix was transferred into a PCR tube (0.2 ml). Fifty µl of worm lysis buffer (WLB) and 10 µl of Proteinase K (20 mg ml-1) were added to each tube (Holterman *et al.* 2006); the tubes were frozen at –80°C for at least 10 min. Then the tubes were incubated at 65 °C for 1 h and 95 °C for 10 min consecutively in a thermocycler. After incubation, the tubes were centrifuged for 1 min at 16400 g and kept at –20 °C until use (Tanha Maafi *et al.* 2003).

For molecular identification, the ITS-rDNA region was amplified. One ng of DNA was added to the PCR reaction mixture containing 23 µl ddH2O, 25 µl 2× DreamTaq PCR Master Mix (Thermo Scientific, Belgium) and 1 µM of each

forward primer (5'-CG TAACAAGGTAGCTGTAG-3') and reverse primer (5'-TCCTCCGCTAAATGATATG-3') (Ferris *et al.* 1993). The DNA thermal cycler program consisted of 5 min at 95 °C; 40 cycles of 94 °C for 30 s, 45 °C for 45 s and 72 °C for 45 s; followed by a final elongation step of 8 min at 72 °C. Following PCR amplification, 5 µl of each PCR product was mixed with 1 µl of 6× loading buffer (Fermentas Life Sciences, Germany) and loaded on a 1.5% standard TAE buffered agarose gel. After electrophoresis (100 V for 40 min) the gel was stained with ethidium bromide (0,1 µg ml-1) for 15 min, visualised and photographed under UV-light. The remaining PCR product was stored at –20 °C.

Ninety µl of PCR product was loaded on a 1% agarose gel for electrophoresis (100 V, 40 min). The purification was carried out as described in the manufacturer's instructions (Wizard® SV Gel and PCR Clean-Up System Kit, Promega). Purified PCR-product from each sample was sequenced (Macrogen, Amsterdam, The Netherlands) in both directions to obtain overlapping sequences of both DNA strands. Species were identified using the BLAST tool on the NCBI-website (www.ncbi.com).

RESULTS

Heterodera filipjevi, H. latipons, Pratylenchus neglectus, and *P. thornei* were identified using molecular and morphological methods to determine the cereal nematodes associated with the wheat growing area of the East Anatolian Region of Turkey. *H. filipjevi* was the most frequently and widely distributed species in this region. However, *H. latipons* was found only in three provinces (Elazığ, Malatya and Erzincan), which have temperate climatic conditions, which was an unexpected result of our project. Among the RLN species, *P. neglectus* was the more prevalent species in soil and root samples, compared to *P. thornei.*

A total of 280 soil samples were investigated for RLN and CCN and the results showed that 33.93% and 32.50% were infested with CCN and RLN, respectively (Table 1). Highest infestation rate in RLN was 42.50% in Erzurum Province, and lowest in 17.14% in Sivas Province. CCN infestation was highest in Elazig Province 60%, and was lower (15%) in fields of Erzincan Province.

DISCUSSION

Cereal cyst and root-lesion nematodes were found to be the most common plant-parasitic nematodes for wheat production areas in the East Anatolian Region of Turkey. Cereal cyst nematode *H. filipjevi* was found in 32.5% of the

surveyed areas. Cereal cyst nematode was identified as *H. filipjevi* except the *H. latipons* was also identified in three provinces; Elazığ, Malatya and Erzincan. In similar climatic conditions, Şahin *et al.* (2009) reported 78% incidence of cyst nematodes belonging to *H. filipjevi* except one location in Yozgat, which was *H. latipons* in the Central Anatolian Region of Turkey.

Table 1. Number of fields and occurrence of cereal cyst nematodes (H. filipjevi and H. latipons) and root-lesion nematodes (Pratylenchus neglectus and P. thornei) in wheat and barley fields surveyed in East Anatolian Region in Turkey

Province	Root-lesion nematodes (P. neglectus and P. thornei)			Cereal cyst nematodes (H. filipjevi and H. latipons)		
	# fields surveyed	Infested fields	Infestation rate (%)	# fields surveyed	Infested fields	Infestation rate (%)
Sivas	70	12	17,14	70	23	32,86
Erzurum	40	17	42,50	40	9	22,50
Erzincan	40	9	22,50	40	6	15,00
Elazığ	30	11	36,67	30	18	60,00
Malatya	35	23	65,71	35	16	45,71
Iğdır	25	13	52,00	25	7	28,00
Kars	40	10	25,00	40	12	30,00
Total	280	95	33,93	280	91	32,50

Some *H. avenae* species which were identified by Yüksel (1973) in the past are today the same species identified as *H. filipjevi* by classical and morphological methods in the East Anatolian Region of Turkey. *H. avenae* was not identified in this survey, unlike the past identifications of that species in this region.

Root-lesion nematodes are also important nematode pests for wheat cropping systems in Turkey. *P. thornei* and *P. neglectus* are the most frequently studied species all over the world. Şahin *et al.* (2005) found that 43% of soil samples were infected with *P. thornei* and/or *P. neglectus* in the Central Anatolian Region of Turkey.

The population densities of CCN and RLN have been found to be above the critical threshold for damage in a proportion of the samples, so some crop losses could be expected.

In conclusion, CCN and two RLN species are widespread in many wheat growing areas of the East Anatolian Region of Turkey. Research is needed to further determine the economic importance and to improve resistant varieties against these pathogens.

ACKNOWLEDGMENTS

This study was funded by TUBITAK project number 112 O 565 of (Turkey Scientific and Technical Research Council).

REFERENCES

- Abidou H, El-Ahmed A, Nicol JM, Bolat N, Rivoal R, Yahyaoui A (2005) Occurrence and distribution of species of the *Heterodera avenae* group in Syria and Turkey. *Nematologia Mediterranea*, 33, 195–201.
- Dababat AA, Imren M, Erginbas-Orakci G, Ashrafi S, Yavuzaslanoglu E, Toktay H, Pariyar SR, Elekcioglu H, Morgounov A, Mekete M (2015) The importance and management strategies of cereal cyst nematodes, *Heterodera* spp., in Turkey. *Euphytica*, 202, 173-188.
- Ferris VR, Ferris JM, Faghihi J (1993) Variation in spacer ribosomal DNA in some cyst-forming species of plant parasitic nematodes. *Fundamental and Applied Nematology*, 16, 177-184.
- Handoo ZA (2002) A key and compendium to species of the *Heterodera avenae* Group (Nematode: Heteroderidae). *Journal of Nematology*, 34, 250-262.
- Holterman M, Van Der Wurff A, Van den Elsen S (2006) Phylum-wide analysis of SSU rDNA reveals deep phylogenetic relationships among nematodes and accelerated evolution toward crown clades. *Molecular Biology and Evolution*, 23, 1792-1800.
- Hooper DJ (1986) Extraction of free living stages from soil. In: 'Labarotory Methods for Work with Plant and Soil Nematodes.' (Ed. JF Southy) pp. 5-30. (Her Majesty's Stationery Office, London).
- İmren M, Toktay H, Özarslandan A, Nicol JM, Elekçioğlu IH (2012) Determination of the cereal cyst nematode species, *Heterodera avenae* group in cereal fields of South East Anatolia. *Turkish Journal of Entomology*, 36, 265-275.
- McDonald AH, Nicol JM (2005) Nematode parasites of cereals. In: 'Plant Parasitic Nematodes in Subtropical and Tropical Agriculture.' (Eds. M Luc, RA Sikora, J Bridge) pp. 131-191. (CAB International, Wallingford, UK).
- Nicol JM, (2002) Important nematode pests. In: 'Bread Wheat: Improvement and Production.' (Eds. BC Curtis, S Rajaram, H **Gó**mez Macpherson) pp. 345-366. (FAO, Rome).
- Rivoal R, Cook R (1993) Nematode pests of cereals. In: 'Plant Parasitic Nematodes in Temperate Agriculture.' (Eds. K Evans, DL Trudgill, JM

Webster). pp. 259-303. (CAB International, Wallingford, UK).
- Rumpenhorst HJ, Elekçioğlu İH, Sturhan D, Öztürk G, Enneli S (1996) The cereal cyst nematode *Heterodera filipjevi* (Madzhidov) in Turkey. *Nematologia Mediterránea*, 24, 135-138.
- Siddiqi MR (2000) Tylenchida Parasites of Plants and Insects (CAB International, Wallingford, UK).
- Subbotin SA, Mundo Ocampo M, Baldwin JG (2010) Systematics of Cyst Nematodes (Nematoda: Heteroderinae). *Nematology Monograps and Perspectives* Volume 8B (Eds. DJ Hunt, RN Perry). (Brill, Leiden, The Netherlands).
- Şahin E, Nicol JM, Elekcioglu IH, Yorgancılar Ö, Yıldırım AF, Tülek A, Hekimhan H, Yorgancılar A, Kılınç AT, Bolat N, Erginbaş G (2009) Frequency and diversity of cereal nematodes on the Central Anatolian Plateau of Turkey. In: 'Cereal Cyst Nematodes: Status, Research and Outlook.' (Eds. IT Riley, JM Nicol, AA Dababat) pp. 100-105. (CIMMYT, Ankara, Turkey).
- Tanha Maafi Z, Subbotin SA, Moens M (2003) Molecular identification of cyst-forming nematodes (Heteroderidae) from Iran and a phylogeny based on ITS-rDNA sequences. *Nematology*, 5, 99-111.
- Toktay H, İmren M, Öcal A, Waeyenberge L, Viaene N, Dababat AA (2015) Incidence of cereal cyst nematodes in the East Anatolia Region in Turkey. *Russian Journal of Nematology*, 23. (in press).
- Yüksel H (1973) Studies on the morphological and biological differences of *Heterodera* species (Nematoda: Heteroderidae) in Turkey. *Atatürk Üniversitesi Ziraat Fakültesi Dergis*i, 4, 53–71.

PLANT PARASITIC NEMATODE SPECIES ASSOCIATED WITH BARLEY (*HORDEUM VULGARE*) AND WHEAT (*TRITICUM* SPP. L.) IN ADIYAMAN PROVINCE

ATILLA ÖCAL[1,*] AND İBRAHIM HALIL ELEKCIOĞLU[2]

[1]Batı Akdeniz Agricultural Research Institute, Muratpaşa, Antalya, Turkey; [2]University of Cukurova, Faculty of Agriculture, Department of Plant Protection, Sarıcam, Balcalı, Adana, Turkey. *Corresponding author email: atilla_ocal@hotmail.com

SUMMARY

In this study, occurance and distribution of plant-parasitic nematode species in barley and wheat fields in Adiyaman Province were investigated. In 2010-2011, during the spring and summer period, a total of 221 soil and plant root samples were taken from different locations and nematodes were extracted by using a modified Baerman Funnel method and Fenwick method. Extracted nematodes were slide-mounted, identified and morphological and allometric measurements were preformed for each specimens. A total of 17 species were determined, of which species were in 11 genera belonging to nine subfamilies within seven families of Tylenchoidea, Anguinoidea, Hoplolaimoidea, Dolichodoridea and Aphelenchoidea superfamilies of Tylenchina, Hoplolaimina and Aphelenchina suborders of Tylenchida and Aphelenchida orders. The most abundant plant-parasitic nematodes detected were *Merlinius brevidens*, *Scutylenchus quadrifer*, *Heterodera latipons*, *Pratylenchus thornei*, and *Aphelenchus avenae*.

* ÖCAL A, ELEKCIOĞLU İH (2015) Plant parasitic nematode species associated with barley (*Hordeum vulgare*) and wheat (*Triticum* spp. l.) in Adiyaman Province. In 'Nematodes of Small Grain Cereals: current status and research.' (AA Dababat, H Muminjanov and RW Smiley) pp. 149-156. (FAO: Ankara, Turkey).

INTRODUCTION

Plant-parasitic nematodes are one of the major biotic agents that cause economical yield losses in barley and wheat growing regions. These nematodes play an important role in limiting the productivity of wheat crops (Sasser 1987). They can cause significant plant damage ranging from negligible injury to total destruction of plant material (Williamson and Gleason 2003). Cereal cyst nematodes (CCN) and root lesion nematodes (RLN) are important plant-parasitic nematodes of barley and wheat. These nematodes occur in most of the cereal growing regions of the world. CCNs are identified by cysts forming on plant root systems. RLNs cause root lesions and help other root pathogens to penetrate roots systems. Nematode damage from both the sedentary CCN and migratory RLN are documented to be economically important nematodes of wheat production systems in several parts of the world, especially under rainfed or water stressed conditions (Williamson & Gleason 2003). Recent studies showed that CCN and RLN cause yield losses between 5-50% and 70% in Turkey, respectively (Nicol 2002, Gözel 2001, Toktay 2008). Wheat (*Triticum aestivum* L.) is the dominant crop in temperate countries, and is used for human food and livestock feed. Turkey is one of the ten largest wheat producers in the world with an average annual production of 21 million tonnes with average grain yield of 2,3 tonnes ha^{-1} (Anon. 2010). It is a staple and strategic crop and an essential food in the Turkish diet, consumed mostly as bread, but also as bulgur, yufka (flat bread) and cookies.

Many researches on the distribution of plant-parasitic nematodes in cereals (Gözel 2001, Nicol *et al.* 2002, İmren *et al.* 2009, 2010, 2011, 2014, Kılıç 2011, Kasapoğlu 2012, Öcal and Elekcioğlu 2012) were conducted in different parts of Turkey and species from Tylenchida and Aphelenchida orders were detected. Documented yield loss from CCN species and their importance have been reviewed (Nicol 2002, Dababat *et al.* 2015). Losses ranged from 15 to 20% on wheat in Pakistan, 40 to 92% on wheat and 17 to 77% on barley in Saudi Arabia, and 20% on barley and 23 to 50% on wheat in Australia.

The present paper reports the results of an investigation conducted to determine the occurrence and distribution of plant-parasitic nematodes in wheat growing areas of Adıyaman Province.

MATERIALS AND METHODS

A survey on the incidence of plant-parasitic nematodes was carried out in barley and wheat fields in Adıyaman, Besni, Çelikhan, Gölbaşı, Gerger, Kahta,

Samsat, Sincik, Tut districts. A total of 221 soil samples (Table 1) were taken between 2010-2011 from different locations.

Nematodes were extracted by using a modified Baerman Funnel Method (Petri Dish) to extract vermiform nematodes and Fenwick Can Method to extract sedentary nematodes (Hooper 1986). Cereal cyst nematode was extracted from 250 g dry soil. In petri dish method, as shown in Fig. 1, 100 g of soil was taken after mixing the soil sample and evenly spreading it on a circle of single-ply paper towel supported on a coarse-meshed plastic screen standing in a 20-cm diameter petri dish. Water was added to the petri dish to get the soil thoroughly wet. Then petri dish was covered with larger petri dish to reduce evaporation of the water. After 48 hours the soil was removed and nematodes

Table 1. Number of soil samples to the plant parasitic nematodes in Adıyaman province

Districts	Samples
Adıyaman	65
Kahta	59
Sincik	6
Tut	9
Çelikhan	2
Besni	43
Gölbaşı	16
Gerger	8
Samsat	13
Total	221

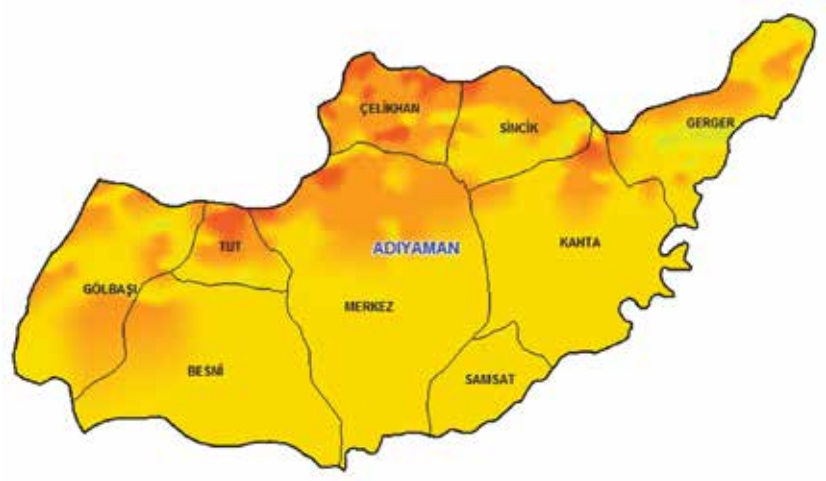


Figure 1. The Map of Adıyaman

Figure 2. A-B) Petri dish method, and **C)** Fenwick method used to extract nematodes from the soil samples.

were collected. Extracted nematodes were fixed according to De Grisse (1969) and specimens were mounted in glycerol as described by Seinhorst (1959). The slides of specimens except root knot nematodes were prepared according to Hooper (1986). Slides of root knot nematodes were prepared according to the modified method of Hartman and Sasser (1985). Finally, after measurements and morphological identifications, the taxonomic classification was followed according to Siddiqi (2000).

RESULTS

A total of 17 species (Table 2) were determined, of which species were 11 genera belonging to nine subfamilies within seven families of Tylenchoidea, Anguinoidea, Hoplolaimoidea, Dolichodoridea and Aphelenchoidea superfamilies of Tylenchina, Hoplolaimina and Aphelenchina suborders of Tylenchida and Aphelenchida orders.

The most abundant plant parasitic nematodes detected were *Merlinius brevidens*, *Scutylenchus quadrifer*, *Heterodera latipons*, *Pratylenchus thornei*, and *Aphelenchus avenae*. *Heterodera latipons* is an important nematode in wheat growing areas, occurring mostly throughout the Mediterranean region but also in Asia and Europe (Peng *et al.* 2007). Yield losses caused by cereal cyst nematodes could be up to 90% in severely infested fields (Rivoal & Cook 1993). This nematode was identified in Turkey by different researchers (İmren *et al.* 2009, 2010, 2011, 2014; Kılıç 2011; Öcal and Elekcioğlu 2012). In our study this nematode was observed in different locations of Gölbaşı and Kahta districts.

DISCUSSION

Cereal cyst (CCN) and root lesion nematodes (RLN) are the most common plant-parasitic nematodes in many parts of the world where barley and wheat plants are produced. These nematodes are also present in the south-east-

Table 2. Determination and identification of plant-parasitic nematodes in Adıyaman province

Species	Location
Amplimerlinius vicia	Adıyaman, Besni
Aphelenchus avenae	Adıyaman, Besni, Gölbaşı, Kahta, Sincik, Tut
Ditylenchus myceliophagus	Adıyaman, Besni, Gölbaşı
Filenchus thornei	Besni
Filenchus cylindricus	Besni
Filenchus cylindricauda	Adıyaman, Gölbaşı
Heterodera latipons	Gölbaşı, Kahta
Merlinius brevidens	Adıyaman, Besni, Gölbaşı, Kahta, Samsat, Sincik
Merlinius microdorus	Adıyaman, Besni, Gölbaşı, Sincik, Tut
Paratrophurus acristylus	Adıyaman, Besni, Kahta, Samsat
Paratrophurus loofi	Kahta
Paratrophurus striatus	Adıyaman, Kahta, Samsat
Pratylenchoides alkani	Adıyaman, Besni, Gölbaşı, Kahta, Samsat, Sincik
Pratylenchus neglectus	Gölbaşı, Kahta
Pratylenchus thornei	Adıyaman, Besni, Kahta
Rotylenchulus macrosoma	Adıyaman, Kahta
Scutylenchus quadrifer	Adıyaman, Besni, Gölbaşı, Kahta, Tut

ern region of Anatolia. The important species of CCN, *Heterodera avenae*, *H. latipons* and *H. filipjevi*, were determined found in wheat fields of the Eastern Mediterranean and the South-eastern Anatolia regions. CCNs have been found in many countries and have caused significant economic damage to wheat, especially under sub-adequate moisture conditions (Nicol *et al.* 2003). In this study, finding *H. latipons* in these areas was unexpected, as the majority of fields were at high elevations in Turkey, and this species is generally found in fields on the Mediterranean coast or regions having a Mediterranean climate (İmren *et al.* 2015). In these growing areas, barley and wheat are continuously cultivated on the same land as monoculture. The intensity of the incidence and impact of CCN depends on the type of host and soil, nematode pathotype, ecotype and climatic conditions of the area (Rivoal & Cook 1993). The growing of cereals as a monoculture has resulted in a gradually increasing population of CCN that influence the amount of yield losses in infected fields. It appears that existence of environmental conditions suitable for the completion of the life cycle of these nematodes can be an important factor for posing a threat to cereal production in Adıyaman Province. To maintain the population densities of these species of nematodes below damaging levels, appropriate management measures are necessary, such as rotational schemes and the use of resistant varieties. A number of resistance

sources for breeding purposes have been found in domestic cereals and their wild relatives, and express resistance to both *Heterodera* species (Dababat *et al.* 2015). In conclusion, The CCN *H. latipons* and the two RLN species (*P. neglectus* and *P. thornei*) are widespread in many barley and wheat breeding areas of the South-east Anatolian region of Turkey.

REFERENCES

- Anon (2010). www.tuik.gov.tr
- Dababat AA, Imren M, Erginbas-Orakci G, Ashrafi S, Yavuzaslanoglu E, Toktay H, Pariyar SR, Elekcioglu H, Morgounov A, Mekete M (2015) The importance and management strategies of cereal cyst nematodes, *Heterodera* spp., in Turkey. *Euphytica*, 202, 173-188.
- De Grisse AT (1969) Redescription ou modification de quelques techniques utilisées dans l'étude des nematodes phytoparasitaires. Meded. RijksfakulteitLandbouwwetenschappen, Gent, 34, 351-369.
- Fenwick DW (1940) Methods for the recovery and counting of cysts *Heterodera schachtii* from soil. *Journal of Helminthology*, 18, 155-172.
- Gozel U (2001) Doğu Akdeniz Bölgesi Buğday Alanlarında Bulunan Bitki Paraziti Nematod Türleri Üzerinde Araştırmalar. Çukurova Üniversitesi Fen Bilimleri Enstitüsü. Doktora tezi, Adana.
- Hartman KM, Sasser JN (1985) Identification of *Meloidogyne* species on the basis of differantial host test and perineal pattern Morphology. In: 'An advanced Treatise on *Meloidogyne*, Vol. II. Methodology.' (Eds. KR Barker, CC Carter, JN Sasser) pp. 69-77. (North Carolina State University Graphics, Raleigh, North Carolina).
- Hooper DJ (1986) Extraction of free living stages from soil. In: 'Laboratory Methods for Work with Plant Soil Nematodes.' (Ed. JF Southey) pp. 5-30, (Her Majesty's Stationary Office, London, UK).
- İmren M, Toktay H, Öcal A, Nicol JM, Elekcioglu İH (2009) Occurence of cereal cyst nematode *Heterodera avenae* in Southeast Anatolia, Turkey. In: 'Cereal Cyst Nematodes: Status, Research and Outlook.' (Eds. IT Riley, JM Nicol, AA Dababat) pp. 82-87. (CIMMYT, Ankara, Turkey).
- İmren M, Özarslandan A, Toktay H, Öcal A, Elekcioglu H, Dababat AA, Nicol J (2010) Preliminary survey study on *Heterodera* species in some provinces of Southeast Anatolia region of Turkey. 30th European Society of Nematologist Congress, Vienna.

- İmren M, Toktay H, Özarslandan A, Öcal A, Elekcioglu İH (2011) Doğu Akdeniz Bölgesi Buğday Alanlarında Tahıl Kist Nematodu (*Heterodera avenae* group) Türlerinin Belirlenmesi. Türkiye IV. Bitki Koruma Kongresi, Kahramanmaraş.
- İmren M, Elekcioglu İH (2014) Effect on yield of cereal cyst nematode: *Heterodera avenae* (Tylenchida: Heteroderidae) in some spring wheat varieties in Adana Province, Turkey. *Turkish Journal of Agriculture & Forestry*, 38, 820-823.
- Kasapoglu EB (2012) Adana ili Tarım Alanlarında Yetiştirilen Önemli Kültür Bitkilerinde Bulunan Bitki Paraziti Nematod Türleri. Çukurova Üniversitesi Fen Bilimleri Enstitüsü, Yüksek Lisans Tezi.
- Kilic M (2011) Mardin İli Buğday Ekiliş Alanlarında Bulunan Bitki Paraziti Nematod Türleri Üzerinde Taksonomik Araştırmalar. Harran Üniversitesi Fen Bilimleri Enstitüsü, Yüksek Lisans Tezi, Şanlıurfa.
- Nicol JM, Rivoal R, Valette S, Bolat N, Aktas H, Braun HJ, Mergoum M, Yildrim AF, Bagi A, Elekcioglu İH, Yahyaoui A (2002) The frequency and diversity of the cyst and lesion nematode on wheat in the Turkish Central Anatolian Plateau. *Journal of Nematology*, 4, 272 (abstract)
- Nicol JM (2002) Important nematode pests. In: 'Bread Wheat Improvement and Production.' (Eds. BC Curtis, S Rajaram, H Gómez Macpherson) pp. 345-366. (FAO Plant Production and Protection Series, FAO, Rome, Italy).
- Öcal A, Elekcioglu İH (2012) Plant parasitic nematode species associated with different cultivations in Adıyaman Province. 31st International Symposium of the European Society of Nematologists, 23-27 September, Adana, Turkey, p. 118.
- Peng D, Zhang D, Nicol JM, Chen S, Waeyenberge L, Moens M, Li H, Tang W, Riley IT (2007) Occurrence, distribution and research situation of cereal cyst nematode in China. In: 'Proc Int Plant Protection Conf 16th' p. 350-351. Glascow, Scotland, UK. 15-18 Oct. 2007. Br. Crop Production Council, Alton, Hampshire, UK.
- Rivoal R, Cook R (1993) Nematode pests of cereals. In: 'Plant Parasitic Nematodes in Temperate Agriculture.' (Eds. K Evans, Trudgill DL, JM Webster) pp. 259–303. CABI Publishing, Wallingford, UK
- Sasser JN (1987) A Perspective on Nematode Problems Worldwide. In: 'Nematode Parasitic to Creals and Legumes in Temperate Semi-Arid Regions.' (Eds. MC Saxena, Sikora RA, JP Sarivastava) pp. 1-12. *Proceedings of a Workshop Held at Larnaca*, Cyprus.

- Seinhorst JW (1959) A rapid Method for the Transfer of Nematodes from Fixative to Anhydrous Glycerin. *Nematologica*, 4; 67-69.
- Siddiqui MR (2000) Tylenchida parasites of plants and insects. CABI Publishing, UK, 833 pp.
- Şahin E (2010) Distribution of the Important Plant Parasitic Nematodes in Wheat Production Area in Central Anatolian Plateau and Studies on The Biology and control of Cereal Cyst Nematode *Heterodera filipjevi*. PhD. Univeristy of Cukurova, Turkey.
- Williamson VM, Gleason, CA (2003) Plant-Nematode Interactions. *Curr Opin Plant Biol*, 6: 327–333.

PART 3

MANAGEMENT THROUGH HOST RESISTANCE





DNA MOLECULAR MARKERS FOR DISEASE RESISTANCE IN PLANT BREEDING WITH EXAMPLE IN WHEAT

FAHEEM S. BALOCH[1], GÖNÜL CÖMERTBAY[2], AND HAKAN ÖZKAN[3,*]

[1]Department of Field Crops, Abant İzzet Baysal University, Bolu, Turkey; [2]Eastern Mediterranean Agricultural Research Institute, Adana, Turkey; [3]Department of Field Crops, University of Çukurova, Adana, Turkey. *Corresponding author email: hozkan@cu.edu.tr

SUMMARY

Development of disease resistant cultivars is one of the most cost-effective and environmentally friendly methods for disease control in crops. In the last few decades, the use of molecular markers has played an increasing role in plant breeding and genetics. Molecular marker technologies gained momentum with the advent of SSR markers and recently with next generation sequencing. These procedures have influenced plant protection methods for breeding resistant cultivars. In Turkey, use of DNA molecular markers in plant breeding is limited particularly in tagging the quantitative trait loci (QTL) for disease resistance. Introduction of resistance genes through plant breeding remains an important and effective method for protecting crops from diseases. Plant species commonly carry genes for disease resistance within their collective germplasm base. Marker-assisted breeding provides an opportunity for wheat breeders to introgress/pyramid genes of interest into breeding lines and to identify genes and/or QTL loci in germplasm to be used as parents. Markers are equally well suited for the pyramiding of resistance genes and many functional markers have been developed and are being used in marker-assisted breeding for diseases.

* Baloch FS, Cömertbay G, Özkan H (2015) DNA molecular markers for disease resistance in plant breeding with example in wheat. In 'Nematodes of Small Grain Cereals: current status and research.' (AA Dababat, H Muminjanov and RW Smiley) pp. 159-166. (FAO: Ankara, Turkey).

INTRODUCTION

One of the main problems for agricultural crop production is the damage caused by biotic stresses including attack by bacteria, fungi, nematodes, or viruses, each of which may cause a serious economic loss for the farmers. Pathogens are mostly controlled by three different ways; 1) Husbandry techniques, 2) Use of agrochemicals, and 3) Use of resistant varieties. Chemical control is the most widely used method to control diseases and other harmful agents, however, chemical control could be the best and effective method for some pathogens but could be poor for other pathogens like bacteria, and to have no effect mostly on viruses. Moreover use of agrochemicals has many limitations for health and could be a source of air pollution. Reduction in applying chemicals to control diseases and pests will benefit both the farmer and consumer. The use of the same chemical for control of a disease or pest may cause development of a resistance mechanism in insects, pests and pathogens. Now our world is moving towards organic farming that is intended to develop agricultural practices without the use of agro-chemicals and fertilizers. Therefore, other means to control pathogens, insects, and pests are indispensable for the farmers and as well as for the consumers. That is why production of high quality and environment friendly products using sustainable production practices requires the development of resistant varieties as a principal tool for reducing damage caused by pathogens, insects and pests.

Disease resistant varieties have been developed in many crops by conventional breeding techniques during the 20th century. Varieties that are resistant to one or more diseases are now available in most agricultural crops of economic importance. For example, wheat breeding resulted in cultivars resistant to many diseases, although they vary in stability and level of resistance because of environment interactions. Conventional breeding had a significant impact on plant breeding and resulted in resistance to many diseases. However, it is time consuming to select parents, make crosses and back crosses, and select desired progeny, making it difficult to react adequately for pathogens.

Since 1980s, DNA markers have been widely used as a principle tool for breeding and in selecting desired parents. DNA markers are now commonly used for identification and tracking the loci and genomic regions in crop breeding programs, as large numbers of molecular markers that are tightly linked to disease resistance traits are available in most major crop species. Molecular markers are utilized in mapping genes, marker-assisted selection, and map-based cloning of

genes of interest. Different types of markers have been developed in the past few decades and are now widely used in plant improvement programs. Of the various classes of existing markers, microsatellites have emerged as the markers of choice for plant breeding applications. In wheat, great work has been realized to find markers linked to disease resistance genes. The application of molecular markers has enabled breeders to select superior genotypes for traits that are difficult to select based solely on phenotype, or to pyramid desirable combinations of genes into a single genetic background. Marker-assisted backcrossing (MAB) also offers the opportunity to improve responses from selection because molecular markers can be applied earlier in the life cycle; for example, gametic selection in the F1 seedling stage. MAB not only contributes to improved precision for selection of specific traits but is also cost-effective compared with conventional plant breeding procedures. MAB also offers the opportunity to hasten transfer of desirable alleles from un-adapted genetic backgrounds into a desirable germplasm through cross-breeding. To date, 30 different loci responsible for traits like resistance to various diseases, quality and agronomic traits (plant height, photoperiod response, grain weight, tolerance to abiotic stress, etc.) have been cloned, and 97 functional markers have been developed to categorize 93 alleles based on gene sequences (Liu *et al.* 2012).

During the past decade, six genes for disease resistance were cloned in wheat (Laroche *et al.* 2000, Feuillet *et al.* 2003, Feuillet and Keller 2004, Huang *et al.* 2003, Yahiaoui *et al.* 2004, Fu *et al.* 2009, Krattinger *et al.* 2009). Among them, functional markers are available for alleles at the *Pm3* locus for reaction to powdery mildew and for the *Lr34*/*Yr18*/*Pm38* locus for resistance to leaf rust, stripe rust and powdery mildew (Tommasini *et al.* 2006, Lagudah *et al.* 2009). These markers have been successfully used in the discriminating the allelic variation for powdery mildew and leaf rust (Table 1).

The effective utilization of resistance genes requires the phenotypic and genotypic characterization of the mapping population under study. This has been widely exploited in many genetic studies, either through the use of classical bi-parental crosses and linkage mapping to determine the number and chromosomal location of stripe rust resistance genes (Yang *et al.* 2003) or the use of recent approaches such as genome-wide association mapping (GWAM) which involves a collection of adapted germplasm. The advantages of GWAM over bi-parental mapping population includes higher mapping resolution, increases in allele number, and time saving in establishing a marker-trait association and immediate application of its results in a breeding program (Flint-Garcia *et al.* 2003).

Table 1. Diagnostic markers for some disease resistance genes in wheat[1]

Locus	Marker	Primer sequence	Allele	Expected PCR Product Size
Pm3[2]	Pm3a	Pm3a/F: GGAGTCTCTTCGCATAGA Pm3a/R: CAGCTTCTAAGATCAAGGAT	*Pm3a*	624
	Pm3b	Pm3b/F: GGCACAGACAAAGCTCTG Pm3b/R: TCGAGTAGCTCGGGAATC	*Pm3b*	1382
	Pm3c	Pm3c/F: CTAGTGGAGGTAGTTGAC Pm3c/R: AGTCGTTCAAGAGAACGGC	*Pm3c*	846
	Pm3d	Pm3d/F: TGACTATTCGTGGGTGCA Pm3d/R: GACTGCGGCACAGTTCAGC	*Pm3d*	1109
	Pm3e	Pm3e/F: GGAATCCCTTTGGCTTGT Pm3e/R: CTAGCAGAGCAGTGCAAG	*Pm3e*	524
	Pm3f	Pm3f/F: GGAGTCTCTTTGCTTAAG Pm3f/R: CAGCTTCTAAGATCAAGGAT	*Pm3f*	624
	Pm3g	Pm3g/F: GAATCCCTTTATCTTGAC Pm3g/R: ATTCCCCTAGCAGAGCAGAA	*Pm3g*	540
Lr34/ Yr18/ Pm38[3]	cssfr1	L34DINT9F: TTGATGAAACCAGTTTTTTTTCTA L34PLUSR: GCCATTTAACATAATCATGATGGA	*+Lr34*	517
	cssfr2	L34DINT9F: TTGATGAAACCAGTTTTTTTTCTA L34MINUSR: TATGCCATTTAACATAATCATGAA	*-Lr34*	523
	cssfr3	Lr34DINT9F: TTGATGAAACCAGTTTTTTTTCTA	*+Lr34* *-Lr34*	517+150 229
		Lr34PLUSR: GCCATTTAACATAATCATGATGGA		
		csLV34F: GTTGGTTAAGACTGGTGATGG		
		csLV34R: TGCTTGCTATTGCTGAATAGT		
	cssfr4	Lr34DINT9F: TTGATGAAACCAGTTTTTTTTCTA	*+Lr34* *-Lr34*	150 523+229
		Lr34MINUSR: TATGCCATTTAACATAATCATGAA		
		csLV34F: GTTGGTTAAGACTGGTGATGG		
		csLV34R: TGCTTGCTATTGCTGAATAGT		
	cssfr5	Lr34DINT9F: TTGATGAAACCAGTTTTTTTTCTA	*+Lr34* *-Lr34*	751 523
		Lr34MINUSR: TATGCCATTTAACATAATCATGAA		
		Lr34SPF: GGGAGCATTATTTTTTTCCATCATG		
		Lr34DINT13R2: ACTTTCCTGAAAATAATACAAGCA		
	cssfr6	cssfr6_f: CTGAGGCACTCTTTCCTGTACAAAG cssfr6_r: GCATTCAATGAGCAATGGTTATC	*+Lr34* *-Lr34*	652 649
	cssfr7	cssfr7_f: GCGTATTGTAATGTATCGTGAGAG cssfr7_r: CATAGGAATTTGTGTGCTGTCC	*+Lr34* *-Lr34*	247 214

[1] This table was taken from Liu et al. (2012).

[2] The Pm3 locus confers resistance to powdery mildew and is located on chromosome 1AS (Tommasini et al. 2006).

[3] The Lr34/Yr18/Pm38 locus confers resistance to leaf rust, stripe rust and powdery mildew, and is located on chromosome 7DS (Lagudah et al. 2009).

Lev-Yadun *et al.* (2000) proposed a "core area" for the origins of agriculture within the Fertile Crescent. This was based on the proposition that wild einkorn and wild emmer from that area are genetically more closely related to the domesticated wheat plants than elsewhere (Özkan *et al.* 2010). Wheat landraces are an important potential source of new resistance genes since relatively few landraces have been used in modern plant breeding. Studies have demonstrated that wheat landraces can be a good source of resistance to diseases. Therefore, it is possible that, through use of some of the landraces, some early and advanced cultivars may contain some of the resistance genes. There are some good examples (Escinas –Alcazar 1993) about the importance of plant genetic resources from the Mediterranean Region and their contribution in the development of elite cultivars throughout the world. One local variety of wheat found in Turkey, collected by J. R. Harlan in 1948, was ignored for many years because of its many negative agricultural characteristics, but it was discovered in the 1980s that this variety carries genes resistant to fungi such as *Puccinia striiformis*, 35 strains of *Tilletia caries* and *T. foetida,* 10 strains of *T. controversa,* and is also tolerant to certain species of *Urocystis*, *Fusarium* and *Typhula*. It has therefore been used as a source of resistance to a whole array of diseases (Kronstad 1986).

Evaluation of landraces from the areas of domestication and diversity of any species can provide useful information for the understanding the pattern of evolution and how socio-economic and geo-ecological factors of those areas have influenced their genetic structuration (Baloch *et al.* 2014). These landraces serve great purpose as: 1) they can serve as the sources of new genes or new variations (alleles), 2) they possess high variation at inter- and intra-population levels and this intra-population diversity is very important because it can provide a buffering capacity against increasing stochastic environmental variation, and 3) their genetic structure could provide very useful insights towards better understanding the role of society, and possess valuable alleles for various diseases and other biotic stresses.

In many cases, it was observed that resistance genes are present in wheat cultivars or genotypes however the cultivar is susceptible to that particular disease. That mean some other genes are responsible for the resistance of the cultivar for that disease. Therefore, it is important to identify more genes. For this there is a need for more collaboration and for more detailed genome-wide association mapping using diverse germplasm from the Fertile Crescent, particularly from its area of diversity. Recent advances in next-generation sequencing

(NGS) and single-nucleotide polymorphism (SNP) genotyping promise to greatly accelerate crop improvement if properly deployed. High-throughput SNP genotyping offers a number of advantages over previous marker systems, including an abundance of markers, rapid processing of large populations, a variety of genotyping systems to meet different needs, and straightforward allele calling and database storage due to the bi-allelic nature of SNP markers. We now genotyping our collection on wheat landraces for DArTseq and will use this data for association studies for important diseases in diverse wheat germplasm from Turkey. We will try to find new linked markers for important diseases that could further be used in marker assisted selection.

To date, around 50 genes having monogenic nature and hundreds of QTL conferring disease resistance have been reported in wheat. Marker-assisted breeding could be easily applied for monogenic traits, but mostly marker-assisted selection is not used because the germplasm is selected phenotypically. However in quantitative disease resistances, MAS would be very useful. Molecular markers linked to many monogenic and a few quantitative disease resistances have been reported; for details please see Miedaner and Korzun 2012. Despite these efforts, there is generally a lack of reports concerning MAS and the usefulness of molecular markers in breeding. The future of MAS looks promising and as the technologies become cheaper there is no doubt that MAS will become more integrated into classical breeding programs.

REFERENCES

- Baloch SF, Alsaleh A, Saenz de Miera LE, Hatipoğlu R, Çiftçi V, Karaköy T, Yıldız M, Özkan H (2015) DNA based iPBS-retrotransposon markers for investigating the population structure of pea (*Pisum sativum*) germplasm from Turkey. *Biochemical Systematic and Ecology*, 61, 244-252.
- Esquinas -Alcázar JT (1993) Plant Genetic Resources, Plant Breeding: Principles and Prospects. (Eds. MD Hayward, NO Bosemark, I Romagosa). (Chapman & Hall, London).
- Feuillet C, Travella S, Stein N, Albar L, Nublat A, Keller B (2003) Map-based isolation of the leaf rust disease resistance gene Lr10 from the hexaploid wheat (*Triticum aestivum* L.) genome. Proceedings of the National Academy of Sciences; USA, 100, 15253–15258.
- Feuillet C, Keller B (2004) Molecular markers for disease resistance: The example wheat biotechnology in agriculture and forestry, Vol. 55. In: 'Molecular Marker Systems.' (Eds. H Lörzand, G Wenzel) (Springer-Verlag, Berlin, Heidelberg).

- Flint-Garcia SA, Thornsberry JM, Buckler ES (2003) Structure of linkage disequilibrium in plants. *Annual Review of Plant Biology*, 54, 357–374.
- Fu DL, Uauy C, Distelfeld A, Blechl A, Epstein L, Chen XM, Sela H, Fahima T, Dubcovsky J (2009) A kinase-START gene confers temperature-dependent resistance to wheat stripe rust. *Science*, 323, 1357–1360.
- Huang L, Brooks SA, Li WL, Fellers JP, Trick HN, Gill BS (2003) Map-based cloning of leaf rust resistance gene Lr21 from the large and polyploid genome of bread wheat. *Genetics*, 164, 655–664.
- Krattinger SG, Lagudah ES, Spielmeyer W, Singh RP, Huerta-Espino J, McFadden H, Bossolini E, Selter LL, Keller B (2009) A putative ABC transporter confers durable resistance to multiple fungal pathogens in wheat. *Science*, 323, 1360–136.
- Kronstad WE (1986) Germplasm: the key to past and future wheat improvement. In: 'Genetic Improvement of Yield in Wheat.' (Ed. E Smith) pp. 41-54. Special Publication 13. (Crop Science Society of America, Madison, Wisconsin).
- Lagudah ES, Krattinger SG, Herrera-Foessel S, Singh RP, Huerta-Espino J, Spielmeyer W, Brown-Guedira G, Selter LL, Keller B (2009) Gene-specific markers for the wheat gene Lr34/Yr18/Pm38 which confers resistance to multiple fungal pathogens. *Theoretical and Applied Genetics*, 119, 889–898.
- Laroche A, Frick MM, Huel R, Nykiforuk C, Conner B, Kuzyk A (2000) Molecular identification of the wheat stripe rust resistance gene Yr10, the first full-length leucine zipper-nucleotide binding site-leucine-rich-repeat resistance gene in cereals.(http://www.ncbi.nlm.nih.gov/entrezviewer.fcgi?db=nucleotide&val= 11990496).
- Lev-Yadun S, Gopher A, Abbo S (2000) The cradle of agriculture. *Science*, 288, 1602–1603.
- Liu Y, He Z, Appels R, Xia X (2012) Functional markers in wheat: current status and future prospects. *Theoretical and Applied Genetics*, 125, 1–10.
- Miedaner T, Korzun V (2012) Marker-assisted selection for disease resistance in wheat and barley breeding. *Phytopathology*, 102, 560–566.
- Özkan H, Willcox G, Graner A, Salamini F, Kilian B (2010) Geographic distribution and domestication of wild emmer wheat (*Triticum dicoccoides*). *Genetic Resources and Crop Evolution*, 58, 11–53.
- Tommasini L, Yahiaoui N, Srichumpa P, Keller B (2006) Development of functional markers specific for seven Pm3 resistance alleles and their validation in the bread wheat gene pool. *Theoretical and Applied Genetics*, 114, 165–175.

- Yahiaoui N, Srichumpa P, Dudler R, Keller B (2004) Genome analysis at different ploidy levels allows cloning of the powdery mildew resistance gene Pm3b from hexaploid wheat. *Plant Journal*, 37, 528–538.
- Yang T, Xie C, Sun Q (2003) Situation of the sources of stripe rust resistance of wheat in the post-CYR32 era in China. *Acta Agronomica Sinica*, 29, 161–168.

CURRENT RESEARCH INTO ROOT LESION NEMATODES (*PRATYLENCHUS* SPP.) IN AUSTRALIAN WHEAT

LEA M. MEAGHER[1,*], GRANT J. HOLLAWAY[2], KIRSTY J. OWEN[1], JOSHUA P. FANNING[2], KATHERINE J. LINSELL[3], JULIE M. NICOL[4], ABDELFATTAH A. DABABAT[5] AND STEPHEN M. NEATE[1]

[1]Centre for Crop Health, University of Southern Queensland, Toowoomba, Queensland 4350, Australia, [2]Department of Economic Development, Jobs, Transport and Resources, Horsham, Victoria 3401, Australia, [3]South Australian Research and Development Institute, Adelaide 5001, Australia, [4]CIMMYT Global Wheat Program and [5]ICARDA-CIMMYT Wheat Improvement Program, Ankara, Turkey. *Corresponding author email: lea.meagher@usq.edu.au

SUMMARY

Root lesion nematodes (RLN) (*Pratylenchus* spp.) are associated with significant yield losses in Australian wheat crops. During the past 40 years research has focussed on RLN impact on yield, their distribution and methods of control. Current research is focussed on developing wheat cultivars with resistance and/or tolerance of the important RLN species in Australia, while the impact of rotational crops on nematode densities is also being examined. Molecular studies are examining the genetic diversity of cultivars and possible sources of resistance. This paper focuses on recent research and results into the epidemiology and control of root lesion nematodes in Australian wheat crops.

INTRODUCTION

During the 1980s there was recognition of losses in Australian wheat crops due to root lesion nematodes (RLN), prompting significant research into the epide-

* Meagher LM, Hollaway GJ, Owen KJ, Fanning JP, Linsell KJ, Nicol JM, Dababat AA, Neate AM (2015) Current research into root lesion nematodes (*Pratylenchus* spp.) in Australian wheat. In 'Nematodes of Small Grain Cereals: current status and research.' (AA Dababat, H Muminjanov and RW Smiley) pp. 167-180. (FAO: Ankara, Turkey).

miology and control of these important soil-borne pests in Australia (Thompson *et al.* 1995). To coincide with the Fifth International Congress of Nematology (5ICN) in 2008, the current status and recent research of RLN in Australian wheat crops was reviewed by Thompson *et al.* (2008) and Vanstone *et al.* (2008). The objective of this paper is to provide an update on the current status of RLN in Australian cropping soils and the current national research activities.

HISTORY

Australian wheat production: With a gross value exceeding A$7.5 billion, and an annual production of 25 million tonnes, wheat is Australia's second largest agricultural commodity grain (ABS 2015). Australia's wheat is produced on 12.6 million hectares of land in an area that extends from central Queensland, through New South Wales, Victoria and South Australia and the south-west of Western Australia (Figure 1). Based on climatic conditions the wheat belt is divided into three cropping regions: the sub-tropical northern region, which receives significant summer rainfall, and the temperate southern and western regions, which have a Mediterranean climate with winter-dominant rainfall (Fisher 1999). Because of the mild climate in wheat growing areas, the Australian bread wheat crop is almost exclusively hard white spring wheat which is planted in autumn and harvested in late spring to early summer.

An important constraint to Australia's annual wheat production is the presence of soil borne root lesion nematodes (*Pratylenchus* spp.) (Castillo and Vovlas 2007, Nicol *et al.* 2011). Their effects are compounded by limited and variable rainfall combined with diminished soil fertility, in relation to nutrients, organic matter and structure, that creates a challenging cropping environment for Australian growers (Webb *et al.* 1997).

Species of RLN and their detection: Nationally, there are four species of root lesion nematode that are known to contribute to production losses (Murray and Brennan 2009). In south-eastern Australia, the important root lesion nematode species are *Pratylenchus thornei* and *P. neglectus,* with the later causing more yield loss due to its wider distribution (Murray and Brennan 2009, Vanstone *et al.* 2008). Conversely, in northern Australia where these two species are also widespread *P. thornei* causes more yield loss (Murray and Brennan 2009) due to a greater presence, and density, in fields (Thompson *et al.* 2010). In Western Australia, in addition to *P. neglectus*, both *P. quasitereoides* (formerly *P. teres*) and *P. penetrans* also occur; however the losses caused by these two species are less understood (Riley and Wouts 2001).

Figure 1. The Australian wheat belt (Fischer et al. 2014).

P. neglectus is the most important and extensively distributed RLN species in the southern and western cropping regions (Riley and Kelly 2002, Vanstone *et al.* 2008). In the Wimmera and Mallee regions of the southern cropping region DNA based diagnostic test (Ophel-Keller *et al.* 2008) results from 355 fields detected *P. neglectus* in 97% and *P. thornei in* 40% of fields without any other RLNs detected during 2014 and 2015 (Fanning *un-published*). Previously, in south-west Victoria *P. crenatus* and *P. penetrans* were also identified in wheat crops, however they were not considered important (Hollaway *et al.* 2008). In the northern cropping region, *P. thornei* is the dominant species and is found in 67% of fields sampled, whereas *P. neglectus* is found in 32% of fields, with both occurring together in 26% of fields (Thompson *et al.* 2010). In the Western cropping region, results from 2,363 paddocks collected since 1997, RLN were found in approximately 65% of cropping fields with the main species identified being *P. neglectus, P. quasitereoides, P. thornei* and *P. penetrans* (Wilkinson and Collins 2014).

Traditionally, nematologists and growers have relied on manual extraction of live nematodes (e.g. Whitehead tray method (Whitehead and Hemming 1965), and visual quantification using nematode morphology. However, the development of commercial DNA based soil testing capability during the late 1990s

(Ophel-Keller *et al.* 2008) has been widely adopted by researchers and growers nationally. This accurate and economical method enables rapid identification and quantification of nematodes in soils and reduces the need for technicians skilled in nematode identification to process large numbers of routine research and diagnostic samples. The tests are species specific and are currently able to detect the most prevalent RLN species *P. thornei, P. neglectus* and *P. quasitereoides* (PIRSA 2015).

In addition to providing estimated nematode numbers to researchers, the diagnostic service allows growers to make more informed decisions about the most suitable crop and cultivar. The prediction of possible losses from soil borne diseases, prior to a crop being planted, is through the allocation of a disease risk category based on the level of DNA obtained from samples (Ophel-Keller *et al.* 2008). Another advantage of this service is that it identifies and quantifies a range of other soil borne pathogens which allows a grower to recognise other potential biotic constraints in their fields.

ECONOMIC IMPACT

The migratory, endoparasitic behaviour of the *Pratylenchus* genus, combined with their short generation time, ability to withstand desiccation and their polyphagous nature, has resulted in them regularly being classified as one of the most economically damaging nematodes (Duncan and Moens 2006, Jones *et al.* 2013, Nicol *et al.* 2011). Murray and Brennan (2009) estimated, through surveys of plant pathologists nationally, that the two primary RLN affecting wheat in Australia, *P. neglectus* and *P. thornei*, caused annual yield losses exceeding A$ 73 million and A$ 50 million, respectively. In Western Australia annual yield losses caused by *P. quasitereoides* were estimated at A$ 9 million and A$ 2 million for *P. penetrans* (Murray and Brennan 2009). The accuracy of these yield loss estimates may be affected by differences in cultivar tolerance, nematode population densities and seasonal conditions (Vanstone *et al.* 2008).

There have been multiple studies demonstrating a negative correlation between the population density of *P. thornei* and *P. neglectus* with wheat yield loss (Nicol *et al.* 1999, Owen *et al.* 2014, Smiley *et al.* 2005, Smiley *et al.* 2005, Taylor *et al.* 1999). Fanning *et al.* (2014) reported a 5 to 20% yield loss in intolerant wheat with every additional 10 nematodes per gram of soil present in a paddock prior to sowing. In the past three decades the implementation of control strategies, such as, the integration of non-host crop rotations, as well as the development of

tolerant and resistant varieties has been effective in managing and reducing the impact of RLN. Nationally, a significant focus of current Grains Research and Development Corporation (GRDC) and state government funding through a national nematology project (DAV00128) is to establish the economic impacts of RLN in each cropping region (Hollaway *et al.* 2014).

STATUS

Chemical control of RLN was studied in field trials in the 90's, but was not recommended due to human toxicity of the nematicides, cost in a low value broad-acre crop and poor efficacy at depth (Taylor *et al.* 1999, Vanstone *et al.* 2008). As a result, the control of RLN is through crop rotation, utilising resistant cereals or other crops. Where high densities of nematodes are present tolerant crops or cultivars can be sown to reduce the subsequent yield and economic losses associated with RLN.

Tolerance of wheat to RLN: Tolerance is defined as the host's response and ability to grow and yield in the presence of high nematode numbers. Whish *et al.* (2014) demonstrated that in fields with high *P. thornei* numbers, plant growth in intolerant cultivars was restricted between 50-70 days after sowing, when plants required increased resources for exponential growth. This period coincided with an increase in nematode numbers and damage to the upper root layers, consequentially decreasing the plant's ability to absorb water and nutrients. Restriction of resources at this early stage in the season resulted in a yield loss of 34% in intolerant varieties, due to the reduction in above ground development (Whish *et al.* 2014).

Each year in the northern and southern cropping regions the tolerance of *P. thornei* and *P. neglectus* of wheat cultivars and advanced breeding lines is assessed in the GRDC National Variety Trials by determining relative grain yield of cultivars grown in high nematode densities in field trials (McKay *et al.* 2014, Thompson *et al.* 2008). From these National Variety Trials a consensus rating for each line is agreed and is provided in the annual State wheat variety guides. In the 2015 Queensland variety recommendations, 55% of varieties were rated as at least moderately tolerant to *P. thornei* and 22% at least moderately tolerant to *P. neglectus* (Lush 2015).

Resistance in wheat: The northern, western and southern cropping regions annually screen wheat and barley varieties, and breeding lines, for resistance

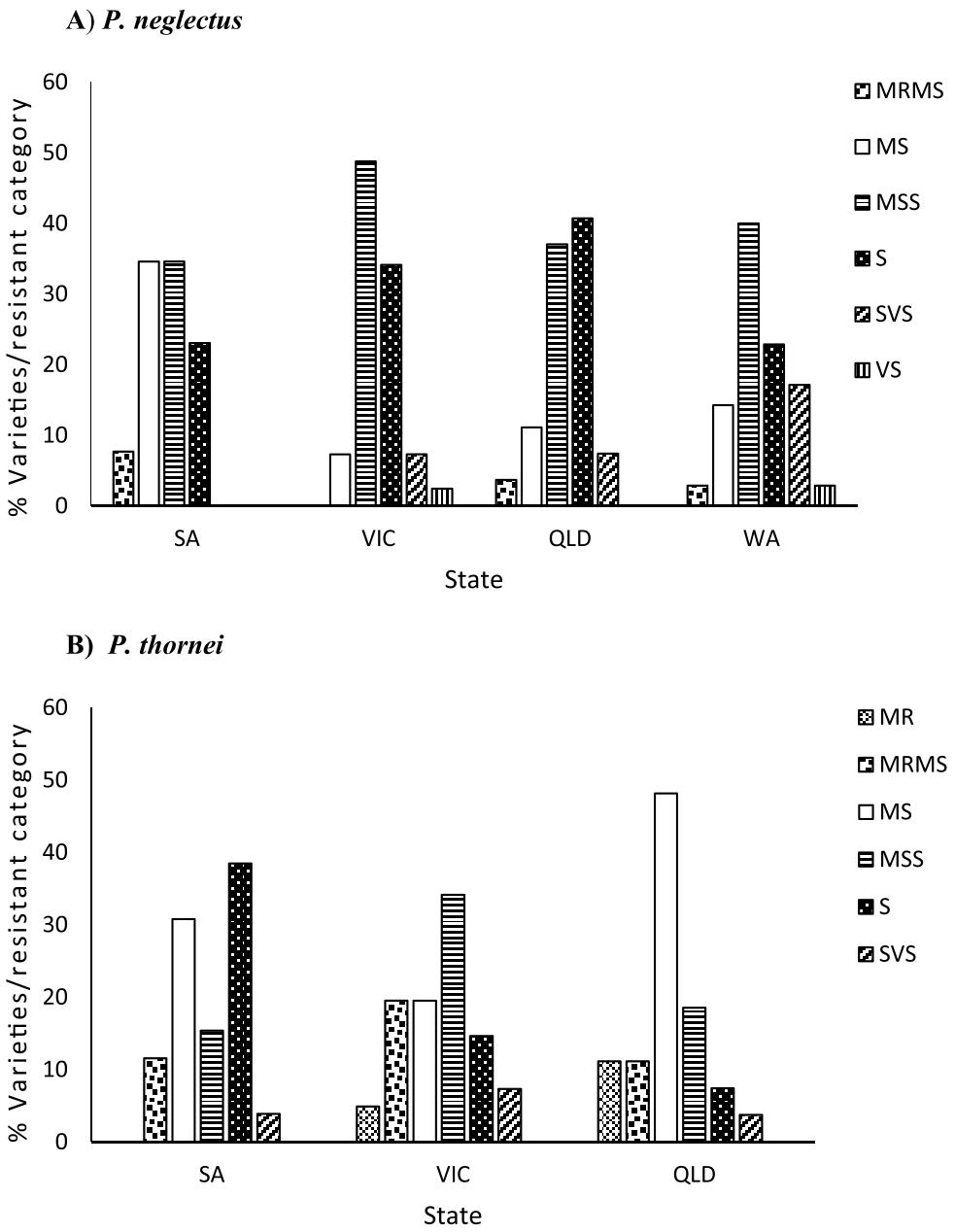


Figure 2. Percentage of varieties in each resistance category after assessment of the varieties in each state to A) P. neglectus and B) P. thornei in the 2014 season. SA = South Australia (26 varieties), VIC = Victoria (41 varieties), QLD = Queensland (27 varieties) and WA = Western Australia (35 varieties). MR = moderately resistant, MRMS = moderately resistant- moderately susceptible, MS= moderately susceptible, MSS= moderately susceptible- susceptible, S = susceptible, SVS = susceptible- very susceptible and VS = very susceptible. Hollaway (2015), Lush (2015), Trainor et al. (2015), Wallwork (2015).

to RLN as part of the GRDC National Variety Trial program. Northern and southern cropping regions test for resistance against *P. thornei* and *P. neglectus* and the western cropping region tests for resistance against *P. neglectus* and *P. quasitereoides*. Standardised data generation, collection and analysis allows cultivars to be assessed on a nine point scale ranging from Resistant to Very Susceptible. Sheedy *et al.* (2014) confirmed that even with different geographic locations and trial management practices, the three cropping regions ranked cultivars consistently. Figure 2A and 2B display the percentage of wheat varieties that were classified in each category for *P. neglectus* and *P. thornei,* respectively, by state for the 2014 season. South Australia has the greatest percentage of varieties that are moderately resistant to moderately susceptible (MRMS) to *P. neglectus*, the most damaging RLN in that state. In Queensland, where *P. thornei* is the more damaging RLN, 11% of varieties are moderately resistant (MR), while another 11% are MRMS. Out of all the varieties available only one variety assessed in South Australian offers MRMS to both *P. neglectus* and *P. thornei*.

This information, along with other disease screening results, is then released as a variety guide for growers to assist in the selection of suitable varieties for different environmental conditions (Hollaway 2015, Lush 2015, Trainor *et al.* 2015, Wallwork 2015). The absence of high levels of resistance is notable in Figure 2. To date only partial resistance to *P. thornei* and *P. neglectus* has been identified in varieties in Australia. Alternative sources of resistance are being investigated in an effort to increase the available levels of resistance. Wild relatives of wheat, such as the diploid and tetraploid accessions examined by Sheedy *et al.* (2012) and Iranian landraces (Sheedy and Thompson 2009), have proven to be valuable sources of novel resistance. Simultaneously there is effort toward ensuring moderate levels of resistance are widely available in cultivars and widely deployed to ensure that populations of RLN decrease over time.

Additional research has focused on identifying quantitative trait loci (QTL) for resistance to *P. neglectus* and *P. thornei* using different mapping populations. Zwart *et al.* (2010) demonstrated that two QTLs for *P. neglectus* resistance are present on chromosomes 2BS and 6DS, and three major QTLs for *P. thornei* resistance are located on chromosomes 2BS, 6DS and 6DL. These results, and those from Thompson and Seymour (2011) indicate that up to six genes could be responsible for resistance to *P. thornei*. This suggests that resistance is polygenic and therefore marker assisted selection is possible. In a doubled haploid (DH) population, Linsell *et al.* (2014a) identified eight QTL associated with

P. thornei resistance, and developed molecular markers for the major QTL on 2B and 6D. These molecular markers could potentially be used in genotypic selection.

The *Rlnn1 P. neglectus* resistance gene was first mapped by Williams *et al.* (2002) and has recently been fine mapped by Jayatilake *et al.* (2013). This recent work confirmed *Rlnn1* is located in the terminal region of chromosome 7AL, and is very closely linked with the rust resistance genes *Sr15* and *Lr20* as well as several molecular markers, which could also be useful for wheat breeding.

Further molecular developments for the potential control of *Pratylenchus* spp. include the finding that *P. thornei* and *Pratylenchus zeae* are amenable to RNA interference (RNAi) gene silencing through soaking the nematodes in double stranded (ds) RNA (Tan *et al.* 2013). Reproduction was reduced by up to 80% when two different genes were silenced. Interestingly dsRNA from either RLN species was effective at silencing the corresponding gene, therefore *in planta* delivery of dsRNA to target nematode genes is potentially a viable source of resistance (Tan *et al.* 2013).

Resistance of rotational crops: As stated previously resistant cultivars are those that impede nematode reproduction. Different crops have been shown to have varying degrees of susceptibility and resistance to *P. thornei* and *P. neglectus* (Hollaway *et al.* 2000, Taylor *et al.* 2000). Owen *et al.* (2014)K. J.</author><author>Clewett, T. G.</author><author>Bell, K. L.</author><author>Thompson, J. P.</author></authors></contributors><titles><title><style face="normal" font="default" size="100%">Wheat biomass and yield increased when populations of the root-lesion nematode (</style><style face="italic" font="default" size="100%">Pratylenchus thornei</style><style face="normal" font="default" size="100%"> recently confirmed that crop rotation can be used to reduce *P. thornei* densities and increase wheat yield. Nematode densities were dramatically decreased, and wheat yield increased after partially resistant canaryseed (*Phalaris canariensis*) and panicum (*Setaria italica*) were grown sequentially, prior to the planting of a susceptible wheat variety. In contrast, nematode densities were more than 15 times greater, and wheat yield 60% less in a field that was first planted with a susceptible wheat variety, followed by a susceptible crop of soybean and finally the susceptible wheat variety. The current GRDC national nematology project (Hollaway *et al.* 2014) has a significant focus on the use of rotational crops for the control of RLN.

Crop rotations can have limitations due to the polyphagous nature of *Pratylenchus.* In addition, mixed populations of *Pratylenchus* are often present in the same field, and crops and cultivars can differ in their resistance to the different *Pratylenchus* species. In the southern cropping region some rotational crops such as field peas and lentils are moderately resistant to both *P. neglectus* and *P. thornei* (Hollaway 2015). A consideration for the northern grain cropping region is the importance of arbuscular mycorrhizal (AM) fungi for some wheat crops. Owen *et al.* (2010) illustrated that although canola was effective in reducing nematode densities, subsequent wheat crops had low AM fungal colonisation which resulted in decreased wheat growth and yield. Stirling (2011) conducted studies in the northern cropping region which suggested biological mechanisms of suppression influence multiplication of *P. thornei* in certain soils. If soils could be economically managed to increase their suppression to *P. thornei*, this could be combined with resistance and rotation to further reduce the impact of RLN on yields.

RESEARCH PROSPECTS

The importance of resistance is evident from the amount of research investment by industry to find a simple and efficient genetic method of control for RLN. Sources of resistance have been identified, QTL have been mapped and the resistance mechanisms have been investigated for one mapping population against *P. thornei* (Linsell *et al.* 2014b). Studies conducted at the University of Southern Queensland, in collaboration with the International Maize and Wheat Improvement Centre (CIMMYT) in Turkey, are investigating the mechanisms of resistance to *P. neglectus* and comparing it to the mechanisms in *P. thornei* so that in the future, resistance genes with different mechanisms can be combined into one wheat variety.

Laboratory and growth chamber experiments, utilising fluorescent microscopy staining as well as nematode extraction and quantification, are being used to examine the attraction and penetration behaviour of *P. neglectus* in susceptible and resistant lines. Additionally, a genome wide association mapping approach will be employed to identify novel QTL for resistance to *P. thornei* and *P. neglectus* in CIMMYT germplasm. Increased knowledge of the biological resistance mechanisms, and the identification of resistance QTL in unique CIMMYT germplasm has the potential to increase the effectiveness of pre-breeding for resistant breeding lines for both Australia and other wheat production regions where RLN is a known constraint of wheat productivity.

ACKNOWLEDGMENTS

Financial support from the Australian Government for an Australian Postgraduate Award, the Grains Research and Development Corporation for a Grains Industry Research Scholarship (GRS10930), and the International Maize and Wheat Improvement Centre in Turkey is gratefully acknowledged.

REFERENCES

- ABS (2015) Australian Bureau of Statistics: Agricultural Commodities Australia, 2013-2014, Commonwealth of Australia.
- Castillo P, Vovlas N (2007) *Pratylenchus* (Nematoda : Pratylenchidae): Diagnosis, Biology, Pathogenicity and Management. *Nematology Monographs and Perspectives* Volume 6 (Brill Academic Publishers: Boston, MA, USA).
- Duncan LW, Moens M (2006) Migratory endoparasitic nematodes. In: 'Plant Nematology.' (Eds. RN Perry, M Moens) pp. 123-142. (CABI Publishing: Wallingford, UK).
- Fanning J, McKay A, Forknall C, Hollaway G (2014) 'Pre-sowing *Pratylenchus thornei* density can be used to determine yield loss risk in cereal crops grown in Victoria', Proceedings of the 8th Australasian Soilborne Disease Symposium, Hobart Function and Conference Centre, Tasmania. p. 51.
- Fischer RA, Byerlee D, Edmeades GO (2014) Crop yields and global food security: will yield increase continue to feed the world?, Australian Centre for International Agricultural Research, Canberra, Australia.
- Fisher BS (1999) Australian grains industry: performance by GRDC agro-ecological zones, Australian Bureau of Agricultural and Resource Economics and Sciences, Canberra.
- Hollaway G, Collins S, McKay A, Owen K, Simpfendorfer S, Taylor S (2014) A national approach to the management of root lesion nematodes in Australian field crops. Proceedings of the 8th Australasian Soilborne Disease Symposium, Hobart Function and Conference Centre, Tasmania. p. 15.
- Hollaway GJ, Taylor SP, Eastwood RF, Hunt CH (2000) Effect of field crops on density of *Pratylenchus* in South Eastern Australia; Part 2: *P. thornei*. *Journal of Nematology*, 32, 600-608.
- Hollaway GJ, Vanstone VA, Nobbs J, Smith JG, Brown JS (2008) Pathogenic nematodes of cereal crops in south-west Victoria, Australia. *Australasian Plant Pathology*, 37, 505-510.
- Hollaway K (2015) Victorian winter crop summary 2015, Grains Research and Development Corporation/ Victorian Department of Economic Development, Jobs, Transport and Resources.

- Jayatilake DV, Tucker EJ, Bariana H, Kuchel H, Edwards J, McKay AC, Chalmers K, Mather DE (2013) Genetic mapping and marker development for resistance of wheat against the root lesion nematode *Pratylenchus neglectus*. *BMC Plant Biology*, 13, 1-12.
- Jones JT, Haegeman A, Danchin EG, Gaur HS, Helder J, Jones MG, Kikuchi T, Manzanilla-Lopez R, Palomares-Rius JE, Wesemael WM, Perry RN (2013) Top 10 plant-parasitic nematodes in molecular plant pathology. *Molecular Plant Pathology*, 14, 946-61.
- Linsell KJ, Rahman MS, Taylor JD, Davey RS, Gogel BJ, Wallwork H, Forrest KL, Hayden MJ, Taylor SP, Oldach KH (2014a) QTL for resistance to root lesion nematode (*Pratylenchus thornei*) from a synthetic hexaploid wheat source. *Theoretical and Applied Genetics*, 127, 1409-21.
- Linsell KJ, Riley IT, Davies KA, Oldach KH (2014b) Characterization of resistance to *Pratylenchus thornei* (Nematoda) in wheat (*Triticum aestivum*): attraction, penetration, motility, and reproduction. *Phytopathology*, 104, 174-87.
- Lush D (2015) Queensland 2015 Wheat varieties guide, Grains Research and Development Corporation/Queensland Department of Agriculture, Fisheries and Forestry.
- McKay A, Linsell K, Davey R, Fanning J, Bogacki P, Gogel B, Hollaway G (2014) Resistance and tolerance of cereal cultivars to root lesion nematode *Pratylenchus neglectus* and *P. thornei* in south-eastern Australia. Proceedings of the 8th Australasian Soilborne Disease Symposium, Hobart Function and Conference Centre, Tasmania. p. 30.
- Murray GM, Brennan JP (2009) Estimating disease losses to the Australian wheat industry. *Australasian Plant Pathology*, 38, 558–570.
- Nicol JM, Davies KA, Hancock TW, Fisher JM (1999) Yield loss caused by *Pratylenchus thornei* on wheat in South Australia. *Journal of Nematology*, 31, 367-376.
- Nicol JM, Turner SJ, Coyne DL, Nijs Ld, Hockland S, Maafi ZT (2011) Current nematode threats to world agriculture. In: 'Genomics and Molecular Genetics of Plant-Nematode Interactions.' (Eds. JT Jones, G Gheyson, C Fenoll) pp. 21-43. (Springer: Dordrecht; New York).
- Ophel-Keller K, McKay A, Hartley D, Herdina, Curran J (2008) Development of a routine DNA-based testing service for soilborne diseases in Australia. *Australasian Plant Pathology*, 37, 243-253.
- Owen KJ, Clewett TG, Bell KL, Thompson JP (2014) Wheat biomass and yield increased when populations of the root-lesion nematode (*Pratylenchus thornei*) were reduced through sequential rotation of partially resistant winter and summer crops. *Crop and Pasture Science*, 65, 227-241.

- Owen KJ, Clewett TG, Thompson JP (2010) Pre-cropping with canola decreased *Pratylenchus thornei* populations, arbuscular mycorrhizal fungi, and yield of wheat. *Crop & Pasture Science*, 61, 399-410.
- PIRSA (2015) PREDICTA B, Primary Industries and Regions South Australia (PIRSA), http://pir.sa.gov.au/research/services/molecular_diagnostics/predicta_b.
- Riley IT, Kelly SJ (2002) Endoparasitic nematodes in cropping soils of Western Australia. *Australian Journal of Experimental Agriculture*, 42, 49-56.
- Riley IT, Wouts WM (2001) *Pratylenchus* and *Radopholus* species in agricultural soils and native vegetation in southern Australia. *Transactions of the Royal Society of South Australia*, 125, 147-153.
- Sheedy JG, McKay AC, Lewis J, Vanstone VA, Fletcher S, Kelly A, Thompson JP (2014) Cereal cultivars can be ranked consistently for resistance to root-lesion nematodes (*Pratylenchus thornei* & *P. neglectus*) using diverse procedures. *Australasian Plant Pathology*, 44, 175-182.
- Sheedy JG, Thompson JP (2009) Resistance to the root-lesion nematode *Pratylenchus thornei* of Iranian landrace wheat. *Australasian Plant Pathology*, 38, 478-489.
- Sheedy JG, Thompson JP, Kelly A (2012) Diploid and tetraploid progenitors of wheat are valuable sources of resistance to the root lesion nematode *Pratylenchus thornei*. *Euphytica*, 186, 377-391.
- Smiley RW, Whittaker RG, Gourlie JA, Easley SA (2005) *Pratylenchus thornei* associated with reduced wheat yield in Oregon. *Journal of Nematology*, 37, 45-54.
- Smiley RW, Whittaker RG, Gourlie JA, Easley SA (2005) Suppression of wheat growth and yield by *Pratylenchus neglectus* in the Pacific Northwest. *Plant Disease*, 89, 958-968.
- Stirling GR (2011) Suppressive biological factors influence populations of root lesion nematode (*Pratylenchus thornei*) on wheat in vertosols from the northern grain-growing region of Australia. *Australasian Plant Pathology*, 40, 416-429.
- Tan JA, Jones MG, Fosu-Nyarko J (2013) Gene silencing in root lesion nematodes (*Pratylenchus* spp.) significantly reduces reproduction in a plant host. *Experimental Parasitology*, 133, 166-78.
- Taylor SP, Hollaway GJ, Hunt CH (2000) Effect of field crops on the densities of *Pratylenchus neglectus* and *P. thornei* in Southeastern Australia; Part 1: *P. neglectus*. *Journal of Nematology*, 32, 591-599.

- Taylor SP, Vanstone VA, Ware AH, McKay A, Szot D, Russ MH (1999) Measuring yield loss in cereals caused by root lesion nematodes (*Pratylenchus neglectus* and *P. thornei*) with and without nematicide. *Australian Journal of Agricultural Research*, 50, 617-622.
- Thompson JP, Clewett TG, Sheedy JG, Reen RA, O'Reilly MM, Bell KL (2010) Occurrence of root-lesion nematodes (*Pratylenchus thornei* and *P. neglectus*) and stunt nematode (*Merlinius brevidens*) in the northern grain region of Australia. *Australasian Plant Pathology*, 39, 254-264.
- Thompson JP, Mackenzie J, Amos R (1995) Root-lesion nematode (*Pratylenchus thornei*) limits response of wheat but not barley to stored soil moisture in the Hermitage long-term tillage experiment. *Australian Journal of Experimental Agriculture*, 35, 1049-1055.
- Thompson JP, Owen KJ, Stirling GR, Bell MJ (2008) Root-lesion nematodes (*Pratylenchus thornei* and *P. neglectus*): a review of recent progress in managing a significant pest of grain crops in northern Australia. *Australasian Plant Pathology*, 37, 235-242.
- Thompson JP, Seymour NP (2011) Inheritance of resistance to root-lesion nematode (*Pratylenchus thornei*) in wheat landraces and cultivars from the West Asia and North Africa (WANA) region. *Crop & Pasture Science*, 62, 82-93.
- Trainor G, Zaicou-Kunesch C, Dhammu H, Shackley B, Shankar M (2015) Wheat variety guide for Western Australia 2015, Grains Research and Development Corporation/Government of Western Australia Department of Agriculture and Food.
- Vanstone VA, Hollaway GJ, Stirling GR (2008) Managing nematode pests in the southern and western regions of the Australian cereal industry: continuing progress in a challenging environment. *Australasian Plant Pathology*, 37, 220-234.
- Wallwork H (2015) Cereal Variety Disease Guide 2015, Grains Research and Development Corporation/South Australian Research and Development Institute.
- Webb AA, Grundy MJ, Powell B, Littleboy M (1997) The Australian sub-tropical cereal belt: soils, climate and agriculture. In: 'Sustainable crop production in the sub-tropics: an Australian perspective.' (Eds. AL Clarke, PB Wylie) pp. 8–23. (Department of Primary Industries: Brisbane, Queensland).
- Whish JPM, Thompson JP, Clewett TG, Lawrence JL, Wood J (2014) *Pratylenchus thornei* populations reduce water uptake in intolerant wheat cultivars. *Field Crops Research*, 161, 1-10.

- Whitehead AG, Hemming JR (1965) A comparison of some quantative methods of extracting small vermiform nematodes from soil. *Annals of Applied Biology*, 55, 25-38.
- Wilkinson CJ, Collins SJ (2014) Yield losses in Western Australian cereal crops caused by root lesion nematodes. Proceedings of the 8th Australasian Soilborne Disease Symposium, Hobart Function and Conference Centre, Tasmania, p. 39.
- Williams J, Taylor P, Bogacki P, Pallotta M, Bariana S, Wallwork H (2002) Mapping of the root lesion nematode (*Pratylenchus neglectus*) resistance gene *Rlnn1* in wheat. *Theoretical and Applied Genetics*, 104, 874-879.
- Zwart RS, Thompson JP, Milgate AW, Bansal UK, Williamson PM, Raman H, Bariana HS (2010) QTL mapping of multiple foliar disease and root-lesion nematode resistances in wheat. *Molecular Breeding*, 26, 107-124.

A NEW CANDIDATE PATHOSYSTEM FOR INVESTIGATING THE INTERACTIONS BETWEEN WHEAT AND THE CEREAL CYST NEMATODE HETERODERA AVENAE

LING-AN KONG[#], DU-QING WU[#], JIANG-KUAN CUI, WEN-KUN HUANG, HENG PENG, AND DE-LIANG PENG*

State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, China; [#] both authors contributed equally to this work. *Corresponding author email: dlpeng@ippcaas.cn

SUMMARY

Cereal cyst nematode (CCN) *Heterodera avenae* is one of the most important cereal pathogens worldwide and causes significant yield losses (Long *et al.* 2013). A better understanding of the mechanisms underlying the CCN-host interactions is important for the development of new control strategies for control this pathogen (Simonetti *et al.* 2010). However, studying the complex molecular interactions between CCN and its host is challenging due to the lack of suitable model pathosystem. The nematode is not amenable to transformation and no system for gene silencing using RNAi with CCN has been reported, meaning that characterization of CCN gene function is dependent on wheat (host)-induced gene silencing (HIGS) (Sindhu *et al.* 2009). However, the extremely low-efficiency of genetic transformation and the large and complex genome of wheat make it difficult to investigate CCN gene function using HIGS and also make study of host gene function in terms of the interactions with CCN challenging (Delporte *et al.* 2012). A model pathosystem for CCN would be of value for the study of the mechanisms of parasitism by CCN and its interactions with the host.

* Kong LA, Wu DQ, Cui JK, Huang WK, Peng H and Peng DL (2015) A new candidate pathosystem for investigating the interactions between wheat and the cereal cyst nematode *Heterodera avenae*. In 'Nematodes of Small Grain Cereals: current status and research.' (AA Dababat, H Muminjanov and RW Smiley) pp. 181-184. (FAO: Ankara, Turkey).

Brachypodium distachyon is widely used as a model cereal plant (Brkljacic *et al.* 2011), and has been used as a model plant to study the interactions with various cereal pathogens including rice blast fungus *Magnaporthe oryzae* (Parker *et al.* 2008), wheat head blight disease *Fusarium graminearum* (*Gibberella zeae*) (Peraldi *et al.* 2011), and wheat root rot *Rhizoctonia solani* (Schneebeli *et al.* 2015). An alternative model may be to use the ancestral diploid wheat lines 2A, 2B and 2D. Compared with *B. distachyon*, these ancestral diploid wheats are likely to be genetically closely related to hexaploid bread wheat (Marcussen *et al.* 2014). The genomes of the ancestral diploid wheat 2A *Triticum urartu* and 2D *Aegilops tauschii* have been sequenced, providing new insights into the genetic background of polyploid wheat species (Ling *et al.* 2013, Jia *et al.* 2013). In this study, we investigated the potential of the model cereal *Brachypodium distachyon* (Bd21-3) and diploid wheat 2A (G1812) and 2D (AL8/78) as model hosts for CCN.

First, we tested whether *B. distachyon* could also be used as a host to study its interactions with *H. avenae.* Nematode infection results showed that although some CCN penetrated Bd21-3 roots, CCN failed to develop into the J3 stage even at 19 dpi within Bd21-3 roots and remained at the J2 stage. By contrast, nematodes that infected susceptible wheat WEN19 gradually developed to the following stages by 14 dpi. In addition, the number of CCNs in Bd21-3 roots was much lower than that in WEN19. The number of J2 nematodes in Bd21-3 roots declined at later stages and no nematode was detected at 25 dpi. No cyst was formed on Bd21-3 roots at 90 dpi.

To rule out the possibility that this incompatibility was a specific feature of the CCN population used in this experiment, we performed similar assays with different CCN populations from a wide geographical range within China (Lan-kao county (Henan province), Da-xing district (Beijing) and Tai-an city (Shandong province). Both populations showed similar results, in that they failed to develop into the J3 stage, and failed to complete their life cycle in Bd21-3 roots with no cyst at 90 d. These results indicated that *B. distachyon* is a non-host for CCN.

To investigate whether the incompatibility of *B. distachyon* to CCN was due to rapid production of reactive oxygen species (ROS), we assayed the ROS content in Bd21-3 roots infected with CCN J2s at 24 hpi, 3 dpi and 8 dpi, and the results showed that there was a strong ROS burst in Bd21-3 roots at 3 days post CCN inoculation. No increased production of ROS was seen at 24 hpi or

8 dpi. Twenty one Class III peroxidase (POX) genes and nine NADPH Oxidase (NO) genes were found in Bd21-3 by alignment with orthologs from rice (*Oryza sativa*). To confirm whether POX and NO were also responsible for the ROS burst in Bd21-3 caused by CCN infection, we examined the relative expressions of these genes by qRT-PCR. The results showed that 7 of 21 POX genes were up-regulated in Bd21-3 roots in response to CCN infection. Four POX genes were significantly up-regulated at 3 dpi compared with those at 24 hpi and 8 dpi. Two NO genes were significantly up-regulated after infection, while the other 7 NO genes showed no clear change in expression. The results indicated that POX is likely to play an important role in ROS generation in response to nematode infection.

Since *B. distachyon* was a non-host for CCN, we investigated the potential of both G1812 (diploid wheat 2A) and AL8/78 (diploid wheat 2D) for use as a CCN-host pathosystem. The nematode infection rate and syncytium formation in G1812 was similar to that in the susceptible wheat line WEN19, although nematode developmental progress in G1812 was somewhat delayed compared to that in WEN19. At 33 dpi, approximately 60% of the CCN within G1812 roots had developed into the J4 stage, whereas approximately 75% of the CCN in WEN19 had reached J4 at this time point. However, the cyst numbers that formed on both G1812 and WEN19 were very similar at 90 dpi. Compared with G1812, AL8/78 was found to show some resistance to CCN, and the number of cysts formed on AL8/78 roots was significantly lower than the number in susceptible wheat WEN19. Moreover, the syncytia formed within AL8/78 roots were smaller than those both G1812 and WEN19. These results showed that both diploid wheat 2A and 2D were compatible with CCN, and that diploid wheat 2A was more susceptible than diploid 2D and may therefore be of use as a model plant for the study of the interactions between CCN and cereal hosts. The availability of an efficient transformation system for diploid wheat 2A can be used in conjunction with the pathosystem established here to allow identification and functional characterization of important genes in both CCN and its hosts.

ACKNOWLEDGEMENT

This project is supported by grants from Natural Science Foundation of China (31171827, 31301645) and 973 project (2013CB127502) and Natural Science Foundation of China.

REFERENCE

- Brkljacic J, Grotewold E, Scholl R *et al.* (2011) *Brachypodium* as a model for the grasses: today and the future. *Plant Physiology* 157, 3-13.
- Delporte F, Jacquemin J, Masson P, Watillon B (2012) Insights into the regenerative property of plant cells and their receptivity to transgenesis: Wheat as a research case study. *Plant Signaling & Behavior* 7:12, 1608-1620.
- Jia JZ, Zhao SC, Kong XY, Li YR, Zhao JY *et al.* (2013) *Aegilops tauschii* draft genome sequence reveals a gene repertoire for wheat adaptation. *Nature* 496, 91-95.
- Marcussen T, Sandve SR, Heier S, Manuel Spannagl, Pfeifer M *et al.* (2014) Ancient hybridizations among the ancestral genomes of bread wheat. *Science* 345, 1250092-1-4.
- Long HB, Peng DL, Huang WK, Peng H, Wang GF (2013) Molecular characterization and functional analysis of two new β-1,4-endoglucanase genes (*Ha-eng-2, Ha-eng-3*) from the cereal cyst nematode *Heterodera avenae*. *Plant Pathology* 62, 953-960.
- Ling HQ, Zhao S, Liu D, Wang JY, Sun H *et al.* (2013) Draft genome of the wheat A-genome progenitor *Triticum urartu*. *Nature* 496, 87-90.
- Parker D, Beckmann M, Enot DP, Overy DP, Rios ZC, Gilbert M, Talbot N, Draper J (2008) Rice blast infection of *Brachypodium distachyon* as a model system to study dynamic host/pathogen interactions. *Nature Protocols* 3, 435-445.
- Peraldi A, Beccari G, Steed A, Nicholson P (2011) *Brachypodium distachyon*: a new pathosystem to study Fusarium head blight and other Fusarium diseases of wheat. *BMC Plant Biology* 11, doi:10.1186/1471-2229-11-100.
- Sindhu AS, Maier TR, Mitchum MG, Hussey RS, Davis EL, Baum TJ (2009) Effective and specific *in planta* RNAi in cyst nematodes: expression interference of four parasitism genes reduces parasitic success. *Journal of Experimental Botany* 60, 315-324.
- Simonetti E, Alba E, Montes MJ, Delibes A, Lopez-Brana I (2010) Analysis of ascorbate peroxidase genes expressed in resistant and susceptible wheat lines infected by the cereal cyst nematode, *Heterodera avenae*. *Plant Cell Reports* 29, 1169-1178.
- Schneebeli K, Mathesius U, Watt M (2015) *Brachypodium distachyon* is a pathosystem model for the study of the wheat disease rhizoctonia root rot. *Plant Pathology* 64, 91-100.

RESPONSE OF BARLEY, OAT AND WHEAT TO THE MEDITERRANEAN CEREAL CYST NEMATODE *H. LATIPONS*

ABDUL MUNEUM JAABARI[1], ADEL AL ABDED[2], ABDELFATTAH A. DABABAT[3], AND LUMA AL-BANNA[4,*]

[1]National Centre for Agricultural Research and Technology Transfer (NCARTT), Ministry of Agriculture, Jordan, Amman, [2]National Centre for Agricultural Research and Technology Transfer (NCARTT), Ministry of Agriculture, Jordan, Amman, [3]ICARDA-CIMMYT Wheat Improvement Program, P.K. 39 Emek, 06511, Ankara, Turkey, and [4]Department of Plant Protection, University of Jordan Amman-11942-Jordan. *Corresponding author email: lalbanna@ju.edu.jo

SUMMARY

We evaluated 12 barley, 5 oat and 23 wheat accessions against a Jordanian isolate of the Mediterranean cereal cyst nematode *Heterodera latipons***.** A total of 100 freshly hatched second stage juveniles of *H. latipons* were added to each plant of the tested accessions. Plants were harvested 45 days post inoculation. Number of cysts recovered from both soils and roots were recorded and were scaled for resistance following Özarslandan *et al.* (2010). The screening test showed that six barley accessions were resistant while five accessions were moderately resistant with less than 4 cysts/plant recovered. Only one barley accession was susceptible. On the other hand, only one oat accession did not support the nematode while the other four accessions varied in their response from moderately resistant to moderately susceptible. The 23 wheat accessions varied in their susceptibility; 10 showed resistance against the tested cyst nematode while the other accessions ranged from moderately resistant to suscep-

* Jaabari AM, Al Abded A, Dababat AA, Al-Banna L (2015) Response of barley, oat and wheat to the Mediterranean cereal cyst nematode *H. latipons*. In 'Nematodes of Small Grain Cereals: current status and research.' (AA Dababat, H Muminjanov and RW Smiley) pp. 185-192. (FAO: Ankara, Turkey).

tible. Our results showed that some cereal accessions that were resistant to the Jordanian isolate of *H. latipons* were also resistant to *H. filipjevi* whereas other accessions did not have the same response.

INTRODUCTION

The Mediterranean cereal cyst nematode (MCCN), *Heterodera latipons*, is considered the most common species of cyst nematodes that limit the production of wheat and barley in several Mediterranean countries such as Cyprus, Jordan, Palestine, Italy, Libya, Spain, and Turkey (Franklin 1969, Cohn and Ausher 1973, Mor *et al.* 1992, Yousef and Jacob 1994, Philis 1995, Rumpenhorst *et al.* 1996, Al-Abed *et al.* 2004). The use of germplasm that possesses resistant genes against cereal cyst nematodes (CCN) is considered the most effective control measures.

Several sources of resistance against CCN have been identified in wheat and its relatives. Very few studies investigated the resistance of wheat against *H. latipons* (Bekal *et al.* 1998, Rivoal *et al.* 2001, Rivoal 2009, Khrfan 2012). Khrfan (2012) investigated the reaction of 52 wheat genotypes comprised of landraces of durum, common bread wheat cultivars, and synthetic hexaploid wheat against a Jordanian isolate (RJHL) of *H. latipons*. Results of this screening test showed that some wheat genotypes such as commercial bread wheat cultivars (Drysdale, Gladius, GS50A and Silverstar) and synthetic hexaploids showed resistance to the RJHL isolate. Further, 19 out of the 23 Australian synthetic hexaploids genotypes were also resistant against the RJHL isolate of MCCN. While other genotypes, including Jordanian landraces, varied in their susceptibility, with some being moderately resistant while others were moderately susceptible to susceptible genotypes. In this study we aimed to evaluate 12 barley, 5 oat, and 23 wheat accessions against (RJHL) of *H. latipons*.

METHODS

A controlled condition experiment was conducted at the University of Jordan, Jubeiha, Amman, Jordan to investigate the susceptibility of barley, oat and wheat germplasm against a Jordanian isolate of the MCCN *H. latipons* (Tables 1,2 and 3). The local wheat cultivar named "Horani Nawawi" was used as a susceptible control cultivar. To perform the assay, cysts were obtained from a soil sample that was collected from a field in Ramtha area at the northern part of Jordan. That isolate was previously identified as *H. latipons* (Shepherd 1970). The recov-

ered cysts were surface sterilized by soaking in a solution containing 0.5% sodium hypochlorite (NaOCl) for 30 minutes then rinsed with sterilized tap water. The surface sterilized cysts were then transferred to a 1.5 ml Eppendorf tube and placed at 4°C for 2 weeks followed by 2 months of incubation at 10°C to achieve hatching of the second stage juveniles (J2) (Al-Abed *et al.* 2004, Scholz and Sikora 2004). Freshly hatched J2 were used in the screening assay.

Cereal seeds were surface sterilized by soaking these seeds in 96% ethanol for six minutes followed by soaking in a solution of 4.5% NaOCl for 10 minutes and then rinsed six times in sterile distilled water. The surface sterilized seeds were placed on wet sterilized filter paper in Petri dishes and incubated at 20°C for 2-4 days to initiate germination. Once the roots reached 2-3 cm, they were each transplanted into a tube (3-cm diameter and 12-cm tall) filled with oven sterilized soil mixture (83% sand, 12% clay, 5% silt; pH 8 and EC of 0.54 dS/m). Each cultivar was replicated three times. The cultivars that showed resistance against MCCN were further screened with three replicates. The transplanted tubes were placed in the growth chamber at the faculty of Agriculture, University of Jordan with 16 hours light and 8 hours dark at a temperature of 20°C. One week after transplanting, a total of 100 J2 of the cyst nematode were added to each plant. Soil in the tubes was kept wet until the end of experiment.

The plants were harvested 45 days after inoculation and the roots were washed by a gentle stream of tap water. Roots were examined for presence of cysts using a dissecting microscope (Nikon, SMZ645). Soils from each tube were processed to extract the cysts using the floatation method. Cysts recovered from soil and from roots were counted and tabulated. The total number of recovered cysts (soil and roots) for every wheat genotype and their replicates was documented. Averages and standard deviations were tabulated. Based on Özarslandan *et al.* (2010) the following scale was followed for determining the resistance and susceptibility of each wheat genotype; resistant (R) = 0-2; moderately resistant (MR) = 3-4; moderately susceptible (MS) = 5-8; susceptible (S) = 9-12; very susceptible (VS) ≥ 13 cysts/plant.

RESULTS

The reactions of the barley, oat, and wheat genotypes against the RJHL isolate of the MCCN are shown in Tables 1, 2, and 3, respectively. The screening test showed that six barley accessions were resistant while five accessions were moderately resistant with less than 4 cysts/plant (Table 1). Only one barley

Table 1. Reactions of barley accessions against RJHL isolate of MCCN.

Barley accession	White females and brown cysts[1]	Resistance rating[2]
VARDE	0.5	R
LA ESTUANZUELA	0.7	R
MARTIN 403 - 2	1.0	R
BAJO ARAGON	1.3	R
MOROCCO	2.0	R
MAROCAINE	2.3	R
HARLAN 43	2.3	MR
ORTOLAN	2.7	MR
SALKA	3.7	MR
KVL 191	4.0	MR
DALMATISCHE	4.3	MR
SIRI	10.3	S

1 Average number of white females on roots and brown cysts recovered from soil; means of 3 replicates.
2 Based on Özarslandan et al. (2010), the following scale was followed for determining the resistance and susceptibility of each genotype; resistant (R) = 0-2; moderately resistant (MR) = 3-4; moderately susceptible (MS) = 5-8; susceptible (S) = 9-12; very susceptible (VS) ≥ 13 cysts/plant.

Table 2. Reactions of oat accessions against RJHL isolate of MCCN.

Oat accession	White females and brown cysts[1]	Resistance rating[2]
MK H.72-646	0.2	R
SILVA	2.3	MR
SUN II	2.3	MR
ANSI	5.0	MS
PUSA HYBRID BS1	8.7	S

1,2 See footnotes for Table 1.

accession was susceptible (Table 1). On the other hand, only the MK H.72-646 oat accession did not support the nematode while the other four accessions varied in their response from moderately resistant to susceptible (Table 2). The 23 wheat accessions varied in their susceptibility, where ten showed resistance against the tested cyst nematode while the other accessions ranged from moderately resistant to susceptible (Table 3).

Table 3. Reactions of wheat accessions against RJHL isolate of MCCN.

Wheat accession	White females and brown cysts[1]	Resistance rating[2]
ID 2150	0.2	R
MILAN	0.3	R
ISKAMISH K-2- LIGHT	0.5	R
T-2003	1.0	R
F130L1.12/ATTILA	1.0	R
CROC _1/AE.SQARROSA(224)// OPATA (20616)	1.3	R
VP 1620	1.7	R
SONMEZ	1.7	R
KATE A-1	2.0	R
F372	2.0	R
LOROS X KOGA	2.7	MR
AUS 4930.7 / 2*PASTOR	3.0	MR
ZHONGYU	3.0	MR
MIRZABEY2000	3.3	MR
PRINS	4.0	MR
CAPA	4.0	MR
PSATHIAS	4.0	MR
VP5053	4.7	MS
TAIKONG	4.7	MS
AUS GS50AT34/SUNCO//CUN-NINGHAM	4.7	MS
AU/CO65233//2*CA8-155/3/ F474S1-1.1	5.0	MS
VL411R	5.0	MS
HORANII NAWAWII	6.0	MS
RAJ 1	7.3	MS

1,2 See footnotes for Table 1

DISCUSSION

Our results showed that some cereal accessions that were resistant to the Jordanian isolate of *H. latipons* were also resistant to *H. filipjevi*. Both oat accessions MK H.72-646 and Silva that showed resistance against the Jordanian isolate of MCCN were also resistant to *H. filipjevi* (Dababat, unpublished). The spring wheat RAJ1 was susceptible to both the Jordanian isolate of *H. latipons* and *H. filipjevi* (Dababat, unpublished). Other accessions did not have the same response against different species of CCN. This finding agreed with previous

screening tests which showed that both wheat lines and cysts nematode pathotypes varied in their susceptibility and virulence, respectively (Slootmaker *et al.* 1974, Rivoal *et al.* 2000 and 2001, Zaharieva *et al.* 2001, Safari *et al.* 2005, Montes *et al.* 2008, Nicol *et al.* 2009). This is the first report investigating resistance of barley and oat genotypes against a Jordanian isolate of *H. latipons*. Our findings and other previous studies will help breeders to identify genes responsible for the resistance against one or several pathotypes of cyst nematodes attacking cereals. It was recorded that more than one species of CCN could occur sympatrically in the regions cropped with cereals; pyramiding different resistance genes into a single genotype will certainly promote durable resistance (Eastwood *et al.* 1991, Safari *et al.* 2005).

ACKNOWLEDGMENT

This research was supported by the University of Jordan, The National Center for Agricultural Research and Extension (NCARE), Ministry of Agriculture, Jordan, and the International Maize and Wheat Improvement Center (CIMMYT).

REFERENCES

- Al-Abed A, Al-Momany A, Al Banna L (2004) *Heterodera latipons* on barley in Jordan. *Phytopathologia Mediterranea*, 43, 311–317.
- Bekal S, Jahier J, Rivoal R (1998) Host responses of different Triticeae to species of the cereal cyst nematode complex in relation to breeding resistant durum wheat. *Fundamental and Applied Nematology*, 21, 359–370.
- Cohn E, Ausher R (1973) *Longidorus cohni* and *Heterodera latipons*, economic nematode pests of oats in Israel. *Plant Disease Reporter*, 57, 53–54.
- Eastwood RF, Lagudah ES, Appels R, Hannah M, Kollmorgen JF (1991) *Triticum tauschii*: a novel source of resistance to cereal cyst nematode (*Heterodera avenae*). *Australian Journal* of *Agricultural Research*, 42, 69–77.
- Franklin MT (1969) *Heterodera latipons* n. sp., A cereal cyst nematode from the Mediterranean region. *Nematologica*, 15, 535–542.
- Khrfan W (2012) Characterization of genetic resistance against Mediterranean cereal cyst nematode (*Heterodera latipons*) in wheat. PhD Thesis, University of Jordan, Amman, Jordan.
- Montes M, Andrés M, Sin E, López-Braña I, Martin-Sánchez J, Romero M, Delibes A (2008) Cereal cyst nematode resistance conferred by the *Cre7* gene from *Aegilops triuncialis* and its relationship with *Cre* genes from Australian wheat cultivars. *Genome*, 51, 315-319.

- Mor M, Cohn E, Spiegel Y (1992) Phenology, pathogenicity and pathotypes of cereal cyst nematodes, *Heterodera avenae* and *Heterodera latipons* (Nematoda: Heteroderidae) in Israel. *Nematologica*, 38, 494–501.
- Nicol JM, Ogbonnaya FC, Singh AK, Bishnoi SP, Kanwar RS, Li H, Chen S, Peng D, Bolat N, Sahin E, Elekcioglu H (2009) Current global knowledge of the usability of cereal cyst nematode resistant bread wheat germplasm through international germplasm exchange and evaluation. In: 'Cereal Cyst Nematodes: Status, Research and Outlook.' (Eds. IT Riley, JM Nicol, AA Dababat) pp.149-153. (CIMMYT, Ankara, Turkey).
- Özarslandan M, Özarslandan A, Nicol JM, Elekcioğlu IH (2010) Determination of the pathotype group of *Heterodera filipjevi* (Madzhidov, 1981) population and resistance of *H. filipjevi* populations against wheat genotypes. *Turkish Journal of Entomology*, 34, 515–527.
- Philis J (1995) An up-dated list of plant parasitic nematodes from Cyprus and their economic importance. *Nematologia Mediterranea*, 23, 307–314.
- Rivoal R, Bekal S, Valette S, Gauthier JP, Bel Hadj Fradj M, Mokabli A, Jahier J, Nicol J, Yahyaoui A (2001) Variation in reproductive capacity and virulence on different genotypes and resistance genes of Triticeae, in the cereal cyst nematode complex. *Nematology*, 3, 581–592.
- Rivoal R, Bel Hadj Fradj M, Valette S, Mokabli A, Jahier AJ, Zaharieva M, Nicol JM (2000) Variability for resistance to cereal cyst nematodes in Triticeae: Potential use for *Triticum turgidum* L. var. *durum* improvement. CIHEAM - Options méditerranéennes 413–416.
- Rumpenhorst HG, Sturhan D, Elekcioglu JH, Ozturk G, Enneli S (1996) The cereal cyst nematode *Heterodera filipjevi* (Mazhidov) in Turkey. *Nematologia Mediterranea*, 24,135–138.
- Safari E, Gororo NN, Eastwood RF (2005) Impact of *Cre*1, *Cre*8 and *Cre*3 genes on cereal cyst nematode resistance in wheat. *Theoretical and Applied Genetics*, 110, 567–572.
- Scholz U, Sikora RA (2004) Hatching behavior and life cycle of *Heterodera latipons* Franklin under Syrian agro-ecological conditions. *Nematology*, 6, 245–256.
- Shepherd AM (1970) Extraction and estimation of *Heterodera*; Extraction of cysts from the sample. In: 'Laboratory Methods for Work with Plant and Soil Nematodes.' (Ed. JB Goodey) pp. 23-33. (Ministry of Agriculture, Fisheries and Food, Her Majesty's Stationary Office, London, UK).
- Slootmaker LA, Lange W, Jochemsen G, Schepers J (1974) Monosomic analysis in bread wheat of resistance to cereal root eelworm. *Euphytica*, 23, 497–503.

- Yousef DM, Jacob JJ (1994) A nematode survey of vegetable crops and some orchards in the Ghor of Jordan. *Nematologia Mediterranea*, 22, 11–15.
- Zaharieva M, Monneveux P, Henry M, Rivoal R, Valkoun J, Nachit MM (2001) Evaluation of a collection of wild wheat relative *Aegilops geniculata* Roth and identification of potential sources for useful traits. *Euphytica,* 119, 33-38.

RESISTANCE TO THE ROOT LESION NEMATODES (*PRATYLENCHUS PENETRANS* AND *P. THORNEI*) IN WHEAT GERMPLASM

FOUAD MOKRINI[1,2,3], NICOLE VIAENE[2,4,*], LIEVEN WAEYENBERGE[2], ABDELFATTAH A. DABABAT[5], FOUAD ABBADANDALOUSSI[6], AND MAURICE MOENS[2,3]

[1]National Institute for Agricultural Research (INRA), Km 9, 14000 Kenitra, Morocco, [2]Institute for Agricultural and Fisheries Research, Plant, Crop Protection, Burg Van Gansberghelaan 96, B-9820 Merelbeke, Belgium; [3]Faculty of Bio-science Engineering, Ghent University, Coupure links 653, B-9000 Ghent, Belgium; [4]Department of Biology, Ghent University, K.L. Ledeganckstraat 35, B-9000 Ghent, Belgium; [5]CIMMYT (International Maize and Wheat Improvement Centre) P.K. 39 06511, Emek, Ankara, Turkey; and [6]National Institute of Agricultural Research, Scientific Division, BP415RP, Rabat, Morocco. *Corresponding author email: nicole.viaene@ilvo.vlaanderen.be

SUMMARY

The root lesion nematodes *Pratylenchus penetrans* and *P. thornei* attack roots of wheat and cause significant yield losses especially under rainfed conditions. Breeding for resistant varieties is one of the most effective methods to control nematodes. Therefore, we screened a collection of 14 spring wheat and 11 of winter wheat lines, developed at CIMMYT, for resistance to both nematode species. Individual plants were grown in sand in small tubes (15 × 20 × 120 mm) placed in a random design with 10 replicates in the greenhouse. The resistance level was evaluated based on the numbers of nematodes extracted from both roots and soil of each line. Trials were terminated nine weeks after nematodes infestation. The numbers of *P. penetrans* and *P. thornei* were determined visually

* Mokrini F, Viaene N, Waeyenberge L, Dababat AA, Abbadandaloussi F, Moens M (2015) Resistance to the root lesion nematodes (*Pratylenchus penetrans* and *P. thornei*) in wheat germplasm. In 'Nematodes of Small Grain Cereals: current status and research.' (AA Dababat, H Muminjanov and RW Smiley) pp. 193-204 (FAO: Ankara, Turkey).

using a microscope. Three lines (L9, L12 and L13) were found resistant to *P. thornei* and one of these (L9) was also resistant to *P. penetrans*. To investigate the durability of this resistance, we co-inoculated juveniles of *Heterodera avenae*, a cereal cyst nematode widely present in Moroccan wheat fields, and assessed the reproduction of both lesion nematodes *P. penetrans* and *P. thornei*. Our results showed that the resistant wheat lines L9 and L9, L12, L13 remained resistant to *P. penetrans* and *P. thornei,* respectively. The outcome of this study is valuable to wheat breeding programs in Morocco and the world. However, the resistant sources should be validated under natural field conditions. These findings are important to understand the background of the source(s) of resistance responsible for inhibition of nematode reproduction in promising wheat lines.

Key words: Root-lesion nematodes, resistance, lines of wheat, Morocco, screening

INTRODUCTION

Root-lesion nematodes (RLNs) are considered among the most important group of plant-parasitic nematodes attacking cereals on a worldwide basis, (Smiley and Nicol 2009). Eight species of RLN are known to be parasitic on small grain cereals. Of these, *P. thornei* and *P. penetrans* are the most economically important (Rivoal and Cook 1993, McDonald and Nicol 2005). In Morocco, *Pratylenchus* spp. are the most important group of nematodes in different cereal growing regions followed by cyst nematodes of the *Heterodera avenae* group (Meskine and Abbad Andaloussi 1984, Rammah 1994, Mokrini *et al.* 2012b). They cause extensive necrosis of the roots with consequent crop losses. *Pratylenchus penetrans* is the most abundant and widespread species in Morocco and was recovered from 70% of soil samples taken during a survey of the wheat producing regions (Mokrini *et al.* 2012b).

The use of resistant accessions is considered the most effective and economical method for managing nematodes as it environmentally sustainable and requires no additional equipment or cost. Several resistant wheat accessions against *P. thornei* have been identified (Vanstone *et al.* 1998, Thompson *et al.* 1999, Toktay *et al.* 2012). The soil-borne pathogens program of CIMMYT-ICARDA screens annually about 1000 accessions from the International Winter Wheat Improvement Program (www.iwwip.org) under conditions for multiple disease resistance, including the root lesion nematodes *P. thornei* and *P. neglectus* (Dababat *et al.* 2014).

Competition between different soil-borne parasitic nematodes has been reported for several crops (Yang 1976, Lasserre *et al.* 1994, Tsai 2008, Melakeberhan and Dey 2003, Brinkman *et al.* 2005). Rivoal *et al.* (1995) recorded suppression of *P. neglectus* in the presence of *H. avenae* on oats. The effect of nematodes on the host plant may be altered when two nematode species attack the plant simultaneously. Surveys of cereal fields in the major wheat and barley cultivating areas of Turkey (Sahin *et al.* 2009) and Morocco (Mokrini *et al.* 2009, 2012) showed that the two species *P. penetrans* and *P. thornei* are often found together with *H. avenae*. It would be interesting to know when introducing or breeding for new cultivars if the infection by both a *Pratylenchus* species and *H. avenae* influences the resistance level.

The objectives of this study were 1) to identify resistant wheat lines against the root-lesion nematodes *P. thornei* and *P. penetrans* in pot experiments under greenhouse conditions, and 2) to investigate the effects of co-inoculating *H. avenae* on the reproduction of *P. penetrans* and *P. thornei* on resistant wheat lines under controlled conditions, hence on the durability of the resistance when more than one nematode species are present.

METHODS

Screening for resistance against *P. thornei* and *P. penetrans*: *Plants* - Twenty-five lines of wheat provided by CIMMYT were screened for resistance against a population of *P. thornei* and *P. penetrans.* The set of germplasm represents a collection of 14 lines of spring wheat (SW) and 11 lines of winter wheat (WW) (Tables 1 and 2). The durum wheat cultivar "Ourgh", susceptible to both *P. thornei* and *P. penetrans,* was used as a standard. Two independent experiments were conducted to phenotype the set of wheat against *P. thornei* and *P. penetrans* in greenhouse conditions. One seedling with three seminal roots was transplanted into a plastic folding tube (15 × 20 × 120 mm) filled with a mixture of sand, field soil and organic matter (70:29:1 v/v). For each cultivar, ten screening tubes were placed together in a pot (15-cm diameter). The spaces around the tubes were filled with sand to keep the tubes upright. Thirty replicates of each line were tested. The 75 pots (3 × 25 lines) containing 10 tubes each were placed in a completely randomized design in a greenhouse with temperatures between 22°C and 24°C. Plants were sprayed daily with water using an atomizer.

Nematode inoculum - Experiments were carried out using two populations of *P. penetrans* and *P. thornei*, collected from Gharb and Zaers regions of Moroc-

co, respectively. These two populations were maintained in vitro on carrot-disc cultures according to Moody *et al.* (1973). Nematodes were extracted by placing infected chopped carrot discs on Baermann funnels in a misting chamber for three days. One week after planting, each seedling was inoculated with nematode suspension containing either 400 *P. thornei* (experiment 1) or 400 *P. penetrans* (experiment 2). Nematodes were applied with a pipet into three holes of 2-cm depth made at 0.5-cm distance from the stem base.

Assessment of resistance - Plants were harvested nine weeks after inoculation and above-ground plant parts were removed. The roots were washed separately for every plant. Nematodes were released from the roots by cutting the root system into 2-cm pieces and macerating them with water during 1 min at high speed in a commercial blender (Waring). Nematodes were extracted from this mixture and also from the soil of each tube using an automated zonal centrifuge (Hendrickx 1995). All vermiform stages of *P. thornei* or *P. penetrans* in the obtained nematode suspensions were counted using a stereomicroscope. For the evaluation of the susceptibility of the wheat lines against *P. penetrans* and *P. thornei*, a reproduction factor (Pf/Pi) was calculated, for each plant, where Pf = total number of nematodes from both soil and roots in each tube at harvest and Pi = initial number of nematode inoculated into the tube. Wheat lines which gave a resistant reaction against *P. thornei* or *P. penetrans* based on their reproduction factor (Rf = Pf/Pi < 1) were re-phenotyped for data validation.

Dual inoculation of *H. avenae* and *P. penetrans* or *P. thornei*: *Nematode inoculum* - The same populations of *P. thornei* and *P. penetrans* were used as in the screening described above. Cysts of *H. avenae* were obtained from soil samples collected from a field in Marchoch, Zaers region, Morocco. Cysts were extracted from soil using the sieving and floatation method, surface sterilized with 0.5% NaOCl for 10 min and rinsed several times in distilled water. The cysts were kept at 4°C and then transferred to 10°C to enhance hatching (Dababat *et al.* 2014). Hatched second-stage juveniles (J2) were used as inoculum. A total of 400 J2 of *H. avenae* were inoculated per tube.

Growth conditions and inoculation procedure - Three wheat lines (L9, L12 and L13) resistant against *P. thornei* and one line (L9) resistant against *P. penetrans* were tested under similar conditions as in the first experiments. Seedlings were placed separately in a conical screening tube (100-mm long × -15mm in diam.) filled with a mixture of sterilized sand, field soil and organic matter (70:29:1 v/v). Two experiments (experiment 3 with *P. thornei*, and experiment 4 with

P. *penetrans*) were conducted at the same time in a growth chamber set at a 16h photoperiod, a temperature of 21°C and 70% relative humidity. The same inoculation method was used as in the screening tests described above. Three wheat lines (L9, L12 and L13) resistant to *P. thornei* were inoculated with 400 P. *thornei* (all stages confounded), or 400 J2 of *H. avenae*, or 400 *P. thornei* + 400 *H. avenae*. Similarly, one line (L9) resistant to *P. penetrans* was inoculated with 400 *P. penetrans* (all stages confounded), or 400 J2 of *H. avenae*, or a mixture of 400 *P. penetrans* + 400 *H. avenae*. A control treatment consisting of the susceptible durum wheat cultivar Ourgh was included and was inoculated with 400 *P. thornei* or 400 *P. penetrans*. Ten replicate tubes and inoculum treatments were arranged in a completely randomized design in tube racks placed above a shallow dish holding water. As the lower tips of tubes were about 2 cm inside the water, plants received water as needed.

Evaluation of resistance - Nine weeks after inoculation, shoots were removed and cysts were extracted from soil on 200-µm sieves by the sieving and floatation method (Shepherd 1986). The soil of each tube was then used to extract the different vermiform stages of *Pratylenchus* and *H. avenae* using an automated zonal centrifuge (Hendrickx 1995). The number of cysts, (cysts on roots and in soil) and vermiform stages of *P. thornei, P. penetrans* or *H. avenae* in the obtained nematode suspensions were counted using a stereomicroscope.

Statistical analysis - Data were analyzed with a one-way analysis of variance (ANOVA) using SPSS software for Windows (SPSS Inc., Illinois, USA). Differences in reproduction of nematodes between wheat accessions were checked with Tukey's test for comparison of means, when the F-value was significant at $P < 0.05$.

RESULTS

Screening for resistance to *P. penetrans* and *P. thornei*: Both species were able to survive and even to increase slightly on most wheat lines. The numbers of vermiform stages of *P. penetrans* and *P. thornei* in the 25 lines of wheat ranged from 240 to 2128 per plant, nine weeks after inoculation (Tables 1 and 2). The final nematode numbers per plant on the susceptible line were 1285 and 1804, for *P. penetrans* and *P. thornei*, respectively. The lines can therefore be ordered from resistant (Rf< 1) to susceptible (Rf>1) over a wide range of values. The lowest average number of nematodes per plant was found in line L9 with 360 *P. penetrans* in soil and root, whereas the highest average number

of nematodes per plant was found in line L24 with 2128 *P. penetrans* (Table 1). The corresponding reproduction factor (Rf) varied from 0.9 to 5.3, for lines L9 and L24 respectively. The total number of nematodes (roots and soil) of L9 was significantly lower than in other lines and this was the only line where fewer specimens of *P. penetrans* were found after nine weeks than the number that was inoculated (Rf = 0.9). The reproduction factor of *P. thornei* on the 25 lines varied from 0.6 (L9) to 5.1 (L8) (Table 2). Three lines (L9, L12 and L13) had a reproduction factor less than 1 and the number of *P. thornei* in roots of L9 (84) as well as in soil (156) were the lowest of all lines.

Interaction between *P. thornei* and *H. avenae*: The total number of *P. thornei* in the 3 lines of wheat tested (L9, L12 and L13) was significantly reduced when *H. avenae* was inoculated with *P. thornei*. The reproduction factor of *P. thornei* in the 3 lines of wheat tested in this experiment declined in the presence of *H. avenae*. When inoculated alone, the numbers *P. thornei* extracted from the three lines of wheat were 143 (L9), 160 (L12) and 303 (L13). This corresponded with reproduction factors of 0.4, 0.4 and 0.8 respectively for L9, L12 and L13. When *P. thornei* and *H. avenae* co-inhabited the root, the number of *P. thornei* extracted from roots was decreased in lines L9 and L12, but not for L13, when compared with *P. thornei* in single inoculation. In mixed inoculations, the total number of *P. thornei* per plant was reduced to 45, 92 and 280 respectively for lines L9, L12 and L13.

Interaction of *P. penetrans* and *H. avenae*: The numbers of *P. penetrans* extracted from soil and roots of line L9 were reduced when *H. avenae* and *P. penetrans* were inoculated simultaneously compared to when *P. penetrans* was inoculated alone. A total of 315 nematodes of *P. penetrans* were found in the soil and roots per plant in the single inoculation compared with 167 nematodes extracted from soil and roots in the concomitant inoculation. Consequently, the reproduction factor of *P. penetrans* was lower in the presence of *H. avenae*: 0.4 instead of 0.8.

DISCUSSION

Use of resistant lines of wheat for the management of *P. penetrans* and *P. thornei* is expected to be a vital management component in the future. There was considerable variation in response against two species of *Pratylenchus* among the different lines of wheat tested. Three lines of wheat, viz. L9 (AUS4930.7/2), L12 (CROC_1/AE. SQUARROSA (224)//OPATA(20615), and L13

Table 1. Number of vermiform *Pratylenchus penetrans* per plant of different wheat lines 12 weeks after nematode inoculation.

Code	Line	Accession[1]	Wheat type[2]	P. penetrans					Reaction[3]
				Root	Soil	Total	Min-Max	Rf (Pf/Pi)	
L1	6R (6D)	30883	SW	261 g	379 k	640±33.7	521-801	1.6	S
L2	FRAME	20591	SW	198 i	522 j	720±71.3	589-840	1.8	S
L3	SILVERSTAR		SW	523 b	997 c	1520±89.5	1287-1677	3.8	S
L4	VP5053	30903	SW	453 c	907 d	1360±68.6	1200-1528	3.4	S
L5	T-2003	20628	SW	437 c	1243 b	1680±35	1548-1796	4.2	S
L6	RAJ 1		SW	503 b	1497 a	2000±32	1891-2126	5	S
L7	ID-2150	20626	SW	209 i	791ef	1000±33.4	893-1130	2.5	S
L8	MILAN	990659	SW	326 e	634 i	960±30.2	885-1087	2.4	S
L9	AUS 4930.7/2 PASTOR	30857	SW	98 k	262 l	360±22.3	269-390	0.9	R
L10	AUS GS50AT34/ SUNCO	30798	SW	201 i	439 j	640±30.5	571-721	1.6	S
L11	VL411R	30898	SW	232 h	608 i	840±51.5	780-892	2.1	S
L12	CROC_1/AE. SQUARROSA (224)	20615	SW	102 k	458 j	560±48.5	519-661	1.4	S
L13	CROC_1/AE. SQUARROSA (224)	20616	SW	283 f	357 k	640±28	590-702	1.6	S
L14	VP1620	30901	SW	204 i	636 i	840±116	791-961	2.1	S
L15	F130L1.12/ATTILA	980872	WW	387 d	773 g	1160±26.8	1002-1326	2.9	S
L16	SONMEZ		WW	178 j	822 e	1000±46.5	927-1056	2.5	S
L17	CPI133859		WW	104 c	416 m	520±35. 3	481-601	1.3	S
L18	CPI133872		WW	204 i	636 i	840± 38.5	791-902	2.1	S
L19	KATE A-1	950590	WW	598 a	1202 b	1800±37.7	1759-1902	4.5	S
L20	PRINS		WW	321 e	439 j	760±29.4	744-802	1.9	S
L21	MIRZABEY2000		WW	309 e	691 h	1000±33.8	956-1122	2.5	S
L22	AU/CO652337// 2CA8-155/ 3/F474S1-1.1	50484	WW	595 a	805 f	1400±25.8	1321-1522	3.5	S
L23	F372		WW	384 d	856 e	1240±31.2	1181-1382	3.1	S
L24	TAIKONG		WW	627 a	1501 a	2128 ±36.7	1998-2320	5.3	S
L25	ZHONGYU		WW	523 b	923 d	1446±41.7	1234-1600	3.6	S
Ls[4]	OURGH			395 d	895 d	1285±29.9	1190-1367	3.2	S

[1]Number assigned by CIMMYT, [2]SW = spring wheat, WW = Winter wheat, [3]R = resistant, S = susceptible, [4]Ls = susceptible control. Means with the same letter in the same column are not significantly different at $P < 0.05$, according to the Tukey test.

Table 2. Numbers of vermiform *Pratylenchus thornei* per plant of different wheat lines 12 weeks after nematode inoculation

Code	Line	Accession[1]	Wheat type[2]	P. thornei					Reaction[3]
				Root	Soil	Total	Min-Max	RF (Pf/Pi)	
L1	6R (6D)	30883	SW	140 k	460 g	600±40	577-620	1.5	S
L2	FRAME	20591	SW	300 g	1220 b	1520±78	1479-1602	3.8	S
L3	SILVERSTAR		SW	390ef	530 f	920±30	878-1001	2.3	S
L4	VP5053	30903	SW	247 h	793 d	1040±41	989-1191	2.6	S
L5	T-2003	20628	SW	366 f	634 e	1000±76	986-1020	2.5	S
L6	RAJ 1		SW	746 a	534 f	1280±56	1202-1425	3.2	S
L7	ID-2150	20626	SW	528 c	432 g	960±54	891-1001	2.4	S
L8	MILAN	990659	SW	610 b	1430 a	2040±44	1901-2199	5.1	S
L9	AUS 4930.7/2 PASTOR	30857	SW	84 l	156 k	240±48	170-393	0.6	R
L10	AUS GS50AT34/ SUNCO	30798	SW	147jk	293 h	440±37	370-563	1.1	S
L11	VL411R	30898	SW	319 g	641 e	960±43	898-1042	2.4	S
L12	CROC_1/AE. SQUARROSA (224)		SW	173 j	107 l	280±27	170-390	0.7	R
L13	CROC_1/AE. SQUARROSA (224)	20616	SW	156jk	204 j	360±36	277-524	0.9	R
L14	VP1620	30901	SW	211 i	789 d	1000±37	941-1062	2.5	S
L15	F130L1.12/AT-TILA	980872	WW	411ed	429 g	840±45	711-989	2.1	S
L16	SONMEZ		WW	433 d	327 h	760±36	536-821	1.9	S
L17	CPI133859		WW	333g	507 f	840±41	790-910	2.1	S
L18	CPI133872		WW	211 i	789 d	1000±35	885-1051	2.5	S
L19	KATE A-1	950590	WW	283 g	237 i	520±36	406-570	1.3	S
L20	PRINS		WW	174 j	306 h	480±40	390-581	1.2	S
L21	MIRZABEY2000		WW	246 h	514 f	760±48	820-663	1.9	S
L22	AU/CO652337// 2CA8-155/3/F474S1-1.1	50484	WW	423 d	897 c	1320±39	1563-1245	3.3	S
L23	F372		WW	321 g	439 g	760±52	811-604	1.9	S
L24	TAIKONG		WW	227ih	293 h	520±65	587-488	1.3	S
L25	ZHONGYU		WW	233 h	760 d	1093±49	937-1123	2.5	S
Ls[4]	OURGH			314 g	1490 a	1804±78	1756-1980	4.7	S

[1]Number assigned by CIMMYT, [2]SW = spring wheat, WW = Winter wheat, [3]R = resistant, S = susceptible, [4]Ls = susceptible control. Means with the same letter in the same column are not significantly different at $P < 0.05$, according to the Tukey test.

(CROC_1/AE.SQUARROSA (224)//OPATA(20616) were rated resistant against *P. thornei*. One of these lines, L9 (AUS4930.7/2), also was found resistant against *P. penetrans*.

Interaction between two nematodes may be harmful to one or both species (antagonistic), have no effect (neutral), or be beneficial to both species (mutualistic). The effects of the nematode populations on each other are generally related to the nature of parasitism; the competition is more severe between species with similar feeding habits (Eisenback 1985). The numbers of *P. thornei* and *P. penetrans* were greater from the single species inoculations than from the inoculations mixed with *H. avenae* in two of the three wheat lines tested (L9 and L12). It is likely that over-crowding and clustering of infective units occurred during penetration at the penetration zone which allowed for less penetration. This reduced penetration influenced the rate of population increase. The mutual inhibitory effects could also be due to competition for feeding sites. Why no such antagonistic effect was observed in L13 is not clear.

Competition in varying degrees between two or more different nematodes species has been demonstrated by others. Estores and Chen (1972) reported that *P. penetrans* and *M. incognita* depressed the population of each other in tomato. Gay and Bird (1973) found that the population of *M. incognita* was inhibited by the presence of *P. brachyurus* on cotton. Brinkman *et al.* (2005) reported that *P. penetrans* suppressed the abundance of *H. arenaria* on natural dune grass.

The main result in this study is that the three lines of wheat (L9, L12 and L13) resistant to *P. thornei* and one line (L9) resistant to *P. penetrans* kept their resistance even in a mixed inoculation with *H. avenae*. In mixed inoculation, *P. thornei* and *H. avenae*, as well as *P. penetrans* and *H. avenae*, were mutually antagonistic in two lines of wheat (L9 and L12). Therefore, this study demonstrates that the sedentary endoparasite *H. avenae* was a competitor to both *P. thornei* and *P. penetrans*. Several studies in agricultural systems have shown that *Pratylenchus* spp. inhibit *Heterodera* spp. and *Meloidogyne* pp. (Eisenback 1993, Lasserre *et al.* 1994), whereas the reverse has been reported depending on host suitability (Eisenback 1993). As both of these nematode species were found in the same wheat fields (Mokrini *et al.* 2012b), our results may indicate competition between naturally co-evolved nematodes species. However, the field performance of these lines against root lesion nematode attacks, alone or together with *H. avenae*, should be evaluated before they are released to the farmers.

ACKNOWLEDGMENTS

Funding was from the Islamic Development Bank, Ghent University. Germplasm of wheat was provided by CIMMYT. Nematode diagnostics were conducted by ILVO laboratory. Technical assistance was provided by Nancy de Sutter. We thank Rachid Tahzima, Soukaina Janati, and Khalid Azim for reviewing this paper.

REFERENCES

- Brinkman EP, Duyts H, Van der Putten WH (2005) Competition between endoparasitic nematodes and effect on biomass of *Ammophilaarenaria* (marram grass) as affected by timing of inoculation and plant age. *Nematology*, 7, 169-178.
- Dababat AA, Pariyar S, Nicol J, Duveiller E (2011) Cereal cyst nematode: an unnoticed threat to global cereal production. CGIAR SP-IPM Technical Innovation Brief 11.
- Eisenback JD (1985) Interactions among concominant populations of nematodes. In: "An Advanced Treatise on Meloiodogyne.' (Eds. JN Sasser, CC Carter) pp. 193-253. Department of Plant Pathology. North Carolina State University, Raleigh, North Carolina, USA, and United States Agency for International Development.
- Eisenback JD (1993) Interactions between nematodes in cohabitance. In: 'Nematode Interactions.' (Ed. AW Khan) pp.134-174. (Chapman and Hall, New York).
- Estores RA, Chen TA (1972) Interactions of *Pratylenchus penetrans* and *Meloidogyne incognita* as cohabitants in tomato. *Journal of Nematology*, 4, 170-174.
- Gay CM, Bird GW (1973) Influence of con-comitant *Pratylenchus brachyrus* and *Meloidogyne* spp. on root penetration and population dynamics. *Journal of Nematology*, 5, 212-217.
- Hendrickx G (1995) An automated apparatus for extracting free-living nematode stages from soil. Nematologica, 41, 308.
- Lasserre F, Rivoal R, Cook R (1994) Interaction between *Heterodera avenae* and *Pratylenchus neglectus* on wheat. *Journal of Nematology*, 26, 336-334.
- McDonald AH, Nicol JM (2005) Nematode parasites of cereals. In: 'Plant Parasitic Nematodes in Subtropical and Tropical Agriculture. (Eds. M Luc, RA Sikora, J Bridge) pp. 131-191. (CABI Publishing, Wallingford, UK).

- Melakeberhan H, Dey J (2003) Competition between *Heterodera glycines* and *Meloidogyne incognita* or *Pratylenchus penetrans*: independent infection rate measurements. *Journal of Nematology*, 35, 1-6.
- Meskine M, Andalousi FA (1984) Importance et répartition des nématodes phytoparasites associés au cultures de blé et orge au Maroc. *Journées Nationales de Phytiatrie*, Rabat, pp. 28-30
- Mokrini F, Abbad Andaloussi F, Alaoui Y, Trocoli A (2009) Importance and distribution of the main cereal nematodes in Morocco. In: 'Cereal Cyst Nematodes: Status, Research and Outlook.' (Eds. IT Riley, JM Nicol, AA Dababat) pp. 40-50. (CIMMYT, Ankara, Turkey).
- Mokrini F, Waeyenberge L, Viaene N, Moens M (2012b) Occurrence of nematodes of the *Heterodera avenae* group and *Pratylenchus* spp. on wheat and barley in Morocco. 31st International Symposium of the European Society of Nematologists, 23-27 September 2012, Adana, Turkey.
- Moody EH, Lownsbery BF, Ahmed JH (1973) Culture of root lesion nematode *Pratylenchus vulnus* on carrot discs. *Journal of Nematology*, 5, 255-226.
- Rammah A (1994) Cereal cyst nematode (*Heterodera avenae*) in Morocco. Fifth Arab Congress of Plant Protection, Morocco p: 199.
- Rivoal R, Cook R (1993) Nematode pests of cereal. In: 'Plant Parasitic Nematodes in Temperate Agriculture.' (Eds. K Evans, DL Trudgill, JM Webster) pp. 259-303. (CABI Publishing, Wallingford, UK).
- Rivoal R, Laserre F, Cook R (1995) Consequences of long term cropping with resistant cultivars on the population dynamics of the endoparasitic nematodes *Heterodera avenae* and *Pratylenchus neglectus* in a cereal production ecosystem. *Nematologica*, 41, 516-529.
- Sahin E, Nicol J, Elekçioğlu IH, Yorgancilar O, Yildimir AF, Tulek A, Hekimhan H, Yorgancilar A, Kilnc AT, Bolat N, Erginbas–Orakci G (2009) Frequency and diversity of cereal nematodes on the Central Anatolian Plateau of Turkey. In: 'Cereal Cyst Nematodes: Status, Research and Outlook.' (Eds. IT Riley, JM Nicol, AA Dababat) pp. 100-105. (CIMMYT, Ankara, Turkey).
- Smiley RW, Nicol JN (2009) Nematodes which challenge global wheat production. In: 'Wheat Science and Trade.' (Ed. BF Carver) pp. 171-187. (Wiley-Blackwell, Ames, Iowa, USA).
- Thompson JP, Seymour NP (2011) Inheritance of resistance to root-lesion nematodes (*P. thornei*) in wheat landraces and cultivars from the West Asia and North Africa. *Crop and Pasture Science*, 62, 82-93.
- Thompson JP, Brennan PS, Clewett TG, Sheedy JG, Seymour NP (1999) Progress in breeding wheat for tolerance and resistance to root-lesion nematode (*Pratylenchus thornei*). *Australasian Plant Pathology*, 28, 45–52.

- Toktay H, Yavuzaslanoğlu E, Imren M, Nicol JM, Elekçioğlu IH, Dababat AA (2012) Screening for resistance to *Heterodera filipjevi* (Madzhidov) Stelter (Tylenchina: Heteroderidae) and *Pratylenchus thornei* (Sher & Allen) (Tylenchida: Pratylenchidae) sister lines of spring wheat. *Turkish Journal of Entomology*, 36, 455–461.
- Tsai B (2008) Effect of temperature on the survival of *Meloidogyne incognita*. *Plant Pathology Bulletin*, 17, 203-208.
- Vanstone VA, Rathjen AJ, Ware AH, Wheeler RD (1998) Relationship between root lesion nematodes (*Pratylenchus neglectus* and *P. thornei*) and performance of wheat cultivars. *Australian Journal of Experimental Agriculture*, 38, 181–188.
- Yang H, Powell NT, Barker KR (1976) Interactions of concomitant species of nematodes and *Fusarium oxysporum f. sp. vasinfectum* on cotton. *Journal of Nematol*ogy, 8, 74-80.

ANALYSIS OF WHEAT RESISTANCE TO CEREAL CYST NEMATODE *HETERODERA FILIPJEVI* THROUGH ASSOCIATION MAPPING

SHREE R. PARIYAR[1], ABDELFATTAH A. DABABAT[2], GUL ERGINBAŞ-ORAKCI[2], SHAHID SIDDIQUE[1], ABDELNASER ELASHRY[1], WIEBKE SANNEMANN[3], ALEXEI MORGOUNOV[2], JENS LEON[3] AND FLORIAN M.W. GRUNDLER[1,*]

[1]Institute of Crop Science and Resource Conservation (INRES), Molecular Phytomedicine, Karlrobert Kreiten Straße 13, D-53115 Bonn, Germany; [2]International Maize and Wheat Improvement Centre (CIMMYT), P.K. 39 06511, Emek, Ankara, Turkey; [3]Institute of Crop Science and Resource Conservation (INRES), Plant Breeding, Katzenburgweg 5, D-53115 Bonn. *Corresponding author email: grundler@uni-bonn.de

SUMMARY

Association mapping identifies quantitative trait loci (QTLs) by examining marker-trait associations based on linkage disequilibrium (LD). To identify loci conferring resistance to the cereal cyst nematode (CCN) *Heterodera filipjevi*, 161 diversified modern winter wheat populations were genotyped using 90K Illumina Infinium Single Nucleotide Polymorphism (SNP) array analyzed through a genome-wide association study. To understand the genetic pattern underlying resistance loci, genetic diversity, population structure and LD, 23364 polymorphic SNPs were analyzed using a mixed linear model MLM (Q+K). Eleven novel QTLs were identified on chromosomes 1A, 2A, 2B, 3A, 3B, 4A, 5B, and 7B at a false discovery rate FDR ($P \leq 0.05$). Two genetic subpopulations were identified by structure analysis. The phenotypic diversity

* Pariyar SR, Dababat AA, Erginbaş-Orakçi G, Siddique S, Elashry A, Sannemann W, Morgounov A, Leon J, Grundler FMW. (2015) Analysis of wheat resistance to cereal cyst nematode *Heterodera filipjevi* through association mapping. In 'Nematodes of Small Grain Cereals: current status and research.' (AA Dababat, H Muminjanov and RW Smiley) pp. 205-220. (FAO: Ankara, Turkey).

of population was accounted 6.16% by principle component analysis (PCA). LD decay across wheat genome was in a range of 3cM ($r^2 > 0.2$). Significant markers explained 43% of total phenotypic variation to nematode resistance. Further analysis identified several SNP markers co-localized with genes for plant disease resistance. The SNP marker wnsp_BE426418A_Ta_2_1 (3A) is linked to a gene coding for NADPH-Quinone oxidoreductase subunit 0 suggested to be involved in NADPH oxidation-reduction process. Tdurum_Contig10380_87 (2B) is linked to an amino acid transporter (AAT) gene supposed to be involved in supplying amino acids to sink tissues of plants and nematode-induced feeding structures. RAC875_c13116_ 943 (2A) is linked to a RING/FYVE/PHD-type gene and BE443588A_Ta_2_1 (1A) is linked to DNA methyl transferase 1-associated protein 1 (DMAP1) genes involved in cell death and disease resistance. The putative novel loci presented in this study can be selected and pyramided in wheat cultivars to develop broad-spectrum resistance via marker-assisted selection.

Keywords: *Triticum aestivum*, linkage disequilibrium, genotypes, population structure, single nucleotide polymorphism

INTRODUCTION

Wheat *(Triticum aestivum)* is a major staple food in the world. Cereal cyst nematodes (CCN) are most important group of plant-parasitic nematodes attacking temperate cereals, including wheat and barley. CCN are a group of several closely-related nematode species reported to cause severe yield losses (Nicol and Rivoal 2007). *Heterodera filipjevi* and *H. avenae* are widely acknowledged as economically important nematodes in rainfed wheat production (Rivoal and Cook 1993). Wheat resistance to CCN includes *Aegilops tauschii* (Coss.) (syn. *T. tauschii* (Coss.) Schmal; syn. *Ae. squarrosa* auct non L.) (Loureiro *et al.* 2009, van Slageren 1994) and *T. turgidum*. Resistance sources and their gene designation against *H. avenae* were reviewed (McDonald and Nicol 2005, Nicol and Rivoal 2007, Rivoal *et al.* 2001). *H. avenae* resistance was first reported in barley (Nilsox-Elhe, 1920) and described later in bread wheat (Holm Nielsen, 1966). Nine single dominant genes known as "*Cre*" have been found in wild wheat relatives and successfully used to control *H. avenae* in Australia, France, India and Sweden (Riley *et al.* 2009). Six of the *"Cre"* genes in wheat (*Cre2* to *Cre7*) were derived from *Aegilops* species (Jahier *et al.* 2001) while *Cre1* and *Cre8* were inherited from *T. aestivum* (Bekal *et al.* 1998; Paull *et al.* 1998) and *CreR* was reported from *Secale cereal* (Taylor *et al.* 1998). The "*Cre*" gene features single-gene inheritance between the host genes and corresponding virulence genes in nematodes.

Mapping traits help to understand genetic background and to provide a baseline for gene cloning. Association mapping (AM) accounts historic linkage disequilibrium (LD) and links the phenotype to the genotype. Large populations are phenotyped, genotyped, and subsequently the alleles at loci contributing to trait are identified (Flint-Garcia *et al.* 2003, Rafalski 2002). AM has been used to identify QTL and characterize candidate genes in several crops, including rice (Agrama *et al.* 2007, Wang *et al.* 2001, Zou *et al.* 2000), maize (Kump *et al.* 2011), barley (Cockram *et al.* 2010, Massman *et al.* 2011) and wheat (Mulki *et al.* 2013, Neumann *et al.* 2011, Tommasini *et al.* 2007, William *et al.* 2003). It has been used to identify the QTLs associated to *Stagonospora nodorum* blotch resistance in modern European winter wheat varieties (Tommasini *et al.* 2007, Yao *et al.* 2009), and *H. avenae* and *Pratylenchus neglectus* in synthetic hexaploid wheat SHW (Mulki *et al.* 2013). Wheat landraces and domesticated genotypes possess genetic variation which has shown resistance to biotic and abiotic stresses (Kimber and Feldman 1987). Single nucleotide polymorphism (SNP) markers have become feasible to identify putative QTL associated with disease (Akhunov *et al.* 2009) and have been used extensively to uncover multiple targets in wheat (Wang *et al.* 2014). To discover new allelic diversity and loci underlying resistance to the CCN *H. filipjevi*, we analyzed 161 diverse wheat accessions from the International Winter Wheat Improvement Program (IWWIP) using a 90K SNP wheat chip and analysis by genome-wide association studies (GWAS).

MATERIALS AND METHODS

Wheat genotypes and resistance assay: To identify QTLs associated with CCN resistance, two independent experiments were conducted in a controlled growth room and use of a mapping panel of 161 modern winter wheat genotypes; 101 breeding lines, 58 cultivars and 2 landraces. These accessions were selected based on geographical distribution and diverse genetic background developed by IWWIP (www.iwwwip.org/nursery/Deatail/18-19). Host status of genotypes were categorized into five groups based on mean number of females and/or cysts present per plant and comparing with the highly susceptible cv. Bezostaya (Dababat *et al.* , 2015).

Genotyping with 90K SNP markers: Genomic DNA was isolated from seven day old leaf tissue of 161 wheat accessions using the cetyl trimethyl ammonium bromide (CTAB) method (Sharp *et al.* 1988). The quality of DNA was evaluated on a 0.8% agarose gel and normalized to 50ng/µl. A 2µl aliquot of

DNA from each sample was used to genotype in 90K Illumina Iselect Wheat Bead Chip, TraitGenetics GmbH, Gatersleben, Germany.

Linkage disequilibrium and population structure: Pair-wise measures of LD analyses uses the squared correlation coefficient (r^2) between two loci and summarizes both mutational and recombination history. The extent of LD across the wheat genome was estimated r^2 using SAS 9.2 (Cavanagh *et al.* 2013) with 11680 polymorphic SNP markers. Genetic sub-populations were analyzed using a model-based Bayesian clustering method implemented software STRUCTURE 2.3.4 with 961 polymorphic markers. These 961 markers were selected based on 5cM grid distances of a total of 11680 markers. Principal component analysis (PCA) was analyzed by TASSEL v.3.0 (http://www.maizegenetics.net) with 22364 polymorphic SNP markers used as a covariance matrix and were used for GWAS (Bradbury *et al.* 2007).

Marker-trait associations: To identify significant QTLs conferring resistance to *H. filipjevi*, the phenotypic variation (R^2) was calculated with a simple regression equation implemented in a multiple QTL model in SAS 9.2 using Proc Mixed, corrected for both population structure and familial relatedness MLM (Q+K) (Bauer *et al.* 2009). Association between SNPs and nematode resistance was considered significant if the p-value was ≤0.01 (Malosetti *et al.* 2007). The flanking sequences of the significant SNP markers were blasted against gene models of *Brachypodium distachyon, Oryza sativa*, and *Sorghum bicolor* available in NCBI. The search was limited to the top hit with an E-value cut off of 1E-10. The possible genes/protein based on significant hit and putative functions were further analyzed. In addition, the putative marker genes were annotated in the Arabidopsis Information Resource (https://www.arabidopsis.org) and predicted functions of protein/gene homologs were described.

RESULTS

Resistance assay with *H. filipjevi*: To identify host response of wheat genotypes to *H. filipjevi*, a collection of 161diversified winter wheat populations were screened. The number of females and cysts per genotype were counted and categorized into five ranks from "resistant" to "highly susceptible" based on host status. The results showed that 2% of 161wheat genotypes were resistant, 28% were moderately resistant, 27% were moderately susceptible, 35% were susceptible, and 25% were highly susceptible.

Density of polymorphic SNP markers differs between the A, B and D genomes: To identify the polymorphic markers for GWAS analysis, we genotyped 161 screened wheat accessions with the 90K Illumina Iselect Wheat Bead Chip. A total of 66634 SNPs call were recorded. All of the monomorphic SNPs, missing data, heterozygous data and markers with minor allele frequency less than 5% were culled. A total of 23364 polymorphic SNPs markers were obtained, of which 11680 (49.99%) with known chromosomal location were used for LD. Among the latter, 961 polymorphic SNP markers, based on 5cM grid distances, were analyzed for population structure. To construct a genetic map, 11680 SNP markers were used with 161 genotypes across 21 chromosomes and a total 2956.5cM genome size was measured. An uneven distribution of mapped markers across the wheat genome was revealed. High marker densities were located on genome A (38.43%) and B (51.71%) while relatively low marker density occurred on genome D (9.85%). On average, one marker was mapped for each 0.25cM on the chromosome.

Population structure and kinship: To infer the population structure in 161 wheat genotypes, 961 polymorphic SNP markers were analyzed using Bayesian clustering model in STRUCTURE 2.3.4. The ad hoc quantity based on the second order rate of change in the log probability (ΔK) showed a clear peak at K= 2 (Figure 1a), indicated two genetic sub-populations. The logarithm of the data likelihood (Ln P(D)) on average continued to increase with increasing numbers of assumed sub-populations (K) from 2 to 20 with the exception of a depression at K10, K13 and K16 (Figure 1b). The first group consisted of 89 wheat accessions mostly originating from South Africa (5), Iran (2) and the USA (1), whereas the second group was composed of 72 wheat genotypes mainly originating from Russia (8), Bulgaria (2), Moldova (2), and Turkey-CIMMYT ICARDA (3). The average distance between sub-populations ranged between 0.011 and 0.155 while mean *Fst* value was 0.0341 indicating very small amount of phenotypic variation due to population stratification. In addition, PCA was used to visualize the genotype data (Figure 2). The first, second and third principal components explained 6.19%, 1.0% and 1.35% of the variation, respectively. The first PC did not exhibit strong genetic variation within the population. No significant PCs could be identified.

Linkage disequilibrium: The analysis of LD decay with 11680 markers showed a strong and significant LD is observed at a very short distance <3cM. The genome-wide LD with r^2 values plotted against the genetic distance in cM.

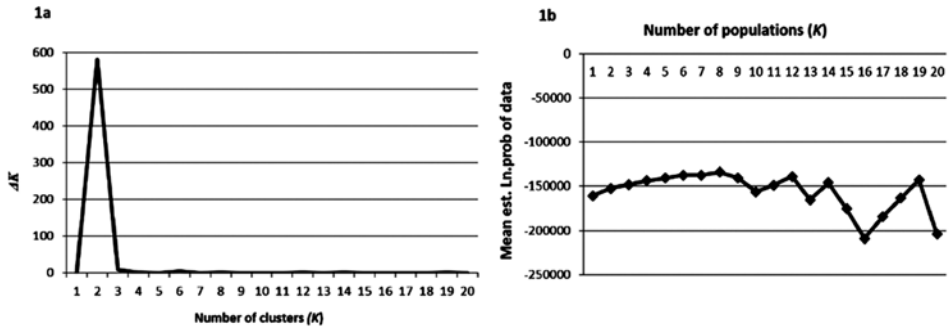


Figure 1. Estimation of number of sub-populations (K) in winter wheat based on 961 SNP markers: a) Estimate of numbers of sub-populations (K) in winter wheat using ΔK values, b) The log probability of data LnP(D) as a function of K for 961 SNP markers, means for each value of K was calculated from 20 independent runs of structure analysis.

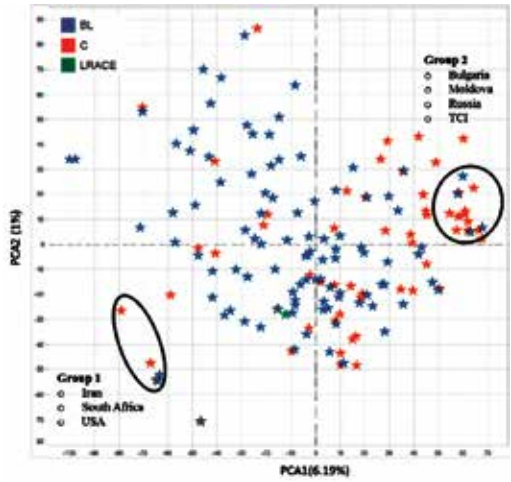


Figure 2. Principal components analysis based on correlation matrix recorded on 161 winter wheat genotypes; BL = breeding line, C = cultivar, LRACE = landraces.

Beyond 3cM, LD became constant at a value of r^2 =0.1 which allowed a precise genetic analysis. Low intra-chromosomal linkage along the wheat chromosomes are calculated for the mean of all chromosomes.

Marker-trait associations: To identify QTL linked to *H. filipevi* resistance were determined by MLM analysis using kinship relationship (K matrix) and population structure (Q matrix) as covariate adjusted FDR at P<0.01. Eleven SNP markers were significantly linked to *H. filipjevi* resistance and were detected on chromosomes 1A, 2A, 3A, 4A, 2B, 3B, 5B and 7B. The phenotypic variation (R^2) of individual SNPs ranged from 6.9 to 12.1% (Table 1). The total phenotypic variation explained by all significant markers was record-

ed as 43.5%. Among eleven SNPs, five markers wsnp_BE443588A_Ta_2_1 (1A, 14.6cM), RAC875_c13116_943 (2A, 64.3cM), Excalibur_c18966_804 (2B, 113.6cM), wsnp_BE426418A_Ta_2_1 (3A, 20cM), Bobwhite_rep_c66630_331 (7B, 79.1cM) were associated with nematode resistance, while six SNP markers Tdurum_contig10380_87 (2B, 74.9cM), Tdurum_contig1208_803 (3B, 74.9cM), Excalibur_c20277_483 (3B, 6.14cM), wsnp_Ex_c55245_57821389 (4A, 53.3cM), Tdurum_contig82236_117 (4A, 155.5cM), Excalibur_c78724_434 (5B, 158.7cM) were associated to support nematode susceptibility in presence of favorable alleles. Two significant markers Tdurum_contig12008_803 and Excalibur_c20277_483, positioned at same position (3B, 6.14cM), were associated to an increase in nematode susceptibility. Highest phenotypic variation was recorded by Tdurum_contig10380_87 (2B, 74.9cM) while lowest was recorded in Excalibur_c18966_804 (2B, 113.6 cM). The density of polymorphic SNP markers among A, B, and D genomes were highly varied. The recombination rate differed in wheat genomic regions. The significant SNP markers were detected only in A and B genome, which could be the reason for low density of polymorphic SNPs located in D genome.

Functional annotation of SNP markers associated with CCN resistance: To analyze the putative biological functions of genes associated to CCN resistance,

Table 1. Marker trait association analysis for H. filipjevi resistance in winter wheat using MLM (Q+K)

SN	Marker	CHR	Pos	Genetic interval	P-LOD	P- FDR	P-value	Allele	Allelic effect	R^2 (%)
1	wsnp_BE443588A_Ta_2_1	1AL	14.6	10.4-15.4	4.24	0.0006	0.00092	A/C	-4.3	10.3
2	RAC875_c13116_943	2AS	64.3	64.3	3.35	0.0016	0.00045	A/C	-3.04	8.5
3	Excalibur_c18966_804	2BL	113.6	113.6	2.91	0.0016	0.00122	G/A	-2.8	6.9
4	wsnp_BE426418A_Ta_2_1	3AL	20	20-26.4	3.01	0.0016	0.00096	T/C	-4.36	8
5	Bobwhite_rep_c66630_331	7BL	79.1	68.6-79.1	2.81	0.0016	0.00154	T/C	-3.31	7.5
6	Tdurum_contig10380_87	2BL	74.9	74.9	4.26	0.0006	0.00006	G/A	4.36	12.1
7	Tdurum_contig12008_803	3BL	6.14	0-6.14	3.61	0.0002	0.00025	T/C	3.11	8.9
8	Excalibur_c20277_483	3BL	6.14	0-6.15	3.64	0.0016	0.00100	G/A	3.4	7.4
9	wsnp_Ex_c55245_57821389	4AS	53.3	53.3	2.98	0.0016	0.00104	T/C	2.77	7.5
10	Tdurum_contig82236_117	4AL	155.5	155.5	2.82	0.0016	0.00152	G/A	3.35	7.6
11	Excalibur_c78724_434	5BL	158.7	158.7	3.06	0.0016	0.00087	G/A	3.65	8.1

an in-silico functional annotation of significant SNP markers were performed. The flanking sequence of significant SNP was blasted against DNA and protein sequences of rice, sorghum, *Brachypodium* and Arabidopsis. Further analysis identified the intra-chromosomal location of many polymorphic SNPs co-localized with genes/QTLs supposed to be involved in plant defense while others were detected in the regions where genes have not yet described. Here we present, the SNPs with biological functions that were previously described, validated or involved to plant defense and disease resistance. However, loci with unknown gene function could provide an opportunity to localize new resistant/susceptible genes. Six different SNP markers on chromosome 1AL, 2AS, 2BL, 3AL and 4AL were linked to the genes supposed to be involved in biotic stress and plant disease resistance. The annotated biological function of closely related SNP markers wsnp_BE426418A_Ta_2_1 and wsnp_BE426418A_Ta_2_2 within same haplotype block with high LD on chromosome 3AL (20 cM and 26.4 cm) were similar. These markers were supposed to code NAD(P)H-Quinone oxidoreductase subunit O are suggested to be involved in NADPH oxidation-reduction process. The SNP marker Excalibur_c18966_804 on chromosome 2BL was also suggested to code proteins involved in NADPH oxidation-reduction process and oxidoreductase activity. The SNP marker RAC875_c13116_943 on chromosome 2AS linked to Zinc finger, a RING/FYVE/PHD-type binding domains supposed to regulate a superoxide-dependent signal and involved in cell death and disease resistance. The SNP marker wsnp_BE443588A_Ta_2_1 on chromosome 1AL linked to DNA methyltransferase 1-associated protein 1 (DMAP1) associated with myb-like transcription factor family protein is supposed to be involved in regulation of very-long-chain fatty acid biosynthesis and activation of hypersensitive cell death response. The SNP marker Tdurum_contig10380_87 on chromosome 2BL linked to amino acid transporter (AAT) genes is supposed to be involved in supplying amino acids to sink tissues of plants and nematode-induced feeding sites. AATs are also reported to be involved in transportation of amino acids across cellular membranes in higher plants and to have an indispensable role in various processes of plant growth and development including responses to pathogens. The SNP marker wsnp_Ex_c55245_57821389 on chromosome 4AS linked to retinoblastoma-binding-protein 5-like/WD40-repeat-containing domain family protein, and the SNP marker Tdurum_contig12008_803 and Excalibur_c78724_434 on chromosome 3BL, are supposed to be involved in biotic and abiotic stress and plant growth.

DISCUSSION

CCN resistance in wheat by marker-trait association: Limited sources of broad-spectrum wheat resistance to cereal nematodes are available. To overcome this limitation, a tremendous effort has been made identify and introduce new CCN resistance in cultivated and wild wheat relatives such as *Ae. tauschii* and *T. turgidum* (Kimber and Feldman 1987). Identification of CCN resistance has been shifted recently towards synthetic hexaploid wheat (SHW, 2n = 6x = 42, AABBDD) and it has become feasible to test for thousands of SNP markers (Mulki *et al.* 2013). The high-throughput and high-density map SNP genotyping enabled GWAS to identify putative QTLs associated with disease resistance (Cavanagh *et al.* 2013). We identified 11 significant markers linked to loci conferring resistance to CCN. Four SNP markers found in this study were located in the same chromosomal regions where a gene/QTL has been previously detected such as a QTL on 5B (Mulki *et al.* 2013), *Cre5*/*CreX* in 2A (Jahier *et al.* 2001), (Barloy *et al.* 2007), *Cre1* on 2BL (Bekal *et al.* 1998) and *CreY* on 3B (Barloy *et al.* 2007). Resistance QTLs on chromosomes 1A and 2A have also identified previously (Singh *et al.* 2010). Six of the significant QTLs found in this study associated with the genes suggested to be involved in host defense to several important diseases and stress management. Significant QTLs located in different chromosomes indicated wheat genotypes potentially posses different novel resistance alleles and could be used as parents to pyramid the resistant loci through marker-assisted backcrossing. This could help to develop resistant wheat genotypes carrying resistance alleles at different loci. The additive effects of combining resistance QTLs on 1B and 6B against *H. avenae* in Trident/Molineux DH wheat population has been reported (Williams *et al.* 2006). Further, the moderate heritability H^2 (46%) in this study suggested nematode resistant alleles could successfully be transmitted to the offspring. The substantial part of the observed variation could be attributed to genetic variation and can be helpful in selecting suitable genotypes.

To reduce the false positives in marker-trait associations, MLM was used to correct false positive generated by population structure (Pritchard *et al.* 2000). We analyzed population structure (Q matrix) as a fixed effect and differences in genetic relatedness among wheat genotypes within subpopulations (Kinship or K matrix) as random effects to control of false positives (Yu and Buckler 2006). While it is not possible to make direct comparisons, similar results with two sub-populations were reported in 81 diversified *Ae. tauschii* accessions

(Sohail *et al.* 2012) and 96 wheat accessions by using DArT markers (Neumann *et al.* 2011). Additionally, PCA was analyzed to determine the variability within the population. PC analysis did not show any subgroups between the populations with low first and second principal component which explained 6.19% and 1%, respectively. The results showed that variability within the population was very low and needed no further correction.

The analysis of LD in 161 wheat populations recorded about <3cM, a smaller distance than previously reported 5cM in wheat (Crossa *et al.* 2007). The high LD observed in this study supports results for self-pollinated plants and could explain by the different levels of historical recombination or effective recombination rate and recombination distance between the loci. LD decay for 157 landraces (<5cM) and a higher LD decay (5–10cM) in 93 modern Chinese bread wheats were reported (Hao *et al.* 2011). Similarly, LD decay <5cM in 189 Canadian bread wheat accessions was reported (Belzile *et al.* 2007). Dense coverage of markers provides detailed insight of LD decay between two loci in closer proximity and will help in identifying the regions influenced by a short, intense breeding history (Benson *et al.* 2012). The high density LD found in the A and B genome could be explained by different levels of historical recombination in the populations. LD blocks detected in QTL regions on A and B genomes might have resulted from favorable selection of phenotypes during the breeding process by IWWIP.

Implications in wheat nematode resistance breeding: The wheat genome possesses significant complexity, and wild and cultivated wheat genotypes exhibit genetic variation for resistance to CCN (Kimber and Feldman 1987). The recent advancement in genome sequencing and genotyping technology helped to exploit natural diversity (Akhunov *et al.* 2009, Allen *et al.* 2011, Cavanagh *et al.* 2013, Somers *et al.* 2003). The 9K SNP markers were used to identify QTL linked to resistance to *Fusarium* head blight, leaf rust, stripe rust and powdery mildew (Bernardo *et al.* 2012, Buerstmayr *et al.* 2009, Gurung *et al.* 2014, Lagudah *et al.* 2009, Würschum *et al.* 2013). In our study, five QTLs linked to CCN resistance suggested multiple putative resistance alleles which can be useful in pyramiding resistance genes through MAS breeding. Combining several putative resistant QTLs and an avoidance of susceptible loci is the other option to control CCN. Four wheat genotypes (Olifants, Lantian 12, T04/17, and Bezenchuskaya 380) with the highest resistant marker allele frequencies showed a significant nematode reduction. These genotypes can be used as parents in CCN resistance breeding, and the novel QTLs/genes can be

introgressed into cultivated wheat lines. However, validation of these QTLs by using bi-parental populations or near-isogenic lines (NILs) and their efficiency test in multi-location field trails are essential. Here we present an opportunity, with high precision, for mining novel resistant alleles/genes from exotic sources to facilitate a more efficient utilization of molecular markers in MAS breeding through association mapping.

ACKNOWLEDGMENTS

The authors would like to thank IWWIP for the wheat genotypes and Ministry of Food, Agriculture and Livestock, Republic of Turkey, and the Transitional Zone Agriculture Research Institute-Eskisehir for technical support. Special thanks to Syngenta Crop Protection AG, Basel, Switzerland for financial support for genotyping. We would also like to thank Traitgenetics, Gatersleben, Germany for genotyping.

REFERENCES

- Agrama H, Eizenga G, Yan W (2007) Association mapping of yield and its components in rice cultivars. *Molecular Breeding*, 19, 341-356.
- Akhunov E, Nicolet C, Dvorak J (2009) Single nucleotide polymorphism genotyping in polyploid wheat with the Illumina GoldenGate assay. *Theoretical and Applied Genetics*, 119, 507-517.
- Allen AM, Barker GL, Berry ST, Coghill JA, Gwilliam R, Kirby S, Robinson, P, Brenchley R C, D'Amore R, McKenzie N (2011) Transcript-specific, single-nucleotide polymorphism discovery and linkage analysis in hexaploid bread wheat (*Triticum aestivum* L.). *Plant Biotechnology Journal*, 9, 1086-1099.
- Barloy D, Lemoine J, Abelard P, Tanguy A, Rivoal R, Jahier J (2007) Marker-assisted pyramiding of two cereal cyst nematode resistance genes from *Aegilops variabilis* in wheat. *Molecular Breeding*, 20, 31-40.
- Bauer AM, Hoti F, von Korff M, Pillen K, Léon J, Sillanpää M (2009) Advanced backcross-QTL analysis in spring barley (*H. vulgare* ssp. *spontaneum*) comparing a REML versus a Bayesian model in multi-environmental field trials. *Theoretical and Applied Genetics*, 119, 105-123.
- Bekal S, Jahier J, Rivoal R (1998) Host responses of different Triticeae to species of the cereal cyst nematode complex in relation to breeding resistant durum wheat. *Fundamental and Applied Nematology*, 21, 359-370.

- Belzile F, Somers DJ, Banks T, DePauw R, Fox S, Clarke J, Pozniak C, McCartney C (2007) Genome-wide linkage disequilibrium analysis in bread wheat and durum wheat. *Genome*, 50, 557-567.
- Benson J, Brown-Guedira G, Paul Murphy J, Sneller C (2012) Population structure, linkage disequilibrium, and genetic diversity in soft winter wheat enriched for fusarium head blight resistance. *The Plant Genome*, 5, 71-80.
- Bernardo,AN, Ma H, Zhang D, Bai G (2012) Single nucleotide polymorphism in wheat chromosome region harboring Fhb1 for Fusarium head blight resistance. *Molecular Breeding*, 29, 477-488.
- Bradbury PJ, Zhang Z, Kroon DE, Casstevens TM, Ramdoss Y, Buckler ES (2007) TASSEL: software for association mapping of complex traits in diverse samples. *Bioinformatics*, 23, 2633-2635.
- Buerstmayr H, Ban T, Anderson JA (2009) QTL mapping and marker-assisted selection for Fusarium head blight resistance in wheat: a review. *Plant breeding*, 128, 1-26.
- Cavanagh CR, Chao S, Wang S, Huang BE, Stephen S, Kiani S, Forrest K, Saintenac C, Brown-Guedira GL, Akhunova A (2013) Genome-wide comparative diversity uncovers multiple targets of selection for improvement in hexaploid wheat landraces and cultivars. *Proceedings of the National Academy of Sciences*, 110, 8057-8062.
- Cockram J, White J, Zuluaga DL, Smith D, Comadran J, Macaulay M, Luo Z, Kearsey MJ, Werner P, Harrap D (2010) Genome-wide association mapping to candidate polymorphism resolution in the unsequenced barley genome. *Proceedings of the National Academy of Sciences*, 107, 21611-21616.
- Crossa J, Burgueno J, Dreisigacker S, Vargas M, Herrera-Foessel SA, Lillemo M, Singh RP, Trethowan R, Warburton M, Franco J (2007) Association analysis of historical bread wheat germplasm using additive genetic covariance of relatives and population structure. *Genetics*, 177, 1889-1913.
- Dababat AA, Imren M, Erginbas-Orakci G, Ashrafi S, Yavuzaslanoglu E, Toktay H, Pariyar SR, Elekcioglu H, Morgounov A, Mekete M (2015) The importance and management strategies of cereal cyst nematodes, *Heterodera* spp., in Turkey. *Euphytica*, 202, 173-188.
- Flint-Garcia SA, Thornsberry JM, Buckler ES (2003) Structure of Linkage Disequilibrium in Plants. *Annual Review of Plant Biology*, 54, 357-374.
- Gurung S, Mamidi S, Bonman JM, Xiong M, Brown-Guedira G, Adhikari TB (2014) Genome-wide association study reveals novel quantitative trait loci associated with resistance to multiple leaf spot diseases of spring wheat. *PloS One*, 9, e108179.

- Hao C, Wang L, Ge H, Dong Y, Zhang X (2011) Genetic diversity and linkage disequilibrium in Chinese bread wheat (*Triticum aestivum* L.) revealed by SSR markers. *PLoS One*, 6, e17279.
- Holm-Nielsen C (1966) Untersuchungen Uber Die Vererbung Der Resistenz Gegen Den Getreidenematoden (*Heterodera avenae*) Beim Weizen. *Nematologica*, 12, 575-578.
- Jahier J, Abelard P, Tanguy M, Dedryver F, Rivoal R, Khatkar S, Bariana H, Koebner R (2001) The *Aegilops ventricosa* segment on chromosome 2AS of the wheat cultivar 'VPM1'carries the cereal cyst nematode resistance gene Cre5. *Plant Breeding*, 120, 125-128.
- Kimber G, Feldman M (1987) Wild wheat. An introduction. *Special Report, College of Agriculture, University of Missouri-Columbia*.
- Kump K L, Bradbury PJ, Wisser RJ, Buckler ES, Belcher AR, Oropeza-Rosas MA, Zwonitzer JC, Kresovich S, McMullen MD, Ware D (2011) Genome-wide association study of quantitative resistance to southern leaf blight in the maize nested association mapping population. *Nature genetics*, 43, 163-168.
- Lagudah ES, Krattinger SG, Herrera-Foessel S, Singh RP, Huerta-Espino J, Spielmeyer W, Brown-Guedira G, Selter LL, Keller B (2009) Gene-specific markers for the wheat gene Lr34/Yr18/Pm38 which confers resistance to multiple fungal pathogens. *Theoretical and Applied Genetics*, 119, 889-898.
- Loureiro I, Loureiro C, Escorial JM, García Baudin C, Chueca (2009) Spontaneous wheat-*Aegilops biuncialis*, *Ae. geniculata* and *Ae. triuncialis* amphiploid production, a potential way of gene transference. *Spanish Journal of Agricultural Research*, 7, 614.
- Malosetti M, van der Linden CG, Vosman B, van Eeuwijk FA (2007) A mixed-model approach to association mapping using pedigree information with an illustration of resistance to *Phytophthora infestans* in potato. *Genetics*, 175, 879-889.
- Massman J, Cooper B, Horsley R, Neate S, Dill-Macky R, Chao S, Dong Y, Schwarz P, Muehlbauer G, Smith K (2011) Genome-wide association mapping of Fusarium head blight resistance in contemporary barley breeding germplasm. *Molecular Breeding*, 27, 439-454.
- McDonald AH, Nicol JM (2005). Nematode parasites of cereals. In: 'Plant-Parasitic Nematodes in Subtropical and Tropical Agriculture(2nd Ed.).' (Eds. M Luc, RA Sikora and J Bridge) pp. 131-191. (CABI Publishing, Egham, UK).
- Mulki MA, Jighly A, Ye G, Emebiri LC, Moody D, Ansari O, Ogbonnaya FC (2013) Association mapping for soilborne pathogen resistance in synthetic hexaploid wheat. *Molecular Breeding*, 31, 299-311.

- Neumann K, Kobiljski B, Denčić S, Varshney R, Börner A (2011) Genome-wide association mapping: a case study in bread wheat (*Triticum aestivum*). *Molecular breeding*, 27, 37-58.
- Nicol J, Rivoal R (2007) Global knowledge and its application for the integrated control and management of nematodes on wheat. In: 'Integrated Management and Biocontrol of Vegetable and Grain Crops Nematodes.' (Eds. A Ciancio, KG Mukerji) pp. 243-287. (Springer Academic Publishing, Dordrecht, The Netherlands).
- Nilsox-Ehle H (1920) The resistance of some varieties of barley to *H. schachtii*. *Hereditas*, 1, 1-34.
- Paull J, Chalmers KJ, Karakousis A, Kretschmer JM, Manning S, Langridge P (1998) Genetic diversity in Australian wheat varieties and breeding material based on RFLP data. *Theoretical and Applied Genetics*, 96, 435-446.
- Pritchard JK, Przeworski M (2001) Linkage disequilibrium in humans: models and data. *The American Journal of Human Genetics*, 69, 1-14.
- Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics*, 155, 945-959.
- Rafalski A (2002) Applications of single nucleotide polymorphisms in crop genetics. *Current Opinion in Plant Biology*, 5, 94-100.
- Riley IT, Nicol JM, Dababat AA (2009) Cereal Cyst Nematodes: Status, Research and Outlook. (CIMMYT, Ankara, Turkey).
- Rivoal R, Bekal S, Valette S, Gauthier J-P, Fradj M B H, Mokabli A, Jahier J, Nicol J, Yahyaoui A (2001) Variation in reproductive capacity and virulence on different genotypes and resistance genes of Triticeae, in the cereal cyst nematode species complex. *Nematology*, 3, 581-592.
- Rivoal R, Cook R (1993) Nematode pests of cereals. In 'Plant Parasitic Nematodes in Temperate Agriculture.' (Eds. K Evans, DL Trudgill, JM Webster) pp. 259-303. (CAB International, Wallingford, UK).
- Sharp P, Kreis M, Shewry P, Gale M (1988) Location of β-amylase sequences in wheat and its relatives. *Theoretical and Applied Genetics*, 75, 286-290.
- Singh K, Chhuneja P, Singh I, Sharma S, Garg T, Garg M, Keller B, Dhaliwal HS (2010) Molecular mapping of cereal cyst nematode resistance in *Triticum monococcum* L. and its transfer to the genetic background of cultivated wheat. *Euphytica*, 176, 213-222.
- Sohail Q, Shehzad T, Kilian A, Eltayeb AE, Tanaka H, Tsujimoto H (2012) Development of diversity array technology (DArT) markers for assessment of population structure and diversity in Aegilops tauschii. *Breeding Science*, 62, 38-45.
- Somers DJ, Kirkpatrick R, Moniwa M, Walsh A (2003) Mining single-nucleotide polymorphisms from hexaploid wheat ESTs. *Genome*, 46, 431-437.

- Taylor C, Shepherd KW, Langridge P (1998) A molecular genetic map of the long arm of chromosome 6R of rye incorporating the cereal cyst nematode resistance gene, CreR. *Theoretical and Applied Genetics*, 97, 1000-1012.
- Tommasini L, Schnurbusch T, Fossati D, Mascher F, Keller B (2007) Association mapping of *Stagonospora nodorum* blotch resistance in modern European winter wheat varieties. *Theoretical and Applied Genetics*, 115, 697-708.
- van Slageren M (1994) Wild wheats: A monograph of *Aegilops* L. and *Amblopyrum* (Jaub and Spach) Eig (Poaceae). Agricultural Univ., Wageningen, The Netherlands, and International Centre for Agricultural Research in the Dry Areas, Aleppo, Syria.
- Wang S, Wong D, Forrest K, Allen A, Chao S *et al.* (2014) Characterization of polyploid wheat genomic diversity using a high-density 90,000 single nucleotide polymorphism array. *Plant Biotechnology Journal*, 12, 787-96.
- Wang Z, Taramino G, Yang D, Liu G, Tingey S, Miao G, Wang G (2001) Rice ESTs with disease-resistance gene or defense-response gene-like sequences mapped to regions containing major resistance genes or QTLs. *Molecular Genetics Genomics*, 265, 302-310.
- William M, Singh R, Huerta-Espino J, Islas SO, Hoisington D (2003) Molecular marker mapping of leaf rust resistance gene Lr46 and its association with stripe rust resistance gene Yr29 in wheat. *Phytopathology*, 93, 153-159.
- Williams KJ, Willsmore KL, Olson S, Matic M, Kuchel H (2006) Mapping of a novel QTL for resistance to cereal cyst nematode in wheat. *Theoretical and Applied Genetics*, 112, 1480-1486.
- Würschum T, Langer SM, Longin CFH, Korzun V, Akhunov E, Ebmeyer E, Schachschneider R, Schacht J, Kazman E, Reif JC (2013) Population structure, genetic diversity and linkage disequilibrium in elite winter wheat assessed with SNP and SSR markers. *Theoretical and Applied Genetics*, 126, 1477-1486.
- Yao J, Wang L, Liu L, Zhao C, Zheng Y (2009) Association mapping of agronomic traits on chromosome 2A of wheat. *Genetica*, 137, 67-75.
- Yu J, Buckler ES (2006) Genetic association mapping and genome organization of maize. *Current Opinion in Biotechnology*, 17, 155-160.
- Zou J, Pan X, Chen Z, Xu J, Lu J, Zhai W, Zhu L (2000) Mapping quantitative trait loci controlling sheath blight resistance in two rice cultivars (*Oryza sativa* L.). *Theoretical and Applied Genetics*, 101, 569-573.

CEREAL NEMATODES MANAGEMENT STRATEGIES IN WHEAT

ABDELFATTAH A. DABABAT[1,*], GUL ERGINBAS-ORAKCI[1], MUSTAFA İMREN[2], HANS-JOACHIM BRAUN[3], ALEXEI MORGOUNOV[1], KARIM AMMAR[3], AND CLIFF WATRIN[4]

[1]CIMMYT Global Wheat Program, Ankara-Turkey; [2]University of Abant Izzet Baysal, Faculty of Agri. and Natural Sciences, Dept of Plant Protection, Bolu, Turkey; [3]CIMMYT Apdo. Postal 6-641, C.P. 06600, D.F. Mexico; [4] Syngenta Crop Protection AG, Basel, Switzerland. *Corresponding author email: a.dababat@cgiar.org

SUMMARY

Soil borne pathogens (SBPs) include the Cereal Cyst Nematode (CCN), caused by *Heterodera* species, and the Root Lesion Nematodes (RLN), caused by *Pratylenchus* species, attack roots of cereal crops and result in high yield losses and reduced grain quality and quantity. The damage caused by these diseases is accelerated in areas where drought exists. A few control options are being used to reduce CCN damage through keeping the population level below damage threshold such as; chemical, biological, cultural, and genetic (resistance/tolerance) practices. Resistance is environmentally friendly and biologically effective once identified. However, up to now, resistance in Turkey has only been identified against one of the CCN nematodes, *Heterodera filipjevi*. The foreign wheat germplasm with this resistance is not yet present in high-yielding cultivars. Resistance to the other nematodes in the CCNs complex is still being sought. Therefore, alternative approaches limiting the damage caused by cereal nematodes (CN) to wheat are needed. As a result of screening wheat germplasm against the CN; hundreds of moderately resistant germplasm in winter and spring wheat to both *Pratylenchus* and *Heterodera* species are avail-

* Dababat AA, Erginbas-Orakci G, İmren M, Braun H-J, Morgounov A, Ammar K, and Watrin C (2015) Cereal nematodes management strategies in wheat. In 'Nematodes of Small Grain Cereals: current status and research.' (AA Dababat, H Muminjanov and RW Smiley) pp. 221-232. (FAO: Ankara, Turkey).

able. The new sources of resistance are found by use of association mapping to be due to new QTL's which were never reported before. The preliminary results of using seed treatments showed that seed treatment of susceptible wheat germplasm gave up to 47% reduction in number of *Heterodera filipjevi* cysts per plant but did not reduce the number of cysts in the resistant germplasm since the cyst number was low and there was no room to decrease it further. *Pasteuria* seed treatment resulted in a significant reduction in RLN but not CCN.

Keywords: Cereal nematodes, Seed treatment, Resistance

INTRODUCTION

Nematodes occur worldwide in nearly all environments and result in losses of approximately 10% of world crop production (Whitehead, 1998). On wheat, the most important soil borne pathogens are cereal cyst nematodes (CCN) and root lesion nematodes (RLN). The RLN *P. thornei* has been reported to cause yield losses in wheat up to 38-85% in Australia and 12-37% in Mexico (Nicol 1996, Nicol and Ortiz-Monasterio 2004, Thompson and Clewett 1986). In southern Australia, grain losses caused by *P. neglectus* ranged from 16-23% and 56-74% in some concomitantly infested areas (Taylor *et al.* 1999). The CCN are caused by *Heterodera* species and the most economically important studied species are *Heterodera avenae*, *H. filipjevi* and *H. latipons*. *H. avenae*, the most damaging species on temperate cereals, is a problem worldwide and is the most studied and reported species while *H. filipjevi* is also an economically important nematode on cereals due to its widespread distribution around the world. Yield losses due to *H. avenae* ranged from 15-20% in Pakistan, 40-92% in Saudi Arabia, and 23-50% in Australia (Brown 1981, Wallace 1965). In Turkey, *H. filipjevi* caused up to 50% crop losses in Central Anatolia Plateau (CAP) conditions and *H. avenae* up to 24% in the Eastern Mediterranean wheat growing regions (Dababat *et al.* 2015, Imren and Elekcioglu 2014, Nicol *et al.* 2006).

So far, many attempts have been made to control CCNs around the world, including cultural practices, chemical control, biological control, and developing resistant wheat varieties (Dababat *et al.* 2011; Smiley and Nicol 2009). Use of resistant crop cultivars is considered the most effective and economical method for managing nematodes in both high and low value cropping systems. The effectiveness of resistance to CCNs depends on the effectiveness and durability of the sources of resistance, and on the correct identification of the nematode species and/or pathotype(s) present in the system. One of the major strategies

of the integrated disease management is the use of genetic resistance against the cereal nematodes. However, one of the key challenges is to identify 'new' sources of genetic resistance in high yielding and adapted germplasm which can be used readily by pre-breeders and breeders with major emphasis to identify highly adapted wheat lines which conveys resistance to more than one pathogen, as it is common to find more than one of these constraints in a given soil. Therefore, the objectives of the study were to: 1) Identify high yielding and potentially novel advanced winter and spring wheat lines with resistance to CCNs (*H. avenae*, *H. filipjevi*, and *H. latipons*) and RLNs (*P. thornei* and *P. neglectus*), 2) Deliver the most promising germplasm in terms of their resistance/tolerance reactions to the international wheat collaborators, 3) Implement known molecular markers linked to resistance genes to CCNs and RLNs for screening landraces and commercial wheat cultivars, 4) Conduct yield loss assessment caused by CCNs and RLNs, and 5) Determine the efficacy of biological – nematicides (*Pasteuria* spp. used as a seed coating on the control of CCN and RLN under the growth room conditions.

MATERIALS & METHODS

Cereal Cyst Nematodes: The soil borne pathogens program at CIMMYT-Turkey annually receives hundreds of winter wheat lines to be screened for the CCN *Heterodera filipjevi*. A core set consisting of 35 lines based on 3 years screening under growth room and greenhouse conditions were planted in the field under both naturally infested and artificially inoculated plots. This core set represents four nurseries; 13 Candidate International Winter Wheat Yield Trial – Semi Arid (13CAND-IWWYT-SA), Candidate 21 Facultative Winter Wheat Observation Nursery – Turkey CIMMYT ICARDA (C21FAWWON-TCI), 13 Candidate 20 Facultative Winter Wheat Observation Nursery –Turkey CIMMYT ICARDA (13C20FAWWON-TCI), Candidate 20 Facultative Winter Wheat Observation Nursery – International (C20 FAWWON-INT). Growth room screening was conducted under controlled conditions at the Transitional Zone Agriculture Research Institute-Eskişehir, Turkey as described by Dababat *et al.* (2014).

Soil samples were collected from Yozgat, Ankara (39° 40′ 10″ N, 35° 16′ 9″ E) located in CAP and cysts were extracted by using the Fenwick-Can technique. Cyst extraction and inoculum preparation and inoculation methods are followed as described by Dababat *et al.* (2014). Wheat genotypes were recorded with mean cyst number and classified into five groups as per Dababat *et al.*

(2014), according to the check lines used. The groups were: Resistant (R) = equal or fewer cysts than in a known resistant check; Moderately Resistant (MR) = slightly more cysts than in a resistant check; Moderately Susceptible (MS) = significantly more cysts than in a resistant check, but not as many as in the susceptible check; Susceptible (S) = as many cysts as in the susceptible check and number of cysts per root system considered damaging; and Highly Susceptible (HS) = more cysts than in the susceptible check.

Root Lesion Nematodes: A total of 211 durum wheat germplasm from Mexico CIMMYT's materials were screened for both *Pratylenchus neglectus and P. thornei* under the growth room conditions in 2014. The most promising resistant lines then were planted in the field in the 2015 growing season in a soil naturally-infested with *P. thornei* to evaluate their resistant and tolerant reactions. Screening for the RLN under the growth room conditions is being conducted by inoculating each plant with 400 *P. neglectus* or *P. thornei* reared on carrot discs. Nine weeks after nematode inoculation, the plants were harvested, shoots were removed, and *P. thornei* and *P. neglectus* individuals were extracted from the roots and soil using the modified Baermann funnel. The total number of *P. thornei* and *P. neglectus* nematodes per plant was calculated based on the number of nematodes counted under a microscope. Genotypes were divided into five groups (as per Dababat *et al.* 2014) based on the number of nematodes per plant, taking into account the reaction of check varieties with known resistant reation to nematodes.Whereas, screening under the field conditions was done in a 2 m^2 plot consisting of 4 rows of 2 m length. In order to measure the nematodes reproduction factor (RF) to evaluate germplasm resistance reactions; the nematode's initial population (Pi) was taken at the sowing time where the nematodes final population (Pf) was taken after wheat heading stages. To determine the tolerance reaction the plots will be harvested and grain will be weighed. The nematode reproduction values obtained were used to classify plant resistance relative to the control varieties.

Seed treatments: In order to study if there is synergetic or additive effect of seed treatments; one susceptible and one moderately resistant germplasm to both RLN and/or CCN were used. Seed treatment was done at Syngenta's facilities in Izmir-Turkey while the germplasm were provided by CIMMYT. After sowing, each tube received 400 nematodes and plants were harvested after 550 DD. Four treatments were used, 1) plant control, 2) nematode control, 3) *Pasteuria* low concentation (5.10^6 spores / seed), and 4) *Pastueria* high concentation and (1.10^7 spores / seed).

Data were analyzed according to standard analysis of variance procedures with the SPSS 14 program for windows. The Tukey test was used to explore differences between treatments, with statistical differences considered significant at $P \leq 0.05$.

RESULTS

After intensive screening of the IWWIP materials, 10 lines are ranging from R to MR as indicated in Table 1, representing germplasm from different countries. The majority of the moderately resistant germplasm originated from Turkey CIMMYT ICARDA program. This set of materials is still being evaluated in an artificially inoculated sick plot. Only 15 lines out of the 211durum wheat lines screened against both *P. neglectus* and *P. thornei* proved to be moderately resistant when compared to the known checks. This set is being screened in a field which is historically infested by *P. thornei*. The nematode's reproduction factor from the field trials showed that most of the lines inhibit the nematode reproduction (Table 2).

Table 1. List of promising lines from the International Winter Wheat Improvement Program (IWWIP) nurseries (13CAND-IWWYT-SA, C21FAWWON-TCI, 13C20FAWWON-TCI, C20 FAWWON-INT) screened against the cereal cyst nematode Heterodera filipjevi in the growth room in 2013-2014

Entry	Cross-Name	CID	OC	Acc No	Cyst #	Reaction
3	SABALAN/3/PVN/BOW//LIVA/4/ MERCAN-2/5/TX96V2427	TCI032546	TCI	110187	9	MR
4	GUN91/3/CROC_1/AE.SQUARROSA (205)//KAUZ/4/IZGI	TCI032143	TCI	110116	7	MR
5	PMF/MAYA//YACO/3/CO693591/ CTK/4/F1-1S-1/CHISHOLM	TCI02-142	TCI	100518	7	MR
15	KS920709-B-5-1-1/4/CHAM6// 1D13.1/MLT/3/SHI4414/CROW	TCI031396	TCI	110347	4	R-MR
23	PFAU/WEAVER/3/MASON/JGR// PECOS	OCW02S369S	OK-TCI	100068	8	MR
26	NALIM-3/ZHETISU/5/Sonmez= ES-98KE14=NALIM-4=BEZ//BEZ/ TVR/3/KREMENA/LOV29/4/KATIA1	TCI022272	TCI	100213	3	R-MR
28	cv. Rodina/Ae.speltoides (10 kR)	179/98w	RUS	101519	8	MR
30	Passarinho//Vee/Nac	1-NS 1590	Iran-Karadj	110479	6	MR
32	NC00-14622/2137	ARS09-382	US-NC	110531	9	MR
34	4WON-IR-257/5/YMH/HYS//HYS/TUR3055/3/DGA/4/VPM/MOS	TCI-02-80	TCI	090082	9	MR

Abbreviations: ACC = Accession number; CID = Cross identification; OC = Origin country, MR = Moderately Resistant

Table 2. List of the promising durum wheat lines screened in the growth room in 2014 for Pratylenchus thornei and P. neglectus, and are being evaluated in the field for P. thornei in Bolu –Turkey in 2015.

No	Cross Name	Cid	GID	Sid	Growth room				Field
					Pt		Pn		Pt
					No.	R	No.	R	RF
1	RANCO//CIT71/CII/3/COMDK/4/ TCHO//SHWA/MALD/3/CREX/5/SNI-TAN/6/YAZI_1/AKAKI_4//SOMAT_3/3/ AUK/GUIL//GREEN	496493	6004486	44	82	MR	65	MR	0
2	MOHAWK/6/LOTUS_5/F3LOCAL(SEL. ETHIO.135.85)/5/CHEN/ALTAR84/3/ HUI/POC//BUB/RUFO/4/FNFOOT	504706	6004811	94	71	MR	55	MR	2
3	GUANAY/3/FULVOUS_1/MFOWL_13// JUPAREC2001/8/R143/RUFF//STIL/3/ YAV79/4/SHWA/MALD/5/ ALTAR84/6/ TILO_1/LOTUS_4/7/YAZI_1/AKAKI_4// SOMAT_3/3/ AUK/GUIL//GREEN	505483	6005080	98	61	MR	42	MR	1
4	MERIDIANO/3/SOMAT_3/PHAX_1// TILO_1/LOTUS_4/5/TATLER_1/TAR-RO_1/3/CANELO_8//SORA/2*PLA-TA_12/4/ARMENT//SRN_3/NIGRIS_4/3/ CANELO_9.1	515598	6139502	112	116	MR	85	MR	0.4
5	AJAIA_12/F3LOCAL(SEL. ETHIO.135.85)//PLATA_13/3/SOM-BRA_20/4/SNITAN/5/SOMAT_4/IN-TER_8/6/ SOMO/CROC_4//LOTUS_1/3/ KITTI/4/JUPARE C 2001	510524	6139776	288	104	MR	85	MR	0.8
6	ZENIT/5/SORA/2*PLATA_12// RASCON_37/4/ARMENT//SRN_3/ NIGRIS_4/3/CANELO_9.1/6/MINI-MUS_4/GRO_2/3/PROZANA/ARLIN// MUSK_6/5/SULA/RBCE_2/3/HUI// CIT71/CII/4/RYPS27_3/ SKARV_3	521621	6421007	58	70	MR	98	MR	1
7	PRECO/6/AJAIA_12/F3LOCAL(SEL. ETHIO.135.85)//PLATA_13/3/POD_9/4/ RASCON_37/TARRO_2//RASCON_37/5/ ARMENT//SRN_3/NIGRIS_4/3/ CANELO_9.1/7/1A.1D 5+1-06/3*MOJO// RCOL/4/ARMENT//SRN_3/ NIGRIS_4/3/ CANELO_9.1	527469	6421062	67	105	MR	84	MR	0.5
8	SOMAT_4/INTER_8/5/CREX//BOY/ YAV_1/3/PLATA_6/4/PORRON_11/6/ MINIMUS_6/PLATA_16//IMMER/3/ SORA/2*PLATA_12/7/RASCON_22/ RASCON_21// MOJO_2/3/ GUANAY/4/ RCOL/5/SORA/2*PLATA_12// SOMAT_3	526447	6421363	154	86	MR	107	MR	1.3
10	KIRKI_1/HIMAN_9/4/LIS_8/FILLO_6/3/ FUUT//HORA/JOR/5/ARMENT//SRN_3/ NIGRIS_4/3/CANELO_9.1/6/ADAM-AR_15//ALBIA_1/ALTAR84/3/SNITAN/4/ SOMAT_4/INTER_8/5/SOOTY_9/ RASCON_37/7/1A.1D 5+1-06/3*MOJO// RCOL/4/ARMENT//SRN_3/NIGRIS_4/3/ CANELO_9.1	527534	6421497	105	65	MR	37	MR	0.5

11	MÂALI/5/LOTUS_5/SORD_1/3/CANELO_8//SORA/2* PLATA_12/4/YAZI_1/AKAKI_4//SOMAT_3/3/AUK/ GUIL//GREEN	521203	6421550	39	85	MR	112	MR	3
12	BELLAROI/6/RASCON_22/RASCON_21//MOJO_2/3/GUANAY/4/RCOL/5/SORA/2*PLATA_12//SOMAT_3/8/PLATA_3//CREX/ALLA/3/YAZI_10/4/JUPARE C2001/ 7/CHEN_11/POC//TANTLO/5/ENTE/MEXI_2//HUI/4/ YAV_1/3/ LD357E/2*TC60//JO69/6/MINIMUS/COMB DUCK_2//CHAM_3	538221	6634315	95	100	MR	75	MR	1
14	1A.1D 5+1-06/3*MOJO//RCOL/4/ARMENT//SRN_3/ NIGRIS_4/3/CANELO_9.1/11/SOOTY_9/RASCON_37/3/SOOTY_9/TARRO_1//AJAIA_2/10/PLATA_10/6/MQUE/4/USDA573//QFN/AA_7/3/ALBA-D/5/AVO/HUI/7/PLATA_13/8/THKNEE_11/9/CHEN/ALTAR84/3/HUI/ POC//BUB/RUFO/4/FNFOOT	537793	6635117	61	54	MR	71	MR	3.5
15	SOMAT_4/INTER_8/3/RASCON_21/KNAR_3//PLATA_8/4/CNDO/PRIMADUR//HAI-OU_17/3/SNITAN/9/GEDIZ/FGO//GTA/3/SRN_1/4/TOTUS/5/ENTE/MEXI_2//HUI/4/YAV_1/3/LD357E/2*TC60//JO69/6/SOMBRA_20/7/JUPARE C2001/8/CS/TH.CU//GLEN/3/GEN/4/MYNA/VUL/5/2*DON87/6/2*BUSCA_3	537883	6635238	86	83	MR	81	MR	1
16	Croc	72726		531	144	MR	122	MR	
17	Seri	167910			759	S	705	S	

Abbreviations: GID = Germplasm identification; CID = Cross identification; SID = Selection identification; Pt = Pratylenchus thornei; Pn = Pratylenchus neglectus; R = Reaction; RF = Reproduction Factor; MR = Moderately Resistant; S = Susceptible

Table 3. Overall comparison among the different treatments on root lesion nematode (Pratylenchus thornei & Pratylenchus neglectus) numbers and plant height (cm) in two spring wheat varieties in short term experiment in the growth room.

No.	Trt	Variety	Pratylenchus thornei		Pratylenchus neglectus	
			Pt	Plant height	Pn	Plant height
1	C	CROC_1/AE.SQUARROSA (224)//OPATA	0	36	0	37,2
2	NC	CROC_1/AE.SQUARROSA (224)//OPATA	850	37	633	41,2
3	Con1	CROC_1/AE.SQUARROSA (224)//OPATA	340	38	480	39,3
4	Con2	CROC_1/AE.SQUARROSA (224)//OPATA	857	36	577	37,7
1	C	Seri	0	34,8	0	35
2	NC	Seri	2010	35,8	2207	36
3	Con1	Seri	1477	34,8	1547	36
4	Con2	Seri	1063	35,5	1903	36

Abbreviations stand for: Trt = Treatment, C = Control, NC = Nematode Control, Con1 = concentration of 5.106 Pasteuria spp. spores/seed, and Con2 = 1.107 spores/seed.

Table 4. Overall comparison among the different treatments on cereal cyst nematode (Heterodera filipjevi) cyst, egg, juvenile numbers and plant height (cm) in two spring wheat varieties in a short-term experiment in the growth room.

No	Trt	Variety	Cereal Cyst Nematode-H. filipjevi (Mean)					
			Cyst/Trt	Egg (big cyst)	Juvenile (big cyst)	Egg (small cyst)	Juvenile (small cyst)	Plant Height
1	C	Katea	0	0	0	0	0	47
2	NC	Katea	6	192	191	51	38	47
3	Con[1]	Katea	4	191	197	50	31	47
4	Con[2]	Katea	7	153	159	44	39	45
1	C	Bezostaja	0	0	0	0	0	42
2	NC	Bezostaja	17	146	97	94	97	42
3	Con[1]	Bezostaja	15	138	90	76	92	42
4	Con[2]	Bezostaja	15	145	93	97	95	43

Abbreviations: Trt = Treatment, C = Control, NC = Nematode Control, Con[1] = concentration of 5.106 Pasteuria spp. spores/seed, and Con[2] = 1.107 spores/seed.

On the other hand, *Pasteuria* seed treatment reduced the number of RLN on both the moderately and susceptible wheat varieties. However, reduction in RLN numbers was more distinct on the susceptible variety (Table 3). No significant differences of *Pasteuria* seed treatment on plant height was recorded (Tables 3 and 4). Cyst nematode was reduced by *Pasteuria* seed treatment on the susceptible variety "Bezostaya" but not the moderately resistant variety "Katea" (Table 4). Juvenile and egg numbers were associated with cyst size.

DISCUSSION

Recent yield trials conducted by different breeding centers around the world have shown that the production of bread wheat is being constrained by several biotic and abiotic stresses. Intensified wheat production, changes in cultural practices including shifts from conventional tillage and stubble burning to reduced tillage practices, and wheat monoculture involving cultivation of susceptible cultivars has resulted in development of wheat diseases to epidemic conditions.

So far, nine resistance genes have been identified as controlling CCNs in bread wheat: *Cre1* and *Cre8* (from *Triticum aestivum)*; *Cre2*, *Cre5*, and *Cre6* (from *Aegilops ventricosa)*; *Cre3* and *Cre4* (from *Triticum tauschii)*; *Cre7* (from *Aegilops triuncialis); and CreR* (from *Secale cereale*) (Barloy *et al.* 2007). However, none of these genes give complete resistance to the winter wheat germplasm in the IWWIP materials. In a recent study conducted by Imren *et al.* (2012) to

identify genetic resistance to the cereal cyst nematodes *Heterodera avenae*, *H. filipjevi*, and *H. latipons*, six *Cre* genes were used to screen some international bread wheat germplasm. The results showed that the resistance genes *Cre1*, *Cre3* and *Cre7* provided some level of resistant reaction against both *H. avenae* and *H. latipons.* The *Cre8* and *CreR* genes gave resistant reactions to *H. filipjevi* only. However, there was no complete resistance by any of the six studied *Cre* genes to the three species. Toktay *et al.* (2012) screened germplasm with *Cre1* genes against *H. filipjevi* and the root lesion nematode *Pratylenchus thornei.* Their results indicated no complete resistance, and no relationship between *H. filipjevi* and *P. thornei* resistance. These most likely have quantitative resistance genes against both CCN and RLN (Sheedy *et al.* 2012). However, it is clear that whilst *Cre* genes provide resistance to *H. avenae*, they do not confirm resistance to the *H. filipjevi* Turkish population.

Seed treatment application could be used especially on susceptible varieties until suitable CCN resistance is bred into the genotype (Dababat *et al.* 2014). Applying seed treatment significantly reduced nematodes on susceptible wheat varieties when compared to the resistant one. This occurred because there was the low room for reducing nematodes on resistant varieties compared to the succeptible varieties. *Pasteuria* reduced the root lesion nematodes numbers but not the cereal cyst nematodes *Heterodera filipevi*. In this study, generally, there was no significant difference of *Pasteuria* seed treatments on growth parameters.

Screening the IWWIP materials is a very fundamental strategy where IWWIP breeds the most promising lines for nematodes and then distributes international nurseries to more than 150 collaborators representing 75 countries. Given that many countries lack the expertise in soil borne diseases, especially nematologists, this program will reduce nematode damage in wheat fields.

Due to climate changes and agricultural practices, wheat production has brought new challenges for farmers, including the increased incidence and severity of soil borne pathogens. These diseases often occur together in the field, where they are often very difficult to distinguish from one another. Hence it is paramount to study their incidence, effect and control together. The complexity of these wheat diseases and the conditions in which they occur makes it essential to seek a holistic approach for their management. Such integrated management approaches include breeding for durable disease resistance, production and distribution of healthy seed, developing appropriate crop manage-

ment practices, monitoring pathogen diversity, and applying recent advances in biotechnology to overcome disease losses. In addition, significant efforts are needed to better understand the cropping systems and their interactions that can influence the spread of these diseases.

ACKNOWLEDGMENTS

The authors would like to thank the Turkish Ministry of Food, Agriculture and Livestock, Syngenta, the Grain Research and Development Corporation (GRDC) and the International Wheat and Maize Improvement Center (CIMMYT) for supporting these studies, and Dr Ravi Singh and the IWWIP for providing the seeds.

REFERENCES

- Brown RA (1981) Nematode diseases. *In* Economic importance and biology of cereal root diseases in Australia. *Report to Plant Pathology Subcommittee of Standing Committee on Agriculture*, Australia.
- Barloy D, Lemoine J, Abelard P, Tanguy AM, Rivoal R, Jahier J (2007) Marker-assisted pyramiding of two cereal cyst nematode resistance genes from *Aegilops variabilis* in wheat. *Molecular Breeding*, 20, 31-40.
- Dababat AA, Pariyar S, Nicol J, Duveiller E (2011) Cereal Cyst nematode: an unnoticed threat to global cereal production. *Technical innovation brief* # (11) CGIAR, SP-IPM.
- Dababat AA, Imren M, Erginbas-Orakci G, Ashrafi S, Yavuzaslanoglu E, Toktay H, Pariyar SR, Elekcioglu H, Morgounov A, Mekete M (2015) The importance and management strategies of cereal cyst nematodes, *Heterodera* spp., in Turkey. *Euphytica*, 202, 173-188.
- Dababat AA, Erginbas-Orakçı G, Toktay H, Imren M, Akin B, Braun HJ, Dreisigacker S, Elekcioğlu IH, Morgounov A (2014) Resistance of Winter Wheat to *Heterodera filipjevi* in Turkey. *Turkish Journal of Agriculture and Forestry*, 38,180-186.
- İmren M, Toktay H, Bozbuga R, Erginbas-Orakci G, Dababat AA, Elekcioglu IH (2013) Identification of genetic resistance to cereal cyst nematodes; *Heterodera avenae* (Wollenweber), *H. filipjevi* (Madzhidov) Stelter and *H. latipons* (Franklin) in some international bread wheat germplasms. *Turkish Journal of Entomology,* 37(3), 277-282.

- İmren M, Elekcioğlu İH (2014) Effect of cereal cyst nematode *Heterodera avenae* (Tylenchida: Heteroderidae) on yield of some spring wheat varieties in Adana Province, Turkey. *Turkish Journal of Agriculture & Forestry*, 38 (6), 820-823
- Nicol JM, Bolat N, Sahin E, Tülek A, Yıldırım AF, Yorgancılar A, Kaplan A, Braun HJ (2006) The cereal cyst nematode is causing economic damage on rainfed wheat production systems of Turkey. *Phytopathology,* 96:169.
- Nicol J, Ortiz-Monasterio I (2004) Effect of the root lesion nematode, *Pratylenchus thornei* on wheat yields in Mexico. *Nematology*, 6 (4), 485–493.
- Nicol JM (1996) The distribution, pathogenicity and population dynamics of *Pratylenchus thornei* (Sher and Allen, 1954) on wheat in south Australia. PhD thesis. Adelaide, Australia, The University of Adelaide.
- Sheedy JG, Thompson JP, Kelly A (2012) Diploid and tetraploid progenitors of wheat are valuable sources of resistance to the root lesion nematode *Pratylenchus thornei*. *Euphytica,* 186, 377-391.
- Smiley RW, Nicol JM (2009) Nematodes which Challenge Global Wheat Production. In: 'Wheat Science and Trade.' (Ed. BF Carver). Oxford, UK: Wiley-Blackwell.
- Taylor SP, Evans ML (1998) Vertical and horizontal distribution of and soil sampling for root lesion nematodes (*Pratylenchus neglectus* and *P. thornei*) in South Australia. *Australasian Plant Pathology*, 27, 90–96.
- Thompson, JP, Clewett TG (1986) Research on root-lesion nematode. In *Queensland Wheat Research Institute Biennial Report 1982-1984*, Qld Dept. Primary Industries, Qld. Govt., Queensland, Australia, p. 32-35. Toowoomba, Queensland, Australia, Wheat Research Institute.
- Toktay H, Yavuzaslanoğlu E, İmren M, Nicol JM, Elekcioğlu IH, Dababat AA (2012) Screening for resistance to *Heterodera filipjevi* (Madzhidov) Stelter (Tylenchina: Heteroderidae) and *Pratylenchus thornei* (Sher & Allen) (Tylenchida: Pratylenchidae) sister lines of spring wheat. *Turkish Journal of Entomology,* 36(4), 455-461.
- Wallace HR (1965) The ecology and control of the cereal root nematode. *Journal of Australian Institute of Agriculture Science*, 31, 178-186.
- Whitehead AG (1998) Plant Nematode Control. New York. NY: CAB International.

EVALUATION OF DIFFERENT WHEAT GENOTYPES FOR RESISTANCE TO *HETERODERA AVENAE* IN SAUDI ARABIA

AHMAD S. AL-HAZMI[1,*], AHMED AM. DAWABAH[1], ABDULLAH A. ALDOSS[2], AND KHALID A. MUSTAFA[2]

[1]Department of Plant Protection, and [2]Department of Plant Production, College of Food & Agric. Sciences, King Saud University, Riyadh, Saudi Arabia.
*Corresponding author email: aslhazmi@ksu.edu.sa

SUMMARY

Forty wheat genotypes (some were developed by King Saud University) were evaluated for their resistance to a local (Saudi) population of the cereal cyst nematode (CCN) *Heterodera avenae* in an outdoors test. Clean plastic pots (15-cm diam.) were filled with CCN naturally-infested field soil (20 eggs + J_2/g soil) and planted with several seeds of the tested lines. The susceptible wheat cultivar "Yecora Rojo" was used as check cultivar. A week after germination, seedlings were thinned to one seedling per pot. Five replicates were used for each genotype. Plants were left outdoor, on a table, for 75 days during the wheat growing season, watered and fertilized as needed. At harvest, plants were washed by tap water to free roots from soil and adhesives. White cysts of each plant were counted and the mean number of white cysts for each genotype was calculated. Genotypes having three, or less, cysts per plant were designated as resistant, while those having more than three cysts were designated as susceptible. Result showed that only seven (17.5%) genotypes were found to be resistant; L11-6, L11-7, L11-8, L11-17, L11-19, L11-21 and L11-23. The other 33 tested genotypes showed various degrees of susceptibility.

* Al-Hazmi AS, Dawabah AM, Aldoss AA, Mustafa KA (2015) Evaluation of different wheat genotypes for resistance to *Heterodera avenae* in Saudi Arabia. In 'Nematodes of Small Grain Cereals: current status and research.' (AA Dababat, H Muminjanov and RW Smiley) pp. 233-238. (FAO: Ankara, Turkey).

INTRODUCTION

The cereal cyst nematode (CCN), *Heterodera avenae* Woll., has a global distribution and causes significant yield losses to wheat and other cereal cops in many countries (Nicol *et al.* 2003, Handoo 2002). In Saudi Arabia, the nematode was first reported from an irrigated wheat field in the central region in 1987 (Youssif 1987). Since then, it has been spreading fast and widely, and becoming a major threat to our wheat production (Al-Hazmi *et al.* 1994, Al-Hazmi *et al.* 2009). Wheat crop losses reached up to more than 90% in a heavily infested field in Riyadh region (Ibrahim *et al.* 1995).

Several local populations of CCN were identified as *H. avenae*, based on morphological and morphometric features (Al-Hazmi *et al.* 1994, Dawabah *et al.* 2012), and DNA markers (Al-Rehiayani 2007). The pathotype of several Saudi Crop rotation, host resistance and host tolerance have proven to be the only economically and environmentally sustainable methods to control damage caused by CCN to cereals. Several years ago, a wheat breeding program was initiated in the Department of Plant Production, King Saud University, and led by wheat breeder Prof. A.A. Al-Doss. Among the several objectives of the program is to search, identify and use different sources of resistance and tolerance to the local pathotype of *H. avenae*. This study is a portion of this program and it aimed to screen 40 wheat genotypes (some were selected from our wheat breeding program) for their resistance to a local population of *H. avenae* under outdoor conditions.

METHODS

Forty wheat genotypes (local and international materials) were evaluated for their resistance to a local (Saudi) population of *H. avenae* in a pot test under outdoor conditions. These genotypes (Table 1) included advanced lines selected from the wheat breading program in the Plant Production Department, College of Food and Agricultural Sciences, King Saud University, and some lines were selected from local CCN-infested wheat fields. Clean plastic pots (15-cm diam.) were filled with CCN naturally-infested field soil (20 eggs + J_2 /g soil) collected from an infected wheat field in Hail region.

Pots were then planted with several seeds of the tested lines. The known susceptible wheat cultivar "Yecora Rojo" was used as a check to test the viability of the nematode inoculum. A week after seed germination, seedlings were

thinned to one seedling per pot. Five replicates were used for each genotype. Plants were kept on a table outdoor during the wheat growing season. Plants were watered and fertilized as needed.

Seventy-five days after germination, plants were very carefully removed from pots. Soil around each root system was gently washed away with a stream of tap water over a 60-mesh sieve. The clean root system of each plant was examined very carefully for the presence of the white cysts. The number of white cysts per each replicate (plant) was counted and the mean number of each genotype was calculated. Genotypes having more than three white cysts/plant were designated as susceptible, while those having three or less cysts/plant were designated as resistant (Mathur *et al.* 1974, Ireholm 1994).

RESULTS AND DISCUSSION

The tested 40 wheat genotypes varied widely in their reaction (0.0 to 32.4 cysts/plant) to our local population of *H. avenae* (Table 1). Among the tested genotypes, only seven (17.5%) genotypes (selected from our wheat breeding program) were found to be resistant (≤ 3cysts/plant). These included the genotypes L11-6, L11-7, L11-8, L11-17, L11-19, L11-21 and L11-23. In fact, the first five of these genotypes were found to be completely resistant (0.0 cysts/plant). The other tested genotypes (82.5%) were designated as susceptible (≥ 3cysts/plant), with various degrees of susceptibility (Table 1). The genotypes L29, L32, KSU 104 and L10-5 were found to be the most susceptible, with ≥30 cysts/plant. The check cultivar "Yecora Rojo", which is the recommended and the most grown (90%) cultivar in Saudi wheat fields, was found to be very susceptible (18.8 cysts/plant).

The initial inoculum density (Pi) used in this study (20 eggs + J_2/g soil) might be considered relatively high. Under field conditions and especially in field soils with lower initial population densities, some of the designated susceptible lines in this study might have exhibited less susceptibility and showed good field tolerance. In a field study (Al-Doss *et al.* 2012) conducted in a wheat field infected with CCN in Hail region, the local line KSU 102, which is designated in our present study as susceptible, produced a relatively high grain yield (7.98 ton/ha), compared to the lines L11-8, L11-7 and L11-21 (designated in our study as resistant), which produced grain yields of 7.13, 7.16 and 8.21 ton/ha, respectively. Developing high yielding wheat cultivars with resistance or tolerance to CCN is one of the major challenges to wheat breeders. Wheat breeders

aim to balance between resistance to CCN and the required agronomic traits in their newly developed wheat cultivars.

Some of the tested lines (such as L11-8, L11-7 and L11-21), which were selected from our breeding program, were found to possess all tested *Cre* genes, namely: *Cre1*, *Cre3*, *Cre5*, *Cre8*, and *CreY*, while the susceptible cultivar "Yecora Rojo" lacked both the *Cre1* and *Cre3* genes (Al-Doss *et al.* 2010). It has been concluded that *Cre3* has the largest impact on reducing the number of cysts, followed by *Cre1* and *Cre8* (Safari *et al.* 2005).

Table 1. Reaction of selected wheat genotypes to a Saudi local population of Heterodera avenae, under outdoor conditions (values are means of five replicates)

Genotype	No. white cyst/plant	Reaction	Genotype	No. white cyst/plant	Reaction
KSU 101	10.0	Susceptible	L 31	20.4	Susceptible
KSU 102	32.4	Susceptible	L 32	30.0	Susceptible
KSU 103	15.2	Susceptible	L 33	13.6	Susceptible
KSU 104	32.0	Susceptible	L 34	32.0	Susceptible
KSU 105	6.4	Susceptible	L 35	27.8	Susceptible
KSU 106	8.4	Susceptible	L 70	11.4	Susceptible
L 10-1	10.4	Susceptible	L 71	15.4	Susceptible
L 10-4	8.4	Susceptible	L 72	13.2	Susceptible
L 10-5	31.4	Susceptible	L 73	11.6	Susceptible
L 10-6	0.0	Resistant	L 74	27.0	Susceptible
L 10-7	0.0	Resistant	L 75	27.2	Susceptible
L 10-8	0.0	Resistant	L 76	11.0	Susceptible
L 11-15	18.6	Susceptible	L 77	16.2	Susceptible
L 11-17	0.0	Resistant	L 100	5.4	Susceptible
L 11-19	0.0	Resistant	L 101	10.0	Susceptible
L 11-21	2.2	Resistant	L 102	6.4	Susceptible
L 11-23	1.8	Resistant	L 103	6.2	Susceptible
L 11-25	7.6	Susceptible	L 104	10.2	Susceptible
L 29	31.0	Susceptible	L 105	5.8	Susceptible
L 30	6.6	Susceptible	Yecora Rojo	18.8	Susceptible

Our efforts are currently underway to introgress the globally known *Cre* resistant genes into our breeding wheat germplasm. In addition, continued efforts are being made to search for new resources.

ACKNOWLEDGMENTS

This work is a portion of a study funded by the research project: CEBR 12 under the Center of Excellence in Biotechnology Research program, King Saud University.

REFERENCES

- Al-Doss AA, Al-Hazmi AS, Dawabah AM, Abdel-Mawgood AA, Al-Rehiayani SM, Al-Otayk S, Moustafa KA, Motawei MI (2010) Impact of Cre and peroxidase genes of selected new wheat lines on cereal cyst nematode (*Heterodera avenae* Woll.) resistance. *Australian Journal of Crop Science*, 4(9), 737-743.
- Al-Hazmi AS, Cook R, Ibrahim AAM (2001) Pathotype characterization of the cereal cyst nematode, *Heterodera avenae*, in Saudi Arabia. *Nematology*, 3, 379–382.
- Al-Hazmi AS, Ibrahim AAM, Abdul-Razig AT (1994) Occurrence, morphology and reproduction of *Heterodera avenae* on wheat and barley in Saudi Arabia. *Pakistan Journal of Nematology*, 12, 117–129.
- Al-Hazmi, AS, Dawabah, AAM (2009) Present status of the cereal cyst nematode (*Heterodera avenae*) in Saudi Arabia. In: 'Cereal Cyst Nematodes: Status, Research and Outlook.' (Eds. IT Riley, JM Nicol, AA Dababat) pp. 56–60 (CIMMYT, Ankara, Turkey).
- Al-Rehiayani SM (2007) Genetic differences among cereal cyst nematode (*Heterodera avenae*) populations in central Saudi Arabia using ITS-rDNA sequences. *International Journal of Nematology*, 17, 124-129.
- Dawabah AAM, Al-Hazmi AS, Al-Rehiayani SM, AbdelMawgood A, Motawei MI, Al-Otayk S, Sadder M, Elgorban AM, Migdadi HM, Moustafa KA, Al-Doss AA (2012) Morphological and molecular characterization of cereal cyst nematode (*Heterodera avenae*) populations from arid environments. *Australian Journal of Crop Science*, 6 (6), 970-979.
- Handoo ZM (2002) A key and compendium to species of the *Heterodera avenae* Group (Nematoda: Heteroderidae). *Journal of Nematology*, 34, 250-262.
- Ibrahim AAM, Al-Hazmi AS, Al-Yahya FA, Alderfasi AA (1999) Damage potential and reproduction of *Heterodera avenae* on wheat and barley under Saudi field conditions. *Nematology*, 1, 625–630.

- Ireholm A (1994) Characterization of pathotypes of cereal cyst nematodes, *Heterodera* spp., in Sweden. *Nematologica*, 40, 399-411.
- Mathur BN, Arya HC, Mathur RL, Handa DK (1974) The occurrence of biotypes of the cereal cyst nematode (*Heterodera avenae*) in the light soils of Rajasthan and Harya, India. *Nematology*, 20, 19–26.
- Nicol JM, Rivoal T, Taylor S, Zaharieva M (2003) Global importance of cyst (*Heterodera* spp.) and lesion nematodes (*Pratylenchus* spp.) on cereals: Distribution, yield loss, use of host resistance and integration of molecular tools, *Nematolology Monographs and Perspectives*, 2, 1-19.
- Safari E, Gororo NN, Eastwood RF, Lewis J, Eagles HA, Ogbonnaya FC (2005) Impact of Cre1, Cre8 and Cre3 genes on cereal cyst nematode resistance in wheat. *Theoretical and Applied Genetics*, 110, 567–572.
- Youssif GM (1987) Effect of *Heterodera avenae* populations in infested field patches of wheat cultivation on plant growth and yield. 10th Symposium on the Biological Aspects of Saudi Arabia, Saudi Biological Society, Jeddah, Saudi Arabia, 20-24 April 1987 (abstract).

RESISTANCE IN SYNTHETIC BREAD WHEAT LINES AGAINST THE CEREAL CYST NEMATODE *HETERODERA FILIPJEVI*

FATEH TOUMI[1,2,*], JAN DE RIEK[3], ALEXEI MORGOUNOV[4], ELLEN DE KEYSER[3], GUL ERGINBAŞ-ORAKÇI[4], NICOLE VIAENE[1,5], ABDELFATTAH A. DABABAT[4], JULIE M. NICOL[6], FRANCIS OGBONNAYA[7,8], LIEVEN WAEYENBERGE[1,2], AND MAURICE MOENS[1,2]

[1]Institute for Agricultural and Fisheries Research (ILVO), Burg. Van Gansberghelaan 96, 9820 Merelbeke, Belgium; [2]Faculty of Bioscience Engineering, Ghent University, Coupure links 653, 9000 Ghent, Belgium; [3]Institute for Agricultural and Fisheries Research (ILVO), Caritasstraat 21, 9090 Melle, Belgium; [4]International Maize and Wheat Improvement Centre (CIMMYT), Ankara, Turkey; [5]Faculty of Sciences, Ghent University, K. L. Ledeganckstraat 35, 9000 Ghent, Belgium; [6]International Maize and Wheat Improvement Centre (CIMMYT); [7]International Center for Agricultural Research in the Dry Areas (ICARDA), PO Box 5466, Aleppo, Syria; [8]Grains Research and Development Corporation (GRDC), Level 1, 40 Blackall Street, Barton ACT 2600, Australia.
*Corresponding author email: fateh.toumi@ilvo.vlaanderen.be

SUMMARY

A total of 230 synthetic winter wheat germplasms representing three groups were screened twice against *Heterodera filipjevi* under growth room conditions. These germplasms were also genotyped using the Amplified Fragment Length Polymorphism (AFLP) technique. Genomic DNA extracted from the young leaves of all germplasms was digested with three restriction enzymes used in two combinations (EcoRI + MseI; PstI + MseI). AFLP was performed

* Toumi F, De Riek J, Morgounov A, De Keyser E, Erginbas-Orakci G, Viaene N, Dababat AA, Nicol JM, Ogbonnaya F, Waeyenberge L, Moens M (2015) Resistance in synthetic bread wheat lines against the cereal cyst nematode *Heterodera filipjevi*. In 'Nematodes of Small Grain Cereals: current status and research.' (AA Dababat, H Muminjanov and RW Smiley) pp. 239-250. (FAO: Ankara, Turkey).

using two sets of primers for each enzyme combination. Within each group, some of the germplasms had relatively lower numbers of cysts compared to the others. The analysis of correlation between different traits resulted in a significant positive but weak, correlation between the number of developed cysts and root length (r^2= 0.136), as well as root surface (r^2= 0.107). Interestingly, the biplot based on the two functions of the discriminate analysis of the AFLP DNA markers, showed clear evidence of the genetic differentiation of the resistant germplasms in all three groups.

Key words: *Heterodera filipjevi*, AFLP, resistant cultivar, wheat

INTRODUCTION

Wheat (*Triticum* spp.), maize and rice are the most important crops in terms of production, acreage and source of nutrition, mainly in developing countries (Nicol and Rivoal 2008). Cereal cyst nematodes (CCN; *Heterodera* spp.) are considered the most important group of plant-parasitic nematodes on cereals worldwide (Sikora 1988) and they can have significant impact in decreasing yield (Brown *et al.* 1985, Nicol and Rivoal 2008, Dababat *et al.* 2015). *Heterodera avenae*, *H. filipjevi* and *H. latipons* are considered the major species among CCN (Yan and Smiley 2009, Dababat *et al.* 2015). *Heterodera avenae* causes yield loss up to 90% on wheat in Saudi Arabia (Ibrahim *et al.* 1999), while *H. latipons* significantly reduced grain yield to 55% in Iran (Hajihasani *et al.* 2010a). The average yield loss caused by *H. filipjevi* on wheat was estimated 42-50% in Turkey (Nicol *et al.* 2006), and 48% in Iran (Hajihasani *et al.* 2010b).

The use of host resistance is considered the most cost efficient, environmentally friendly and accessible option for nematode management. The effectiveness and usefulness of resistance to CCN depends on: (1) the efficacy and durability of the sources of resistance, (2) the interaction of the specific putative resistant accession and the CCN pathotype found in a specific field/region, and (3) the correct identification of the nematode species and/or pathotype(s) present in the system. In the last four decades, due to the absence of variability for CCN resistance in *T. durum*, led to the use of resistance genes from alien cultivated or wild species (Bekal *et al.* 1998) and various species of *Triticum*, *Aegilops* and *Secale*, which have been screened through wheat breeding programs as potential sources of germplasm with resistance to CCN (Ogbonnaya *et al.* 2001). The International Winter Wheat Improvement Program (IWWIP, www.iwwip.org) is a cooperative breeding program

of the Turkish Ministry of Food, Agriculture, and Livestock, the International Maize and Wheat Improvement Center (CIMMYT), and the International Center for Agricultural Research in the Dry Areas (ICARDA). IWWIP aims to develop broadly adapted germplasms for irrigated and semi-arid areas of Central and West Asia. The new advanced lines from IWWIP and germplasms submitted by cooperators are distributed annually to around 130 breeding programs in 50 countries through the international Facultative and Winter Wheat Observation Nursery (FAWWON). In cooperation with these programs we initiated a study to: (1) to phenotype and genotype synthetic wheat lines for resistance to *H. filipjevi*, (2) to study genetic variability for some root traits and nematode responses, (3) to investigate the genetic diversity using AFLP markers and relate it to nematode responses, and (4) to identify new sources of resistance to *H. filipjevi* in bread wheat germplasms.

MATERIAL AND METHODS

Nematode inoculum: Cysts were collected from fields located in Haymana, Ankara, Turkey in July 2014. They were extracted as described by Fenwick (1940) and identified as *H. filipjevi* using species-specific PCR primers (Toumi *et al.* 2013). The extracted cysts were surface sterilized with 0.5% NaOCl, then washed several times with distilled water, and kept in a refrigerator at 4°C till the start of the experiment (Dababat *et al.* 2014). When needed, the cysts were transferred to an incubator (15°C) to enhance and obtain the greatest hatch (Sahin *et al.* 2010). Freshly hatched second-stage juveniles (J2) were used as inoculum in the screening tests.

Wheat materials: A total of 230 synthetic winter wheat lines from the IWWIP were examined. Those lines were divided in three different groups (nurseries): 14SYNT-PYT-HAND (Group 1) consisting of 75 lines originating from crosses between winter durum × *Aegilops* developed at CIMMYT-Mexico; 14SYNT-PYT (Group 2) consisting of 106 lines originating from crosses between winter durum × *Aegilops* × bread wheat developed in Turkey; 14SYN-JAPAN (Group 3) consisting of 48 lines originating from crosses between Langdon durum × *Aegilops* developed in Japan. Four widely grown winter wheat check lines (Katea, Bezostaya, Kutluk, and Sönmez) were included in each run.

Growth chamber experiment: All lines were screened twice against *H. filipjevi* under growth chamber conditions at the Transitional Zone Agricultural Research Institute, Eskisehir, Turkey. Standard small tubes with a closed bottom

(16-cm high × 2.5-cm diam.) were filled with a sterilized mixture (70:29:1) of sand and field soil sterilized at 110°C for 2 hours on 2 successive days, and organic matter sterilized at 70°C for 5 hours. One seedling with 10-cm height from all lines, including the four check lines were inoculated with 300 freshly hatched J2 (4 J2 cm^{-3}). Plants were grown in a growth chamber at 22 ± 3°C, 16 hour artificial photoperiod and 70% relative humidity. The experiments were arranged in a randomized complete block design. Plants were harvested nine weeks after the inoculation. Cysts were extracted from both soils and roots and counted under a stereomicroscope. Washed roots were scanned, and the data of roots were collected (length, surface, volume and number of tips). Based on the number of cysts per plant, the lines were divided into five groups, i.e. Resistant (R), Moderately Resistant (MR), Moderately Susceptible (MS), Susceptible (S) and Highly Susceptible (HS) (Dababat *et al.* 2014).

DNA preparation and AFLP analysis: DNA was extracted from fresh young leaves according to the modified CTAB protocol (Doyle & Doyle 1987). The analyses of AFLP markers were performed according to the protocol described by Vos *et al.* (1995) with some modifications (De Riek *et al.* 2001). Genomic DNA was digested for 2 hours at 37°C with a combination of three restriction enzymes (*EcoRI* + *MseI*; *PstI* + *MseI*). The restricted fragments were ligated with appropriate oligonucleotide adapters. The resulted restriction-adapter ligation mixes were pre-amplified using primers with one selective nucleotide (*EcoRI*+A, *MseI*+C, *PstI*+G and *PstI*+A). Then, a selective amplification was performed using the pre-amplification products, with 13 primer combinations (Table 1); each primer had three selective nucleotides. AFLP fragments were separated by Genetic Analyser.

Statistical analysis and AFLP data visualising: The collected root data were analysed according to standard procedures (analysis of variance). The AFLP data were visualised and scored using GeneMapper-4.1, and the amplified fragments were scored as (1) for presence or (0) for absence for all cultivars and primer combinations. The obtained binary matrix was used as an input for the study of the relationship among the germplasm based on principal component analysis using SPSS-22.

RESULTS

Germplasm resistant reaction*:* The evaluation of all germplasms resulted in the identification of 21 R genotypes (9%), 44 MR genotypes (12.5%), 46 genotypes

Table 1. Adapters and primers sequences used for AFLP analysis

Adapters/primers	Sequence
MseI- adapter (14)	5'-TAC TCA GGA CTC AT-3'
MseI adapter (16)	5'-GAC GAT GAG TCC TGA G-3'
MseI universal primer (M00)	5'-GAT GAG TCC TGA GTA A-3'
M00 + 3 selective bases	M00 + CTT, M00 + CAA, M00 + CAT and M00 + CAG
EcoRI adapter (17)	5'-CTC GTA GAC TGC GTA CC-3'
EcoRI adapter (18)	5'-AAT TGG TAC GCA GTC TAC-3'
EcoRI universal primer (E00)	5'-GAC TGC GTA CCA ATT C-3'
E00 + 3 selective bases	E00 + AGG, E00 + ACG, E00 + AGC, E00 + AAC and E00 + ACC
PstI-ad 15	5'-TGT ACG CAG TCT ACG-3'
PstI-ad 21	5'-CTC GTA GAC TGC GTA CAT GCA-3'
PstI universal primer (P00)	5'-ACT GCG TAC ATG CAG-3'
P00 + 3 selective bases	P00 + ACA, P00 + ACG, P00 + GCT and P00 + GGT

of MS (20%), 85 genotypes of S (37%) and 33 genotypes of HS (14%) (Fig. 1). The highest frequency of R genotypes was observed in the germplasms from 14SYN-JAPAN group (20.8%). The 14SYNT-PYT-HAND group and 14SYNT-PYT group were represented by 6.6% and 4.7% R genotypes, respectively. Among the three groups of germplasms, the R and MR genotypes were most frequently recorded for 14SYN-JAPAN (Fig. 2). The ANOVA of the root data showed significant differences. Also, the descriptive statistics for the collected root data of all germplasms showed high variation for all variables. The cysts number ranged between 0-40 cysts/plant with an average of 13.77. There was a positive correlation between the cysts number and root length ($r^2 = 0.136$) and root surface ($r^2 = 0.107$); the correlation between the root volume and the cysts number was insignificant ($r^2 = 0.76$), and low, negative and insignificant with number of tips ($r^2 = -0.009$). Within the group of 14SYNT- JAPAN a significant correlation between existed between cysts and root surface ($r^2 = 0.143$).

AFLP analysis: In general, all 13 primer combinations detected polymorphisms within the studied germplasms. They amplified a total 552 of AFLP fragments of which 49 (8.9%) were polymorphic. In general, GeneMapper did not show that much polymorphism; some germplasms showed polymorphisms (Fig. 3). AFLP was more effective with wheat genomic DNA using *Pst*I than *Eco*RI. The biplot based on the two functions of the discriminant analysis of the AFLP DNA markers (Fig. 4), showed a clear evidence of the genetic differentiation of the resistant germplasms in the three groups. Moreover, promising

lines derived from 14SYNT-JAPAN group were the most distinct regarding the cysts number when compared to the other two groups. Principal component analysis (PCA) showed that some germplasms which showed a low number of cysts belonged to the three groups; these are pointed out in the biplot (Fig. 4).

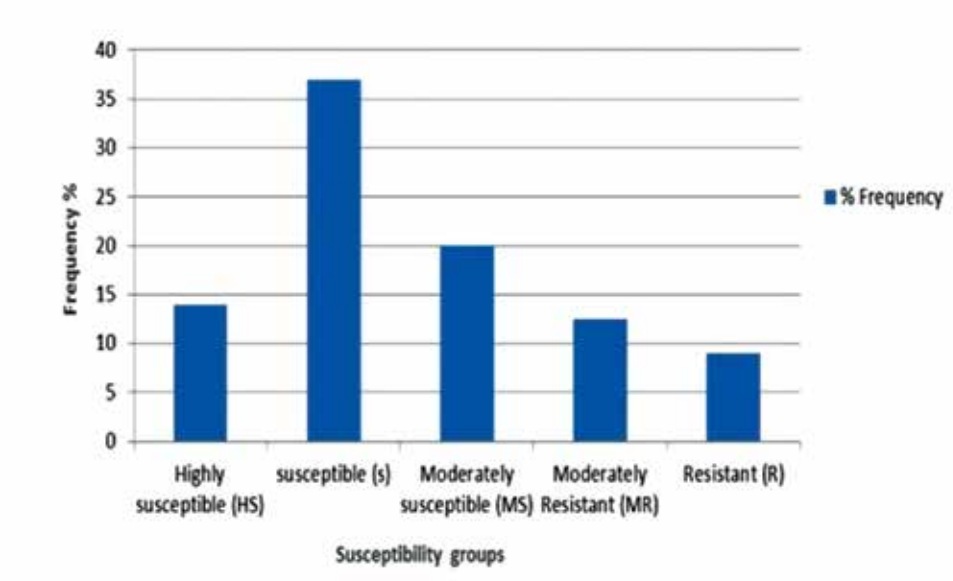


Figure 1. Frequency phenotype (resistance) of all studied synthetic wheat germplasms against Heterodera filipjevi

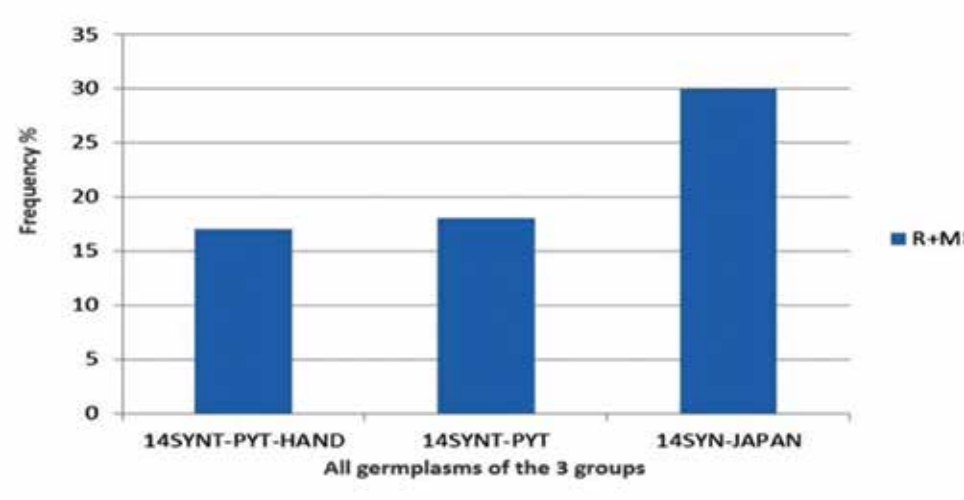


Figure 2. Frequency distribution (%) of the variables resistant (R) and moderately resistant (MR) among the three groups as hosts for Heterodera filipjevi

DISCUSSION

Sometimes only a low level of resistance against cyst nematodes is present in commercial cultivars (Riggs and Schuster 1998). Moreover, breeding in some cultivars resulted in the increase of other nematode problems; e.g. cereal cyst nematode with the associated build-up of *Pratylenchus neglectus* (Turner and Rowe 2006). Hence, using resistant cultivars to control CCN should be checked and evaluated with an accurate breeding scheme.

In this study, the evaluation of all breeding lines from the three groups showed wide variation among genotypes in their responses to *H. filipjevi*. The host efficiency can be described as a continuum from susceptible to resistant. Our results are similar to results obtained during the screening of oat cultivars (*Avena sativa*) against *H. avenae* (Cook and Starr 2006). However, the variation within the highly susceptible (HS) germplasms was lower than in the susceptible ones. This could be due to our newly established rating scale (Dababat *et al.* 2014).

The resistant (R) and moderately resistant (MR) germplasms could be explained by the presence and expression of one or more cereal root eelworm (*Cre*) genes. Two genes, *Cre*8 and *Cre*R, were reported to give a resistance reaction to *H. filipjevi* (Dababat *et al.* 2014). Hence, those germplasms should be crossed with high-yielding cultivars. Many locally adapted wheat varieties are

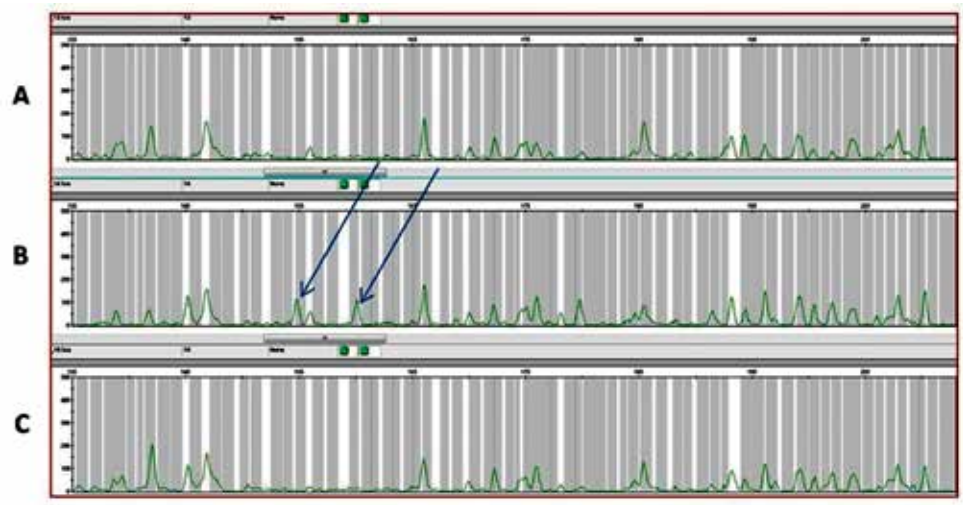


Figure 3. AFLP patterns of wheat germplasms. A, B and C are three patterns of three independent germplasms. Arrows indicate additional fragments (polymorphism). The electropherograms shown are from selective amplification with restriction enzyme/primer combination EcoRI/MseI. Vertical scales: relative fluorescent units; horizontal scales: size of fragment in nucleotides.

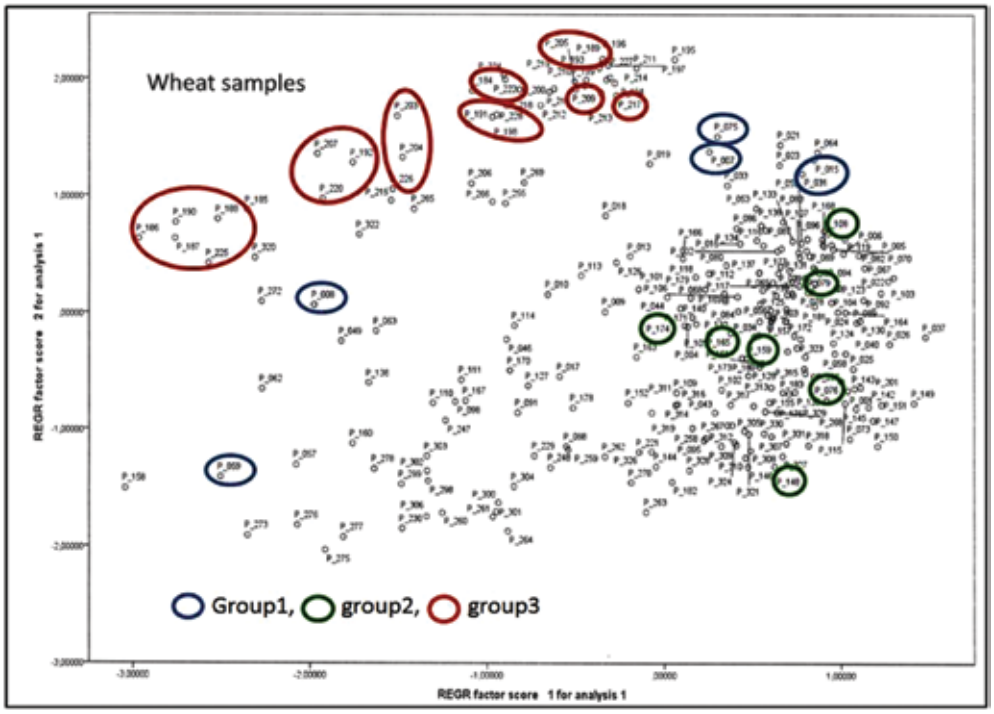


Figure 4. Principal component analysis (PCA) of 230 genotypes from the synthetic wheat germplasms based on AFLP data. Ellipses indicate some germplasms of the three groups that showed a low number of cysts.

susceptible to CCN; the newly found resistant wheat germplasms will allow us to obtain new crosses with local varieties, thereby improving their genetic resistance to CCN.

Usually, and due to the narrow genetic base, synthetic wheat shows relatively low levels of polymorphism for RFLP loci (Chao *et al.* 1989); less than 10% of all RFLP loci are polymorphic in an intraspecific context (Röder *et al.* 1998). The detected polymorphism between all screened germplasms using different AFLP primer combinations clearly shows that genetic differentiation occurred between wheat germplasms during the cross. The better AFLP effectiveness of *Pst*I rather than *Eco*RI is probably due to the high G + C content of *Pst*I recognition sites relative to *Eco*RI resulting in preferential targeting of low-copy/gene-rich regions of the genome (Langridge and Chalmers 1998).
Further study using advanced techniques such as single-nucleotide polymorphisms (SNPs) detection, second-generation sequencing (SGS), or genotyping by sequencing (GBS) is still needed to characterise and identify the location of the genes involved in the resistant lines against *H. filipjevi.*

ACKNOWLEDGMENT

The first author appreciates Monsanto's Beachell-Borlaug International Scholars Program – MBBISP for the financial support for his PhD research. Also, the first author would like to thank Mrs. Veerle Buysens and Mrs. Laurence Desmet for their technical help in the lab.

REFERENCES

- Bekal S, Jahier J, Rivoal R (1998) Host responses of different Triticeae to species of the cereal cyst nematode complex in relation to breeding resistant durum wheat. *Fundamental and Applied Nematology,* 21, 359-370.
- Brown RH (1985) The selection of management strategies for controlling nematodes in cereals. *Agriculture, Ecosystem and Environment,* 12, 371-388.
- Chao S, Sharp PJ, Worland AJ, Warham EJ, Koebner RMD, Gale MD (1989) RFLP-based genetic maps of wheat homoeologous group 7 chromosomes. *Theoretical and Applied Genetics*, 78, 495–504.
- Cook R, Starr JL (2006) Resistant Cultivars. In: 'Plant Nematology' (Eds. RN Perry, M Moens) (CAB International, Wallingford, UK).
- Dababat AA, Imren M, Erginbas-Orakci G, Ashrafi S, Yavuzaslanoglu E, Toktay H, Pariyar SR, Elekcioglu HI, Morgounov AI, Mekete T (2015) The importance and management strategies of cereal cyst nematodes, *Heterodera* spp., in Turkey. *Euphytica*, 202, 173-188.
- Dababat AA, Erginbas-Orakçı G, Toktay H, Imren M, Akin B, Braun HJ, Dreisigacker S, Elekcioğlu IH, Morgounov A (2014) Resistance of winter wheat to *Heterodera filipjevi* in Turkey. *Turkish Journal of Agriculture and Forestry*, 38,180-186.
- De Riek J, Calsyn E, Everaert I, Van Bockstaele E, De Loose M (2001) AFLP based alternatives for the assessment of distinctness, uniformity and stability of sugar beet varieties. *Theoretical and Applied Genetics*, 103, 1254-1265.
- Doyle J, Doyle JL (1987) Genomic plant DNA preparation from fresh tissue – the CTAB method. *Phytochemical Bulletin*, 19, 11-15.
- Fenwick DW (1940) Methods for the recovery and counting of cysts of *Heterodera schachtii* from soil. *Journal of Helminthology,* 18, 155–172.
- Hajihasani A, Tanha Maafi Z, Nicol JM, Rezaee S (2010b) Effect of the cereal cyst nematode, *Heterodera filipjevi* on wheat in microplot trials. *Nematology*, 12, 357–363.

- Hajihasani A, Tanha Maafi Z, Nicol JM, Seraji A (2010a) Relationships between population densities of the cereal cyst nematode, *Heterodera latipons* and yield losses of winter wheat in microplots. *Australasian Plant Pathology*, 39, 330–355.
- Ibrahim AM, Al Hazmi AS, Al Yahya FA, Alderfasi AA (1999) Damage potential and reproduction of *Heterodera avenae* on wheat and barley under Saudi field conditions. *Nematology*, 1, 625-630.
- Langridge P, Chalmers K (1998) Techniques for marker development. In: 'Proceedings of the Ninth International Wheat Genetics Symposium - Volume 1.' (Ed. AE Slinkard). pp. 107–117. (University Extension Press, University of Saskatchawan, Saskatoon, Saskatchawan, Canada).
- Nicol JM, Rivoal R (2008) Global knowledge and its application for the integrated control and management of nematodes on wheat. In: 'Integrated management and biocontrol of vegetable and grain crops nematodes. Vol. 2.' (Eds. A Ciancio, KG Mukerji) pp. 243-287. (Springer, Dordrecht, The Netherlands).
- Nicol JM, Bolat N, Sahin E, Tülek A, Yıldırım AF, Yorgancılar A, Kaplan A, Braun HJ (2006) The cereal cyst nematode is causing economic damage on rainfed wheat production systems of Turkey. *Phytopathology*, 96, S169. [abstract].
- Ogbonnaya FC, Seah S, Delibes A, Jahier J, Lopez-Brana I, Eastwood RF, Lagudah ES (2001) Molecular-genetic characterisation of a new nematode resistance gene in wheat. *Theoretical and Applied Genetics*, 102, 623-629.
- Riggs RD, Schuster RP (1998) Management. In: 'The Cyst Nematodes.' (Ed. Sharma SB). pp. 388-416. (Kluwer Academic Publishers, Dortrecht, The Netherlands).
- Röder MS, Korzun V, Wendehake K, Plaschke J, Tixier MH, Leroy P, Ganal MW (1998) A microsatellite map of wheat. *Genetics*, 149, 2007–2023.
- Sahin E, Nicol JM, Elekcioglu H, Rivoal R (2010) Hatching of *Heterodera filipjevi* in controlled and natural temperature conditions in Turkey. *Nematology*, 12, 193–200.
- Sikora RA (1988) Plant parasitic nematodes of wheat and barley in temperate and semi-arid region. A comparative analysis. In: 'Nematodes Parasites to Cereals and Legumes in Temperate Semi-arid Regions.' (Eds. MG Saxena, RA Sikora, JP Srivastava) pp. 46-48. (ICARDA: Aleppo, Syria).
- Toumi F, Waeyenberge L, Viaene N, Dababat A, Nicol JM, Ogbonnaya F, Moens M (2013) Development of two species-specific primer sets to detect the cereal cyst nematodes *Heterodera avenae* and *Heterodera filipjevi*. *European Journal of Plant Pathology*, 136, 613-624.

- Turner ST, Rowe JA (2006) Cyst Nematodes. In: 'Plant Nematology.' (Eds. RN Perry, M Moens). (CAB International, Wallingford, UK).
- Vos P, Hogers R, Reijans M, van de Lee T, Hornes M, Friters A (1995) AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research*, 23, 4407–4414.
- Yan GP, Smiley RW (2009) Distinguishing *Heterodera filipjevi* and *H. avenae* using polymerase chain reaction-restriction fragment length polymorphism and cyst morphology. *Phytopathology*, 100, 216-224.

INVESTIGATING THE RESISTANCE MECHANISMS OF WHEAT VARIETIES AGAINST ROOT LESION NEMATODES (*PRATYLENCHUS THORNEI* AND *P. NEGLECTUS*) AND CYST NEMATODES (*HETERODERA AVENAE* AND *H. FILIPJEVI*)

ECE B. KASAPOĞLU[1,*], MUSTAFA İMREN[2], ADEM ÖZARSLANDAN[3], TOHID BEHMAND[1], AND İBRAHIM HALİL ELEKCİOĞLU[1]

[1]Cukurova University, Faculty of Agriculture, Department of Plant Protection, 01330-Sarıcam, Adana, Turkey; [2]Abant İzzet Baysal University, Faculty of Agriculture and Natural Science, Department of Plant Protection, Gölköy Kampüs, 14280 Bolu; [3]Biological Control Research Institute, Adana, Turkey. *Corresponding author email: ecekasapoglu@gmail.com

SUMMARY

Plant-parasitic nematodes affect wheat production worldwide and cause yield reduction of 10-20%. Root lesion nematodes (*Pratylenchus* spp.) and cyst nematodes (*Heterodera* spp.) are the most important plant-parasitic nematodes attacking wheat crops. Important species of those two nematode genera in wheat fields were identified and quantified in various production areas in Turkey. Especially, root lesion nematodes (*Pratylenchus thornei* and *P. neglectus*) and cereal cyst nematodes (*Heterodera avenae*, *H. filipjevi* and *H. latipons*) have been detected as the most important wheat nematodes. Wild landrace and national wheat varieties which were moderately resistant to these nematodes have been studied and promising results have been obtained. However, studies on

* Kasapoğlu EB, İmren M, Özarslandan A, Behmand T, Elekçioğlu İH (2015) Investigating the resistance mechanisms of wheat varieties against root lesion nematodes (*Pratylenchus thornei* and *P. neglectus*) and cyst nematodes (*Heterodera avenae* and *H. filipjevi*). In 'Nematodes of Small Grain Cereals: current status and research.' (AA Dababat, H Muminjanov and RW Smiley) pp. 251-256. (FAO: Ankara, Turkey).

molecular- and cellular-level resistance mechanisms are not fully understood. Hence, resistant, tolerant and susceptible varieties will be investigated *in-vitro* and under natural conditions in the Eastern Mediterranean and Central Anatolia Regions, and plant- nematode interactions at molecular- and cellular-levels will be assessed.

INTRODUCTION

Turkey is an important wheat growing country with 9.5 million ha production area and 20 million tons of grain produced per year. Plant-parasitic nematodes are an important threat to this production in that they are estimated to cause 7-15% yield loss (Sasser and Freckman 1987, Whitehead 1998). Among this group, the root lesion nematodes (RLN; *Pratylenchus* spp.) and cereal cyst nematodes (CCN; *Heterodera* spp.) are the most important plant-parasitic nematodes in wheat fields. Economically important parasitic species have already been determined in Turkey. The RLNs *Pratylenchus thornei* and *P. neglectus*, and the CCNs *Heterodera avenae*, *H. filipjevi* and *H. latipons* have been determined to be the most important species.

Screening for resistance against these nematodes has been increased in Turkey during recent years. Especially, there has been a focus on resistance to *H. avenae, H. filipjevi* and *P. thornei.* Promising results were obtained from those studies.

In this study, the resistance reactions of wheat varieties against RLN and CCN under *in-vitro* conditions will be re-investigated, the reactions of varieties found to be resistant and tolerant will be assessed under natural conditions, and the resistant genes and the CCN feeding sites (syncytium cells) will be studied.

METHODS

We will extract CCN from wheat fields known to be infested near Adana (*H. avenae*) and Bolu (*H. filipjevi*). Seed of resistant and tolerant wheat varieties will be obtained (Toktay 2008, İmren *et al.* 2013). Cysts will be surface sterilised (0.5% NaOCl for 10 min), rinsed in distilled water, and stored at 4°C. To initiate hatching, stored cysts will be moved to room temperature (range between 10 to 15°C) (İmren 2013). Freshly hatched second-stage juveniles (J2) will be used as inoculum *in-vitro* and under natural conditions. The popu-

lation of RLN will be from a single nematode collected from a wheat field at Adana, which will then be cultured on carrot discs.

Following seed germination in petri dishes, a single wheat seed will be planted in standard tubes (13-cm high × 3-cm diam.) filled with a mixture of sterilised sand, field soil, and organic matter. The field soil and sand will be sieved and sterilized at 121°C. After plant emergence, five tubes will be selected per genotype and will be inoculated with 175 freshly-hatched J2 of *H. avenae* or *H. filipjevi*, or with 175 mixed stages (J2, J3, J4 and adult) of *P. thornei* or *P. neglectus*. Inoculum will be placed in three holes around the stem base. Some trials will be performed with mixtures of pathogens. Plants inoculated with a CCN will be harvested nine weeks after inoculation and the number of cysts will be counted. *P. thornei* and *P. neglectus* will be extracted from roots and soil using the modified Baermann funnel and mist extraction methods (Southey 1986) and the number of nematodes/plant will be calculated.

Experiments will be conducted under natural field conditions in the Eastern Mediterranean and Central Anatolia regions.

The defence mechanisms for resistant or tolerant reactions will be carried out under *in-vitro* and natural conditions. The durability of resistant and/or tolerant genotypes against the RLN and CCN will be investigated by using *Cre* genes for CCN and sources of multiple resistance alleles for RLN. The plant DNA will be isolated from leaves and Polymerase Chain Reaction (PCR) will be performed.

PROSPECTUS

Resistant, tolerant and susceptible wheat varieties will be validated against CCN and RLN under *in-vitro* conditions, and their reactions under natural conditions will also be determined. Molecular and cellular evaluations of nematode-plant interactions will also be assessed. Resistant, tolerant, susceptible national wheat varieties which were previously identified as resistant against CCN and RLN are the main focus of our work in Turkey. The necessary nematode inoculum sources will be acquired from the Eastern Mediterranean and Central Anatolia regions. The reactions of different populations of the same nematode species are known to affect the resistance outcome. Therefore, different nematode populations from the same nematode species in previous studies will be tested under *in-vitro* conditions to examine both the pathogen

effects and the interactions. The findings of previous studies under *in-vitro* conditions will be examined by our testing under natural conditions. Thus, in this study, the resistant and tolerant wheat varieties will be examined for reactions to plant-parasitic nematodes of wheat not only under *in-vitro* but also under natural conditions. The resistance mechanism and the histological changes in plant tissues will be investigated. Resistant and tolerant wheat varieties will also be evaluated for breeding programs.

ACKNOWLEDGMENTS

This work has been supported by the TUBITAK- 2140419 project.

REFERENCES

- İmren M, Toktay H, Bozbuğa R, Dababat A, Özkan H, Elekçioğlu İH (2013) 'Bazı buğday genotiplerinin Tahıl kist nematodu *Heterodera avenae* (Wollenweber, 1924) karşı dayanıklılıklarının araştırılması', Türkiye Entomoloji Dergisi, 37, 229-238.
- İmren M (2013) 'Doğu Akdeniz ve Güneydoğu Anadolu Bölgeleri Buğday Alanlarında Tahıl Kist Nematodu Türlerinin (*Heterodera avenae group*) Belirlenmesi ve Bazı Buğday Genotiplerinin *Heterodera avenae* (Wollenweber, 1924)'ye Karşı Dayanıklılığın Araştırılması', Doktora Tezi, Fen Bilimleri Enstitüsü, Çukurova Üniversitesi, Adana.
- İmren M, Bozbuğa R, Kasapoğlu EB, Toktay H, Dababat A, Elekcioğlu İH (2014) 'Doğu Akdeniz Bölgesi Koşullarında Tahıl Kist Nematodu, *Heterodera avenae*'nin (Wollenweber, 1924) Buğdayda Meydana Getirdiği Ürün Kaybının Belirlenmesi', 3-5 Şubat, 2014, Antalya, Türkiye.
- Sasser JN and Freckman DW (1987) A world perspective on nematology: the role of the Society. In: 'Vistas on Nematology.' (Eds. JA Veech, DW Dickson) pp. 7-14. (Society of Nematologists, Hyattsville, Maryland, USA).
- Southey JF (1986) Principles of sampling for nematodes. In: 'Labarotory Methods for Work with Plant and Soil Nematodes.' (Ed. JF Southey) pp. 1-4. (Her Majesty's Stationery Office, London, UK).
- Şahin E (2010) 'Orta Anadolu Buğday Alanlarında Önemli Bitki Paraziti Nematodların Belirlenmesi ve Hububat Kist Nematodu *Heterodera filipjevi* nin Biyolojisi İle Mücadelesi Üzerine Çalışmalar.' Doktora tezi, Çukurova Üniversitesi, Fen Bilimleri Enstitüsü, Adana.

- Toktay H (2008) Bazı Yazlık Buğday Çeşitlerinin *Pratylenchus thornei* Sher et Allen (Tylenchida: Pratylenchidae)'ye Karşı Dayanıklılığının Araştırılması. Çukurova Üniversitesi Fen Bilimleri Enstitüsü, (Basılmamış) Doktora Tezi, Adana, Turkey.
- Whitehead AD (1998) Plant Nematode Control. (CABI International, Wallingford, UK).

RESISTANCE SCREENING OF WHEAT ACCESSIONS CHARACTERIZED FOR *CRE1* AND *CRE3* AGAINST *HETERODERA FILIPJEVI* (MADZHIDOV, 1981) STELTER (TYLENCHIDA: HETERODERIDAE)

ELIF YAVUZASLANOĞLU[1,*], I. HALIL ELEKÇIOĞLU[2], AND JULIE M. NICOL[3]

[1]University of Karamanoglu Mehmetbey, Technical Sciences Vocational School, Department of Plant and Animal Production, Karaman, Turkey. [2]Cukurova University, Faculty of Agriculture, Dept. of Plant Protection, Adana, Turkey. [3]International Maize and Wheat Improvement Centre (CIMMYT). *Corresponding author email: eyavuzaslanoglu@kmu.edu.tr

SUMMARY

A set of wheat material which was characterized for presence of *Cre1* and *Cre3* genes was tested for resistance to *Heterodera filipjevi* in 2006-2007 under controlled conditions at 20°C and 70% humidity. The 70 accessions contained Turkish bread and durum wheat, both modern varieties and land races, introduced and International Winter Wheat Improvement Program (IWWIP) developed cultivars, and wild relatives of wheat. Two of each *Cre1* and *Cre3* positive and one negative check cultivar were also tested. The nematode population was obtained from Haymana, Ankara from a naturally-infested field. Wheat accessions were screened with seven replications using a complete randomized block design. Two hundred 2nd-stage juveniles were inoculated per tube in a sand:soil:organic matter medium (70:29:1). Wheat accessions were divided into five groups according to produced cyst numbers. Thirteen accessions produced means of

* Yavuzaslanoğlu E, Elekçioğlu IH, Nicol JM (2015) Resistance screening of wheat accessions characterized for *Cre1* and *Cre3* against *Heterodera filipjevi* (Madzhidov, 1981) Stelter (Tylenchida: Heteroderidae). In 'Nematodes of Small Grain Cereals: current status and research.' (AA Dababat, H Muminjanov and RW Smiley) pp. 257-264. (FAO: Ankara, Turkey).

0-2 cysts and were inferred as resistant, eight accessions produced means of 3-4 cysts and were inferred as moderately resistant, and other accessions produced averages of five to 16 cysts and were moderately susceptible to highly susceptible. *Cre1* check cultivar Silverstar showed a resistant reaction and Goroke was susceptible. The only wheat accession positive for *Cre1*, Sardari, was moderately resistant. None of the wheat accessions had *Cre3* and the *Cre3* positive check cultivars were highly susceptible to *H. filipjevi.* This experiment clearly indicated that the *Cre1* gene provided some level of resistance against the *H. filipjevi* Haymana population while the *Cre3* gene was not effective.

INTRODUCTION

Wheat is the most common commodity in Turkey; producing 22 million tonnes in 2013 (FAO 2015). The Central Anatolian Plateau is the main wheat production area in Turkey and provides 17,4 % of total wheat production in Turkey (TUIK 2015). The main nematode constraints causing yield loss on wheat are cereal cyst and root lesion nematodes in Turkey (Nicol 2004).

The cereal cyst nematode *Heterodera filipjevi* is a widespread nematode species causing damage on wheat in the Central Anatolian Plateau (Rumpenhorst *et al.* 1996; Oztürk *et al.* 1998; Abidou *et al.* 2005; Yavuzaslanoglu *et al.* 2012). The principle control strategy for this nematode damage is to keep the nematode population under economical damage levels in large areas. Due to the economical and environmental concerns, the best control option is the use of resistant cultivars. Therefore it is necessary to find the most effective plant genetic resources effective against the present nematode constraints in local production areas considering the pathotype and ecotype forms of these plant-parasitic nematodes.

Resistance genes were identified for *H. avenae*, which is the most widespread cereal cyst nematode species in the world (Ogbonnaya *et al.* 2001). However it is necessary to investigate these *Cre* genes for availability among germplasm that can be used by the local farmers. *Cre1* and *Cre3* genes were molecularly characterized for a set of wheat materials available for growers in Turkey by Akar *et al.* (2009). None of the tested material had *Cre3* gene but one of the IWWIP developed cultivar, Sardari, was found to have *Cre1* gene. Nevertheless it is not completely useful without knowing the reaction of resistance gene carrying wheat sources against local nematode isolates. Therefore these molecularly characterized wheat materials were tested against a local nematode isolate of the cereal cyst nematode; *H. filipjevi,* Haymana population under controlled conditions.

MATERIAL AND METHODS

Wheat accessions: A total of 70 accessions were tested, consisting of 22 bread wheat (*Triticum aestivum* spp. *aestivum*), nine durum wheat (*Triticum aestivum* spp. *durum*), 18 cultivars developed by the International Winter Wheat Improvement Program (IWWIP) (*Triticum aestivum* spp. *aestivum*), six introduced cultivars (*Triticum aestivum* spp. *aestivum*) and 15 wild relatives including one accession of *Aegilops vavilovi*, one accession of *Aegilops speltoides aucheri*, one accession of *Aegilops speltoides lugisticon*, one accession of *Aegilops tauschii*, two accessions of *T. dicoccum*, 9 accessions of *T. dicoccoides*. Reference cultivars were two positive checks for *Cre1* (Silverstar and Goroke), two positive checks for *Cre3* (92.001E7.32.5 and VM272), and one negative check for *Cre1* and *Cre3* (Hartog).

Experimental procedure: Wheat accessions were grown in test tubes (30 × 130 mm) in the glasshouse at 20 ± 5 °C, 70% humidity, and a 16 hour light and 8 hour dark period for nine weeks. Nematodes were inoculated as 200 newly-hatched second stage juveniles per test tube at two times over a day, just after planting onto sterilized sand:soil:organic matter mixture (70:29:1 w/w %). Each wheat accession was replicated seven times and arranged in a completely randomized block design in containers. Results were evaluated as mean number of cysts per tube both on roots and in soil. Accessions were arranged as five groups according to cyst numbers produced (Table 1).

Table 1. Adapters and primers sequences used for AFLP analysis

Adapters/primers	Sequence
MseI- adapter (14)	5'-TAC TCA GGA CTC AT-3'
MseI adapter (16)	5'-GAC GAT GAG TCC TGA G-3'
MseI universal primer (M00)	5'-GAT GAG TCC TGA GTA A-3'
M00 + 3 selective bases	M00 + CTT, M00 + CAA, M00 + CAT and M00 + CAG
EcoRI adapter (17)	5'-CTC GTA GAC TGC GTA CC-3'
EcoRI adapter (18)	5'-AAT TGG TAC GCA GTC TAC-3'
EcoRI universal primer (E00)	5'-GAC TGC GTA CCA ATT C-3'
E00 + 3 selective bases	E00 + AGG, E00 + ACG, E00 + AGC, E00 + AAC and E00 + ACC
PstI-ad 15	5'-TGT ACG CAG TCT ACG-3'
PstI-ad 21	5'-CTC GTA GAC TGC GTA CAT GCA-3'
PstI universal primer (P00)	5'-ACT GCG TAC ATG CAG-3'
P00 + 3 selective bases	P00 + ACA, P00 + ACG, P00 + GCT and P00 + GGT

RESULTS AND DISCUSSION

Reaction of 70 tested wheat accessions ranged from resistant to highly susceptible (Table 2). The most resistant accession was Kirmizi Misri (durum wheat), on which no cyst formed, while the most susceptible accession was Long Yuan 994 (IWWIP developed cultivar), on which an average of 16 and a maximum of 33 cysts formed per plant. Most of the durum varieties showed a resistant reaction against the *H. filipjevi* Haymana population except Akbugday, a variety which had a moderately susceptible reaction (mean: 6,14 ± 1,62 (max-min : 0-12) cysts/plant). The only resistant wild relative was *Aegilops speltoides aucheri* (mean: 1,86 ± 1,26 (max-min : 0-9) cysts/plant), and others showed a moderately resistant to highly susceptible reaction.

The positive check cultivars for *Cre1* differed, with Silverstar being resistant (mean: 2,43 ± 0,75 (max-min : 0-6) cysts/plant) and Goroke being susceptible with (mean: 7 ± 2,79 cysts/plant). The only cultivar positive for *Cre1* gene, Sardari, produced a mean of 2,57 ± 1,48 (max-min : 0-11) cysts/plant and was ranked as moderately resistant to *H. filpjevi*. Both of the *Cre3* positive check varieties, 92.001E7.32.5 (mean: 8,75 ± 3,90 (max-min: 2-20) cysts/plant) and VM272 (mean: 9,14 ± 2,70 (max-min : 1-21) cysts/plant) were highly susceptible. The negative check for *Cre1* and *Cre3*, Hartog, had a moderately resistant reaction with mean: 3,57 ± 1,67 (max-min : 0-12) cysts/plant.

The reaction of a wide spectrum of wheat material was determined with the current study. The tested wheat materials are used in breeding programs in Turkey mainly for winter wheat and also for spring wheat development. It is useful to have information about nematode resistance sources in the wheat material used in those programs. Even though none of the material had the *Cre3* gene, the susceptible reaction of *Cre3* positive check cultivars indicates the usefulness of *Cre3* sources for breeding for resistance against the *H. filipjevi* Haymana population. Only one wheat accession, cultivar Sardari, had *Cre1* and it showed a level of resistance. The *Cre1*-containing check cultivar Silverstar also had a level of resistance while another *Cre1* check cultivar, Goroke, was susceptible. Toktay *et al.* (2012 and 2013) reported susceptibility of different *Cre1* sources against different populations of *H. filipjevi* in Turkey. The three populations of *H. filipjevi* from Afsin, Elbistan and Yozgat were determined as pathotype Ha33 by Toktay *et al.* (2013) and were shown to be susceptible against *Cre1* sources of AUS10894 and LoroxKoga. However the pathotype of the Haymana population is yet to be determined. Determination of pathotypes

Table 2. The resistance reaction of accessions against Heterodera filipjevi Haymana population.

Accession Name	Triticum Accession species	ww/sw	Cysts/plant[1]	Reaction[2]
Kirmizi Misri	T. aestivum spp. durum	ww	0 ± (0-0)	R
Sari Bursa	T. aestivum spp. durum	ww	0 ± (0-0)	R
Sorgül	T. aestivum spp. durum	ww	0,29 ± 0,18 (0-1)	R
Yelken	T. aestivum spp. durum	ww	0,33 ± 0,33 (0-2)	R
Tam-107	T. aestivum spp. aestivum IWWIP	ww	0,43 ± 0,30 (0-2)	R
AUS4930.7.2	T. aestivum spp. aestivum (introduced)	sw	0,5 ± 0,29 (0-1)	R
Kunduru	T. aestivum spp. durum	ww	0,6 ± 0,24 (0-1)	R
MV17/3/CROC_1/ AE.SQUARROSA (205) // KAUZ	T. aestivum spp. astivum IWWIP	ww	0,66 ± 0,49 (0-3)	R
Altindane 12	T. aestivum spp. durum	ww	0,83 ± 0,31 (0-2)	R
Germir	T. aestivum spp. durum	ww	1,5 ± 0,70 (0-6)	R
Aegilops speltoides aucheri	Wild relative	sw	1,86 ± 1,26 (0-9)	R
Silverstar	T. aestivum spp. aestivum (positive check for Cre1)	sw	2,43 ± 0,75 (0-6)	R
Üveyik	T. aestivum spp. durum	ww	2,43 ± 1,94 (0-14)	R
Sardari	T. aestivum spp. astivum IWWIP (positive check for Cre1)	ww	2,57 ± 1,48 (0-11)	MR
T. dicoccoides-3	Wild relative	sw	2,57 ± 1,31 (0-109)	MR
T. dicoccoides-5	Wild relative	sw	2,71 ± 1,30 (0-9)	MR
Aegilops vavilovi	Wild relative	sw	3,43 ± 0,69 (1-6)	MR
Hartog	T. aestivum spp. aestivum (negative check for Cre1 & Cre3)	sw	3,57 ± 1,67 (0-12)	MR
Aegilops speltoides lugis-ticon	Wild relative	sw	3,57 ± 1,70 (0-10)	MR
Aegilops tauschii	Wild relative	sw	3,67 ± 0,88 (2-4)	MR
Yakar	T. aestivum spp. aestivum	ww	3,71 ± 1,34 (0-9)	MR
T. dicoccum-1	Wild relative	sw	4,57 ± 2,22 (0-14)	MS
Altay	T. aestivum spp. aestivum	ww	4,86 ± 2,11 (0-17)	MS
HN7/OROFEN//BJN8/3/ SERI82/4/74CB462/TRAP-PER//VONA	T. aestivum spp. aestivum IWWIP	ww	4,88 ± 1,51 (0-14)	MS
Çetinel	T. aestivum spp. aestivum	ww	5 ± 1,19 (1-12)	MS
T. dicoccoides-7	Wild relative	sw	5 ± 1,31 (0-9)	MS
SHARK/F4105W2.1	T. aestivum spp. aestivum IWWIP	ww	5,25 ± 4,31 (0-18)	MS
T. dicoccoides-4	Wild relative	sw	5,29 ± 1,95 (0-13)	MS
Göksu	T. aestivum spp. aestivum	ww	5,43 ± 1,41 (1-10)	MS
Burbot-6	T. aestivum spp. aestivum IWWIP	ww	5,43 ± 0,72 (3-8)	MS
AUS4930 5.3/Spear DH#44	T. aestivum spp. aestivum (introduced)	sw	5,71 ± 2,25 (0-15)	MS
Gallya-aral1	T. aestivum spp. aestivum IWWIP	ww	5,86 ± 1,18 (3-11)	MS
Süzen	T. aestivum spp. aestivum	ww	6 ± 0,87 (3-8)	MS
Sakin	T. aestivum spp. aestivum	ww	6 ± 2,01 (0-16)	MS
Akbugday	T. aestivum spp. durum	ww	6,14 ± 1,62 (0-12)	MS
VEE/TSI//GRK/3/ SU-ZEN97	T. aestivum spp. aestivum IWWIP	ww	6,25 ± 1,69 (1-17)	MS
T. dicoccoides-2	Wild relative	sw	6,33 ± 2,74 (0-18)	MS
Bayrak	T. aestivum spp. aestivum	ww	6,57 ± 2,69 (0-19)	S

Accession Name	Triticum Accession species	ww/ sw	Cysts/plant[1]	Reaction[2]
Pehlivan	T. aestivum spp. aestivum	ww	6,71 ± 1,66 (1-11)	S
T. dicoccoides-1	Wild relative	sw	6,71 ± 2,30 (0-16)	S
JUP/4/CLLF/3/II14-53 / ODIN//CI134431/ SEL6425/ WA00477/5/CROC_1/ AE.SQUAR-ROSA (213)// PGO	T. aestivum spp. aestivum IWWIP	ww	6,86 ± 1,50 (3-15)	S
Goroke	T. aestivum spp. aestivum (positive check for Cre1)	sw	7 ± 2,79 (0-17)	S
T. dicoccoides-9	Wild relative	sw	7 ± 2,26 (2-17)	S
Zincirci	T. aestivum spp. aestivum	ww	7 ± 1,41 (1-12)	S
Konya	T. aestivum spp. aestivum	ww	7 ± 1,02 (3-10)	S
AUS 4930.7/2* PASTOR-2	T. aestivum spp. aestivum (introduced)	sw	7 ± 1,62 (2-12)	S
AUS 4930.7/2* PASTOR-1	T. aestivum spp. aestivum (introduced)	sw	7,14 ± 2,31 (1-18)	S
Bezostaya	T. aestivum spp. aestivum	ww	7,14 ± 2,03 (1-14)	S
Lanser	T. aestivum spp. aestivum	ww	7,43 ± 2,52 (0-16)	S
Sultan	T. aestivum spp. aestivum	ww	7,5 ± 1,69 (0-12)	S
Jagger	T. aestivum spp. aestivum IWWIP	ww	7,57 ± 2,72 (1-18)	S
Gerek	T. aestivum spp. aestivum	ww	7,57 ± 1,94 (0-13)	S
Ikizce	T. aestivum spp. aestivum	ww	7,71 ± 1,54 (1-14)	S
Harmankaya	T. aestivum spp. aestivum	ww	7,86 ± 1,60 (3-14)	S
T. dicoccum-2	Wild relative	sw	8,14 ± 2,42 (1-20)	S
Sabalan	T. aestivum spp. aestivum IWWIP	ww	8,5 ± 1,43 (4-17)	HS
92.001E7.32.5	T. aestivum spp. aestivum (positive check for Cre3)	sw	8,75 ± 3,90 (2-20)	HS
ID800994.W/VEE/3/ CHEN/AE.SQUAR-RO-SA(TAUS)//BCN	T. aestivum spp. aestivum IWWIP	ww	9 ± 3,79 (0-28)	HS
PYN/BAU	T. aestivum spp. aestivum IWWIP	ww	9 ± 2,05 (2-18)	HS
Gun	T. aestivum spp. aestivum	ww	9 ± 2,67 (1-22)	HS
Boema	T. aestivum spp. aestivum IWWIP	ww	9,14 ± 2,59 (3-17)	HS
VM272	T. aestivum spp. aestivum (positive check for Cre3)	sw	9,14 ± 2,70 (1-21)	HS
Mizrak	T. aestivum spp. aestivum	ww	9,43 ± 2,19 (4-18)	HS
T. dicoccoides-6	Wild relative	sw	9,57 ± 2,40 (1-18)	HS
Demir	T. aestivum spp. aestivum	ww	9,57 ± 2,19 (0-18)	HS
Türkmen	T. aestivum spp. aestivum	ww	9,71 ± 2,33 (3-21)	HS
T. dicoccoides-8	Wild relative	sw	10,43 ± 2,93 (2-20)	HS
Atli	T. aestivum spp. aestivum	ww	10,86 ± 1,50 (5-17)	HS
TAM200	T. aestivum spp. aestivum IWWIP	ww	11,86 ± 2,81 (4-27)	HS
AUS4930 5.3/Spear DH#43	T. aestivum spp. aestivum (introduced)	sw	11,86 ± 1,84 (8-22)	HS
AUS 493053 / Spear	T. aestivum spp. aestivum (introduced)	sw	12,13 ± 2,22 (4-22)	HS
Lamar-R32	T. aestivum spp. aestivum IWWIP	ww	12,29 ± 3,54 (1-27)	HS
Karlygash	T. aestivum spp. aestivum IWWIP	ww	12,57 ± 3,26 (4-27)	HS
Menceki	T. aestivum spp. aestivum	ww	13 ± 2,18 (6-24)	HS
Long Yuan 994	T. aestivum spp. astivum IWWIP	ww	16,14 ± 3,95 (2-33)	HS

[1]Mean number of cysts/plant ± SE (range) [2]Resistance reaction: R=resistant, MR=moderately resistant, MS=moderately susceptible, S=susceptible, HS=highly susceptible)

of all populations found in Turkey and resistance genetics against *H. filipjevi* will improve the breeding studies for development of resistant cultivars.

ACKNOWLEDGEMENT

The authors kindly thank to CIMMYT for providing wheat material for the study and TUBİTAK for supporting the study financially (Project Number: 105G013).

REFERENCES

- Abidou H, El-Ahmed A, Nicol JM, Bolat N, Rivoal R, Yahyaoui A (2005) Occurence and distribution of species of the *Heterodera avenae* group in Syria and Turkey. *Nematologia Mediterranea,* 33, 195-201.
- Akar T, Calıskan M, Nicol JM, Uranbey S, Sahın E, Yazar S, Willıam M, Braun HJ (2009) Molecular characterization of Cereal Cyst Nematode diagnostic markers *Cre1* and *Cre3* in some winter wheat germplasm and their potential use against *Heterodera filipjevi*. *Field Crops Research,* 114, 2, 320-323.
- FAO (2015) Turkey 2013 Wheat Production Statistics. http://faostat3.fao.org/
- Nicol JM, Bolat N, Bagcı A, Hekımhan H, Elekcıoglu IH, Tunalı B, Yıldırım AF, Sahın E, Kaplan A, Yorgancılar A (2004) Research on root rots and nematodes-progress update of Turkey- CIMMYT collaboration from 2003, Manhattan, Kansas State University, USA. *Annual Wheat Newsletter,* 50, 172-176.
- Ogbonnaya FC, Subrahmanyam, NC, Moullet O, De Majnik J, Eagles HA, Brown JS, Eastwood RF, Kollmorgen J, Apples R, Lagudah ES (2001) Diagnostic DNA markers for cereal cyst nematode resistance in bread wheat. *Australian Journal of Agricultural Resources,* 52, 1367- 1374.
- Özturk G, Yıldırım, AF, Enneli S (1998) Distribution and frequency of cereal cyst nematodes (*H. avenae* Wollenweber) in Konya wheat growing area. *Proceedings of Turkey Phytopathology Congress*, Ankara, Turkey, pp. 260-264.
- Rumpenhorst HJ, Elekcıoglu IH, Sturhan D, Öztürk G, Eneli S (1996) The cereal cyst nematode *Heterodera filipjevi* (Madzhidov) in Turkey. *Nematologia Mediterranea,* 24, 135-138.
- Toktay H, Yavuzaslanoglu E, Imren M, Nicol J, Elekcioglu IH, Dababat A (2012) Screening for resistance to *Heterodera filipjevi* (Madzhidov) Stelter (Tylenchida: Heteroderidae) and *Pratylenchus thornei* (Sher & Allen) (Tylenchida: Pratylenchidae) sister lines of spring wheat. *Turkish Journal of Entomology,* 36, 4, 455-461.

- Toktay H, Imren M, Bozbuga R, Ergınbas Orakc, G, Dababat A, Elekcıoglu IH (2013) Pathotype characterization of the cereal cyst nematode *Heterodera filipjevi* (Madzhidov, 1981) Stelter in Turkey. *Turkish Journal of Entomology*, 37, 2, 213-219.
- TUİK (2015) Turkey 2013 Wheat Production Statistics, http://tuikapp.tuik.gov.tr/
- Yavuzaslanoglu E, Elekcıoglu HI, Nicol JM, Yorgancılar O, Hodson D, Yıldırım AF, Yorgancılar A, Bolat N (2012) Distribution, frequency and occurrence of cereal nematodes on the Central Anatolian Plateau in Turkey and their relationship with soil physicochemical properties. *Nematology*, 14, 7, 839-854.

RESISTANCE AND TOLERANCE OF SPRING WHEAT AND BARLEY TO *HETERODERA AVENAE* IN THE USA

RICHARD W. SMILEY[1,*] AND JULIET M. MARSHALL[2]

[1]Oregon State University, Columbia Basin Agricultural Research Center, Pendleton, Oregon 97801, USA, and [2]University of Idaho, Idaho Falls, Idaho 83402, USA. *Corresponding author email: richard.smiley@oregonstate.edu

SUMMARY

Spring wheat (39 cultivars) and spring barley (46 cultivars) were evaluated for resistance and tolerance to *Heterodera avenae* (CCN) over a 2-year period. Replicated plots consisted of two 9-m-long plant rows. Paired plots of individual cultivars were either treated with aldicarb (banded in seed rows during planting) or left untreated. During plant anthesis, roots of plants were evaluated for numbers of swollen white females. A plot combine was used to measure grain yields and the difference of yield in treated versus control plots was used to determine relative rates of tolerance. Aldicarb reduced the number of white females on individual plants as much as 93% in wheat and 96% in barley. Aldicarb treatment improved grain yields as much as 77% in wheat and 52% in barley. Several cultivars of wheat and barley were notable in having acceptably balanced levels of both resistance and tolerance. Other cultivars were noted for having a high level of either resistance or tolerance, but not both.

INTRODUCTION

The cereal cyst nematode *Heterodera avenae* Woll., 1924 reduces yields of wheat and barley in localized regions of the Pacific Northwest USA (Smi-

* Smiley RW, Marshall, JM (2015) Resistance and tolerance of spring wheat and barley to *Heterodera avenae* in the USA. In 'Nematodes of Small Grain Cereals: current status and research.' (AA Dababat, H Muminjanov and RW Smiley) pp. 265-274. (FAO: Ankara, Turkey).

ley 2009). Extended rotational intervals between susceptible cereal crops are seldom profitable in this region. Cereals are therefore usually produced as i) 2-year monocultures of wheat alternated with non-planted fallow, ii) 2-year rotations of wheat with a non-host such as potato, pulse or brassica species, or iii) 3-year rotations of winter wheat, spring wheat or spring barley, and either a fallow or a broadleaf non-host.

Adequate management of cereal cyst nematodes in short rotations requires the production of cultivars that express both resistance and tolerance (Cook and Evans 1987). Resistance suppresses or prevents reproduction of the nematode, thereby reducing the density of inoculum available for invading roots of the next-planted cereal crop. Tolerant cultivars are characterized as having an ability to withstand or recover from nematode invasion and to yield well in comparison with non-invaded plants. Roots of both resistant and tolerant cultivars are initially invaded by 2nd-stage juveniles of *H. avenae*, which may then cause an intolerant reaction before the resistance trait becomes expressed in resistant cultivars. This contributes to the inability of some resistant cultivars to produce grain yields that are competitive with more susceptible cultivars. Growers are often reluctant to plant resistant but intolerant cultivars that produce yields that are lower than some susceptible cultivars that are more tol*erant. Cultivars with both resistance and tolerance are therefore required for optimal* yield performance in existing plantings as well as reducing the level of disease risk to subsequent plantings of intolerant cultivars. We were not aware of any commercial North American wheat or barley cultivars that were resistant as well as tolerant of *H. avenae*. The objective of this research was to evaluate spring wheat and spring barley cultivars to determine if this dual resistant-plus-tolerant trait could be identified.

METHODS

Details of these experiments will be published in Marshall and Smiley (2015) and Smiley and Marshall (2016). Briefly, we evaluated 39 spring wheat cultivars and 45 spring barley cultivars in naturally-infested fields near St. Anthony, Idaho (latitude 43.922N, longitude -111.638W) during each of two years (2013 and 2014). The continental climate is semi-arid (352 mm annual precipitation) with cold winters and warm, dry summers. The soil is a gravelly sandy loam and supplemental water was applied to each field by sprinkler irrigation. The trials were performed during the spring grain cycle of a 2-year rotation of potato and a spring cereal. Seed was planted soon after the soil thawed following

the winter freeze. Fields were cultivated by disking after the potato harvest. Pre-plant density of *H. avenae* was determined at the time of planting.

Five experimental blocks were planted during April each year; i) 15 soft white spring wheats, ii) 18 hard red plus six hard white spring wheats, iii) 16 2-row feed barleys, iv) 19 2-row malting barleys, and v) four 6-row feed plus six 6-row malt barleys. Each block consisted of four replicates of each cultivar planted into split plots. Each plot consisted of two adjacent 9-m-long drill rows. Cultivars were randomized within each replicate and each cultivar was split as an adjacent nematicide-treated (Temik 15G, 4.2 kg of aldicarb/ha) and a control plot. Aldicarb was banded with the seed into two rows on one side of the 4-row seed drill. Untreated controls and aldicarb treatments therefore each consisted of two rows to provide side-by-side comparisons of cultivar performance in treated and untreated plots. Fertilizer was banded under all four seed rows at the time of planting. Seed treated with fungicides (difenoconazole, mefenoxam and ipconazole) and insecticide (thiamethoxam) was dispensed through a cone-seeder. The primary objective of this research was to identify cultivars that exhibited an acceptable balance among the resistance and tolerance traits even if they did not exhibit the highest levels of either individual trait. Roots were dug to a 15-cm depth at the time of plant anthesis. After washing, numbers of *H. avenae* swollen white females were counted on roots of five plants per plot. Grain was harvested using a plot-size combine. Tolerance was characterized as the percentage yield increase resulting from application of the nematicide. Data grouped over two years were used to identify cultivars that were balanced by being at least moderately resistant (≤6 swollen females/plant) plus moderately tolerant (≤15% yield increase with nematicide).

RESULTS

Wheat: Initial density of *H. avenae* across the two wheat blocks during 2013 was 22,176 eggs plus juveniles/kg of soil. During 2014 the mean densities for the two blocks were 11,880 eggs plus juveniles/kg of soil for soft white wheat cultivars and 3,309/kg for hard red plus hard white wheat cultivars. The mean number of white females was much higher on roots in the control plots than in the nematicide-treated plots; 19 vs. 4 ($HSD_{0.05}$ = 5) for soft wheats and 19 vs 1 ($HSD_{0.05}$ = 8) for hard wheats. Cultivars differed greatly in susceptibility to *H. avenae*, with numbers of newly produced white *H. avenae* females ranging from <5 to 70 per plant. Aldicarb reduced the number of white females as much as 93% on the most susceptible culti-

var (Westbred 936) and increased grain yield as much as 77% for the least tolerant cultivar (Cataldo). Two hard wheat cultivars exhibited an acceptable balance of both resistance and tolerance; Klasic and WB Rockland (Table 1). These cultivars also produced grain yields that were statistically equivalent to the highest-yielding cultivar in their experimental block. The hard red cultivar BWB9576 was also ranked as tolerant but it was moderately susceptible. None of the soft white wheat cultivars met this dual-trait criteria but two intolerant cultivars were ranked as resistant (08SB0658-B) or moderately resistant (Cataldo). Cultivar 08SB0658-B was not considered both resistant and tolerant because the tolerance rating of 15.5 did not strictly meet our maximum value of 15.0 to achieve that rating.

Barley: The initial density of *H. avenae* across the three barley blocks during 2013 was 22,176 eggs plus juveniles/kg of soil. During 2014 the densities for the three blocks were 3,516, 27,000 and 4,980 eggs plus juveniles/kg of soil for 2-row feed, 2-row malt and 6-row barleys, respectively. Cultivars differed greatly in susceptibility, with numbers of newly produced white *H. avenae* females ranging from <1 to 93 per plant. Aldicarb reduced the number of white females as much as 96% on the most susceptible cultivar (ABI Voyager) and increased grain yield as much as 52% for the least tolerant cultivar (B1202). Seven feed-type barley cultivars were ranked as having a balance of being at least moderately resistant and moderately tolerant (Table 2). These cultivars included the 2-row barleys Champion, Lenetah, Xena, Idagold II and Transit, and the 6-row barleys Millenium and Goldeneye. No malting-type cultivar met this dual-trait criteria. The 2-row malt barley Odyssey was the only cultivar that exhibited resistance but not tolerance; it ranked as very resistant with a mean of <1 swollen female/plant over the 2-year test period. This cultivar was not considered both resistant and tolerant because the tolerance rating of 15.5 did not strictly meet our maximum value of 15.0 to achieve that rating. Four cultivars also limited reproduction of *H. avenae* and were ranked as moderately resistant, including the feed barleys CDC Fibar and Steptoe, and the malt barleys Legacy and Tradition, with means of 4.4, 5.3, 3.8 and 5.6 swollen females/plant, respectively. Eighteen barley cultivars (five 2-row feed types, ten 2-row malt types, one 6-row feed type, and two 6-row malt types) were ranked as being tolerant or very tolerant, but not resistant or moderately resistant. Overall, 69% of the barley cultivars in these trials (31 of 45 entries) were at least moderately tolerant to *H. avenae*.

Table 1. Spring wheat tolerance and resistance to Heterodera avenae

Market class and cultivar	White females[1]	Resistance rating[2]	Yield increase[3]	Tolerance rating[4]	MR + MT5
Soft white					
08SB0658-B	2.6	R	15.5	MI	
11SB0096	16.1	S	24.1	MI	
Alpowa	8.9	MS	7.1	T	
Alturas	25.0	S	16.1	MI	
Babe	15.7	S	20.4	MI	
Cataldo	5.2	MR	39.1	I	
IDO 851	26.6	VS	18.0	MI	
IDO 852	10.6	MS	17.8	MI	
IDO 854	23.0	S	27.9	MI	
Penawawa	21.7	S	17.0	MI	
UI Petit	19.7	S	17.7	MI	
UI Stone	11.1	MS	18.7	MI	
WA 8162	26.6	VS	17.2	MI	
WB6121	20.5	S	11.6	MT	
WBexp-125	28.9	VS	14.5	MT	
Hard red					
Alzada	21.4	S	12.5	MT	
Bullseye	24.2	S	16.6	MI	
BWB9576	7.8	MS	5.8	T	
Cabernet	15.2	S	21.7	MI	
Choteau	11.9	MS	18.3	MI	
Glee	12.4	S	16.9	MI	
IDO 649C	8.9	MS	20.7	MI	
IDO1202S	18.3	S	27.6	MI	
IDO862E	23.9	S	25.0	MI	
IDO862T	15.2	S	16.1	MI	
Jefferson	7.7	MS	38.7	I	
Kelse	13.0	S	18.3	MI	
SY 40240R	24.7	S	23.8	MI	
UI Winchester	19.3	S	19.7	MI	
Volt	31.2	VS	35.4	I	
WB Rockland	1.5	R	14.5	MT	X

WB9229	10.4	MS	26.6	MI	
Westbred 936	60.8	VS	41.9	I	
Hard white					
Blanca Grande	20.8	S	10.4	MT	
Dayn	7.5	MS	14.3	MT	
Klasic	4.4	MR	15.0	MT	X
Snow Crest	26.0	VS	23.7	MI	
WB-Idamax	16.2	S	22.4	MI	
WB-Paloma	26.2	VS	25.8	MI	

[1]Number of H. avenae white females produced per plant in the control (no-nematicide) treatment. [2]Cultivars were very resistant (VR; ≤1 swollen female/plant), resistant (R; 1.1-3), moderately resistant (MR; 3.1-6), mod. susceptible (MS; 6.1-12), susceptible (S; 12.1-25), or very susceptible (VS; >25). [3]Percentage increase in grain yield due to application of nematicide. [4]Cultivars were very tolerant (VT; <5% yield increase), tolerant (T; 5-10%), moderately tolerant (MT; 10-15%), mod. intolerant (MI; 15-30%), intolerant (I; 30-50%), or very intolerant (VI; >50%). [5] Moderately resistant (≤6% swollen females/plant) plus moderately tolerant (≤15% yield increase).

DISCUSSION

Our results supported the generally accepted understanding that there is a comparatively higher level of tolerance among spring barley than spring wheat cultivars (Smiley and Nicol 2009). An increasing order of yield damage caused by *H. avenae* on rye and winter barley, spring barley, winter wheat, spring wheat, winter oats and spring oats has been reported (Andersson 1982, Fisher 1982). This research also supports the prediction (O'Brien and Fisher 1977) that a combination of resistance and tolerance is more likely to be achieved in barley than in wheat or oats because barley is generally more tolerant of *H. avenae* than the other two crop species.

We ranked nine of 39 wheat entries (23%) as tolerant (2 entries) or moderately tolerant (7 entries) and no entries as very tolerant. We also ranked two wheat entries as resistant and two as moderately resistant. A balance of agronomically acceptable resistance plus tolerance occurred in only one cultivar of hard red wheat (WB Rockland) and one cultivar of hard white wheat (Klasic). Similar results were reported earlier for WB Rockland (Smiley *et al.* 2013). We were unsuccessful in identifying these dual traits in the 15 soft white wheat cultivars we examined.

We ranked 69% of the 45 barley entries as very tolerant, tolerant or moderately tolerant; 11, 11 and 9 entries, respectively. We also ranked 12 barley entries as very resistant, resistant or moderately resistant; 10, 1 and 1 entry, respectively. A balance of agronomically acceptable resistance plus tolerance occurred in seven feed barleys (five 2-row and two 6-row types) but not in any malt barleys. However, one malt type (Odyssey) nearly met this dual-trait criterion, in that it was very resistant and nearly moderately tolerant. Neither the resistant cultivars nor the tolerant cultivars were always the highest-yielding cultivars in each trial. However, the long-term goals of producing a dual-trait cultivar are to simultaneously minimize the potential yield suppression caused by the nematode in the current crop and, at the same time, reduce the post-harvest density of eggs to decrease the level of disease risk for the next host crop that will be planted in that field. Six of the seven cultivars with balanced levels of resistance and tolerance (Xena, Champion, Goldeneye, Idagold II, Millenium and Lenetah) produced grain yields in control plots that did not differ significantly from the highest yielding cultivar in the experiment during either year. However, when compared to the highest-yielding cultivars in the 2-row feed barley trial, the cultivar Transit was categorized as resistant and tolerant but produced low yields each year. Transit, Julie, CDC Fibar, and CDC McGwire are hulless high ß-glucan cultivars developed for human food, not as a feed barley which are hulled. Likewise, each of the four cultivars that were rated as susceptible but moderately resistant (CDC Fibar, Legacy, Steptoe and Tradition) were not consistently among the highest-yielding cultivars within each of their experiments. Of the 18 cultivars that were rated as very tolerant or tolerant but not resistant, seven yielded among the top-ranked within their experiments both years, including the 2-row feed-types Baronesse, RWA 1758, Tetonia and Vespa, the 2-row malt-types 2AB04-X001084-27 and 2Ab07-X031098-31, and the 6-row feed-type Herald.

These experiments contributed new information regarding relative tolerances of North American spring wheat and spring barley cultivars to *H. avenae*. This research was conducted in Idaho but it should also be applicable to *H. avenae*-infested fields elsewhere in the PNW because we previously determined that wheat cultivars responded equally to *H. avenae* populations in Idaho, Oregon and Washington (Smiley *et al.* 2011, 2013). It is anticipated that small grain cultivars that are both resistant and tolerant to *H. avenae* will become more profitable than cultivars that are susceptible and intolerant when planted into fields infested with *H. avenae*.

Table 2. Spring barley tolerance and resistance to Heterodera avenae

Market class and cultivar	White females1	Resistance rating2	Yield increase3	Tolerance rating4	MR + MT5
2-row feed barley					
Julie	6.2	MS	1.5	VT	
RWA 1758	6.3	MS	4.7	VT	
Tetonia	13.1	S	4.6	VT	
Vespa	10.1	MS	3.0	VT	
Baronesse	6.2	MS	8.7	T	
Champion	5.9	MR	7.2	T	X
Lenetah	2.6	R	9.8	T	X
Xena	3.4	MR	5.4	T	X
08ID2661	7.1	MS	12.4	MT	
CDC McGwire	8.6	MS	11.8	MT	
Idagold II	4.5	MR	10.6	MT	X
Spaulding	14.5	S	12.1	MT	
Transit	4.5	MR	10.5	MT	X
08ID1549	6.3	MS	16.1	MI	
CDC Fibar (hull-less)	4.4	MR	18.2	MI	
Clearwater	7.3	MS	23.1	MI	
2-row malt barley					
2Ab04-X001084-27	21.5	S	3.0	VT	
2B05-0811 (B0811)	12.2	S	1.5	VT	
Copeland	19.4	S	4.4	VT	
Merit	15.3	S	3.8	VT	
Merem	11.8	MS	5.6	T	
2Ab07-X031098-31	11.4	MS	5.7	T	
LCS1820	7.9	MS	8.9	T	
Merit 57	26.4	VS	6.5	T	
Overture	17.1	S	9.1	T	
Pinnacle	19.1	S	6.9	T	
Genie	33.6	VS	10.7	MT	
Harrington	20.8	S	10.3	MT	
Meredith	13.8	S	13.6	MT	
ABI Voyager	38.6	VS	19.0	MI	
Conrad	9.8	MS	16.8	MI	

Hockett	24.1	S	16.6	MI	
Metcalf	16.5	S	16.8	MI	
Odyssey	0.9	VR	15.5	MI	
B1202	23.8	S	30.2	I	
6-row feed barley					
Millenium	5.0	MR	2.6	VT	X
Herald	10.5	MS	5.6	T	
Goldeneye	5.5	MR	13.8	MT	X
Steptoe	5.3	MR	17.2	MI	
6-row malt barley					
01Ab9663	7.8	MS	0.8	VT	
Quest	6.4	MS	3.0	VT	
Legacy	3.8	MR	27.2	MI	
Morex	10.2	MS	17.6	MI	
Tradition	5.6	MR	20.8	MI	
Celebration	6.4	MS	30.5	I	

1-5 See descriptions of footnotes on Table 1.

ACKNOWLEDGMENTS

Funding was from the Idaho Wheat Commission, Oregon Wheat Commission, Washington Wheat Commission, Idaho Agricultural Experiment Station, Oregon Agricultural Experiment Station, and USDA-Agricultural Research Service. Land and crop management assistance was provided by D Daw, St. Anthony, ID. Nematodes were quantified by Western Laboratories, Parma, ID. Technical assistance was provided by S Arcibal, L Beck, M Carrillo, J Gourlie, A Hitzman, C Jackson, K Rhinhart, E Serna, T Shelman, P Thorgersen, N Webster, and G Yan. The author thanks A Boettcher and H Peter-Contesse for reviewing this paper.

REFERENCES

- Andersson, S (1982) Population dynamics and control of *Heterodera avenae* – A review with some original results. *European Plant Protection Organization Bulletin*, 12, 463-475.
- Cook R, Evans K (1987) Resistance and tolerance. In: 'Principles and Practice of Nematode Control in Crops.' (Eds. RH Brown, BR Kerry) pp. 179-231. (Academic Press: Sydney).
- Fisher JM (1982) Problems with the use of resistance in wheat to the Australian pathotypes of *Heterodera avenae*. *European Plant Protection Organization Bulletin*, 12, 417-421.

- Marshall JM, Smiley RW (2015) Spring barley resistance and tolerance to the cereal cyst nematode *Heterodera avenae*. *Plant Disease*, 99, (in press).
- O'Brien PC, Fisher JM (1977) Development of *Heterodera avenae* on resistant wheat and barley cultivars. *Nematologica*, 23, 390-397.
- Smiley RW (2009) Occurrence, distribution and control of *Heterodera avenae* and *H. filipjevi* in the western USA. In: 'Cereal Cyst Nematodes: Status, Research and Outlook.' (Eds. IT Riley, JM Nicol, AA Dababat) pp. 35-40. (CIMMYT: Ankara, Turkey).
- Smiley RW, Marshall JM (2016) Identification of balanced resistance plus tolerance to *Heterodera avenae* in spring wheat. *Plant Disease*, 100, (submitted).
- Smiley RW, Marshall JM, Gourlie JA, Paulitz TC, Kandel SL, Pumphrey MO, Garland-Campbell K, Yan GP, Anderson MD, Flowers MD, Jackson CA (2013) Spring wheat tolerance and resistance to *Heterodera avenae* in the Pacific Northwest. *Plant Disease*, 97, 590-600.
- Smiley RW, Nicol JM (2009) Nematodes which challenge global wheat production. In: 'Wheat: Science and Trade.' (Ed. BF Carver) pp. 171-187. (Wiley-Blackwell: Ames, Iowa).
- Smiley RW, Yan GP, Pinkerton JN (2011) Resistance of wheat, barley and oat to *Heterodera avenae* in the Pacific Northwest USA. *Nematology*, 13, 539-552.

PART 4

OTHER MANAGEMENT STRATIGIES





MANAGEMENT OF CEREAL CYST NEMATODE (HETERODERA AVENAE) IN A LARGE SCALE WHEAT PRODUCTION

AHMED AM DAWABAH*, AHMAD S AL-HAZMI, AND FAHAD A AL-YAHYA

Plant Protection Department, College of Food and Agricultural Sciences, King Saud University. P. O. Box 2460, Riyadh 11451, Saudi Arabia. *Corresponding author email: adawabah@ksu.edu.sa

SUMMARY

The cereal cyst nematode (*Heterodera avenae* Woll., 1924) is a very destructive pathogen to wheat production in Saudi Arabia since 1987. The most affected area is Hail region which is located in the north of the Kingdom. Thus, we selected a large wheat-producing farm in a major agricultural company located in this region to be our case study. The farm has a wheat-cultivated area comprised of 110-130 pivots (72 ha each). *H. avenae* was detected in some pivots of the farm in 1990, and rapidly spread to the other pivots. From 1992 and upward, a granular carbamate nematicide was used by the company as a pre-plant treatment every year. In 2005, this nematicide alone became unable to solve the problem, and the average wheat yield in the farm has dropped from 7.5 tons/ha to 2.5 tons/ha in some pivots. We used a specific kit test to determine the stability period of the nematicide in the soil. Results of this test proved that the repeated use of the nematicide has enabled the soil microorganisms to break-down the nematicide very rapidly. So, we started a management program to manage *H. avenae* on the farm from 2005 to 2010. The program included both short- and long-term strategies. The short-term strategy included the application of a foliar spray nematicide (oxamyl 24% @ 6 l/ha, on two applications). The long-term strategy included; sanitation, crop rotation

* Dawabah AAM, Al-Hazmi AS, Al-Yahya FA (2015) Management of cereal cyst nematode (*Heterodera avenae*) in a large scale wheat production. In 'Nematodes of Small Grain Cereals: current status and research.' (AA Dababat, H Muminjanov and RW Smiley) pp. 277-284. (FAO: Ankara, Turkey).

with alfalfa, cultural practices and the application of a herbicide to control the alternative weed hosts (*Hordeum murinum* L. and *Lolium multiflorum* Lam). All treatments greatly increased wheat growth and productivity which are back again to the normal average (7-8 tons/ha). Crop rotation with alfalfa was superior in increasing wheat growth and grain yield, and decreasing *H. avenae* populations in soil.

Key words: Crop rotation, management, nematicides, nematode, Saudi Arabia, *Triticum aestivum*.

INTRODUCTION

The cereal cyst nematode, *Heterodera avenae* Woll. 1924, is one of the most dangerous plant pathogens attacking wheat worldwide (Rivoal and Cook 1993, Smiley and Nicol 2009, Dababat *et al.* 2015). Losses of wheat yield caused by this nematode can be enormous when they occur in a disease complex, especially in the arid and semi-arid environments, where heat and water stresses are significantly high (Dababat *et al.* 2015). In Saudi Arabia, the nematode was first reported from a wheat field in the Al-Gassim region in 1987 (Youssif 1987). Since then, it has been increasingly spreading in the wheat and barley fields in the Kingdom (Al-Hazmi *et al.* 1994, Dawabah and Al-Hazmi 2007, Al-Hazmi and Dawabah 2009). Under the field conditions of Saudi Arabia, *H. avenae*-caused wheat yield losses reached up to 92% in some heavily-infested sites in a wheat field at Al-Kharj, Riyadh region (Ibrahim *et al.* 1999). As the yield losses increased, the problem became national. In 2003, the Saudi Agricultural Bank and the Ministry of Agriculture decided to cooperate with the wheat growers in scheduling their debts and solving the problem (Al-Hazmi and Dawabah 2009). The Saudi populations of *H. avenae* have been identified, based on morphological and molecular basis (Al-Hazmi *et al.* 1994, Al-Rehiayani 2007, Dawabah *et al.* 2012). The pathotype of the nematode was also identified as to be very close to the European pathotype Ha21 (Al-Hazmi *et al.* 2001).

Heterodera avenae can be effectively managed by resistant cultivars, crop rotation and/or the use of nematicides. Other management strategies include; biological control agents, physical measures and cultural practices such as quarantine, sanitation, bare fallowing, weed control, organic and inorganic fertilizers, and others (Al-Hazmi and Dawabah 2009, 2014, Dababat *et al.* 2015).

When the nematode populations in the soil are too high, and the other management approaches are inadequate, the chemical control strategies would be of great necessity to bring the *H. avenae* populations below the damage threshold levels (Hague and Gowen 1987). Crop rotation with non-cereal crops is also a very effective and rapid method to reduce *H. avenae* populations in the soil (Brown 1984). Under bare fallowing, *H. avenae* populations can decline by 70-80% annually through spontaneous hatching and mortality of the second-stage juveniles (J_2s) due to starvation (Barker *et al.* 1998, Singh *et al.* 2009, Smiley *et al.* 1994).

Since the eradication of *H. avenae* from soil is nearly impossible, it is very important to minimize the transmission of soil from infested to non-infested fields. The soil containing viable eggs and larvae can be transmitted to non-infested areas through its adhering to the agricultural equipment, vehicles wheels, animals, humans (boots), and plant propagative materials such as roots and tubers growing in *H. avenae*-infested soil (Dawabah and Al-Hazmi 2007, Smiley 2005). Cysts with viable eggs can be also carried from infested fields by dust blowing and water moving either by erosion or in return ditches at the discharge end of flood-irrigated fields (Smiley and Yan 2010).

The aim of this study was to determine and suggest an integrated management strategy to manage *H. avenae* in a large wheat producing farm under the conditions of the arid environment in Saudi Arabia.

MATERIALS AND METHODS

Soil samples were collected from fields naturally-infested by *H. avenae* in a large agricultural farm in the Hail region, Saudi Arabia in the 2005 growing season. Samples were collected from those fields that have the relatively highest nematode infestation (≈ 142 eggs + J_2/g of soil), and minimal wheat yield (2.5 tons/ha). A total of 50 random samples were collected from each field (72 ha). Nematodes were extracted using sieving and floatation methods (Shepherd 1986), and identified on the basis of morphometrics and morphological features of nematodes and vulval cone structures (Handoo 2002). Cysts were then crushed, and eggs and second-stage juveniles (J_2s) were counted and expressed as number of eggs + J_2/g of soil.

A three-year field experiment was initiated in the 2005 growing season to determine the effect of five different treatments on the *H. avenae* population

density in soil and wheat grain yield. The experiment was conducted in a completely randomized design with three replicates. Each replicate included a 72 ha field. The treatments included: 1) cultivation of alfalfa for two consecutive years (2005 & 2006 as a crop rotation) then wheat cv. "Yecora Rojo" in 2007, 2) bare fallow for two consecutive years (2005 & 2006) then wheat in 2007, 3) cultivation of wheat and the use of oxamyl 24% as a foliar application @ 6 l/ha on two split equal doses (at three and seven weeks after wheat seedlings' emergence) every year (from 2005-2007), 4) cultivation of wheat with no nematode control treatment (as a nematode control treatment), and 5) cultivation of wheat in non-infested fields (as a healthy control treatment). All treatments (fields) received the recommended fertilizers (NPK) as needed plus the sanitation protocol precautions (cleaning the agricultural equipment, vehicles wheels, laborer's boots, etc.). One month before harvest, soil and root samples were collected as aforementioned to determine the final nematode population. Grain yield (tons/ha) was also recorded after harvest.

The nematode management protocols including the short- (nematicides) and long-term strategies (crop rotation, sanitation, herbicide application and the cultural procedures) started from the 2008 until the 2010 growing seasons. These protocols were applied in 10 fields as a trial to be applied in all the farm fields thereafter. The protocols included:

1. A two year crop rotation with alfalfa (2006 & 2007).
2. Wheat + sanitation + herbicide + NPK (2008).
3. Wheat + sanitation + oxamyl 24% @ 6 l/ha on two split equal doses (at three and seven weeks after wheat seedlings' emergence) + herbicide + NPK (for two years; 2009 and 2010).

At the end of each growing season, from 2008-2010, soil and root samples were collected one month before harvest to determine the final nematode population. Grain yield (tons/ha) was also recorded after harvest. Data were subjected to the analysis of variance (ANOVA) using SAS (2013).

RESULTS AND DISCUSSION

After two consecutive years of alfalfa cultivation (crop rotation) and bare fallowing, numbers of *H. avenae* eggs + J_2s were greatly reduced in the soil, leading to a great increase in wheat grain yield in the 2007 growing season, compared to the nematode control treatment. Foliar application with oxamyl also reduced

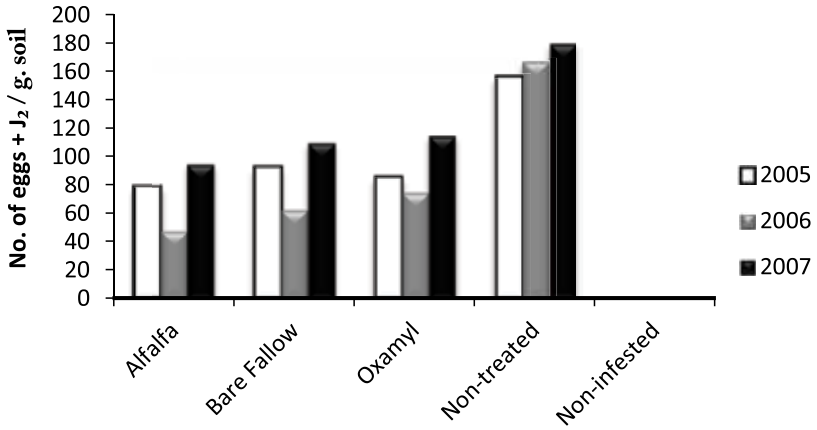


Figure 1. The effect of crop rotation with alfalfa, bare fallowing, oxamyl 24% @ 6 l/ha on the number of *H. avenae* population (eggs + J_2/g soil) in the soil

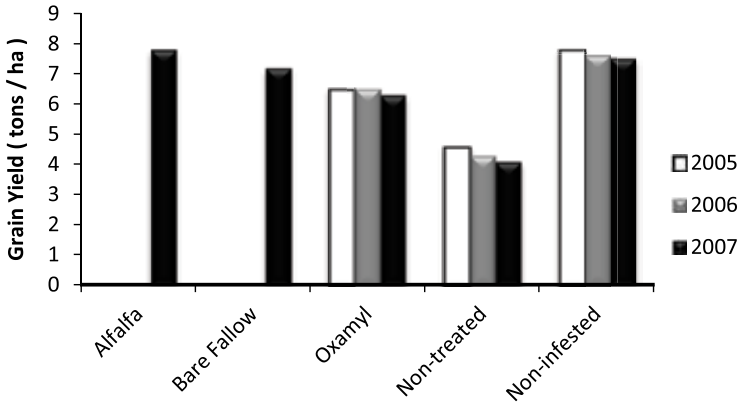


Figure 2. The effect of crop rotation with alfalfa, bare fallowing, oxamyl 24% @ 6 l/ha on the grain yield of wheat cv. "Yecora Rojo"

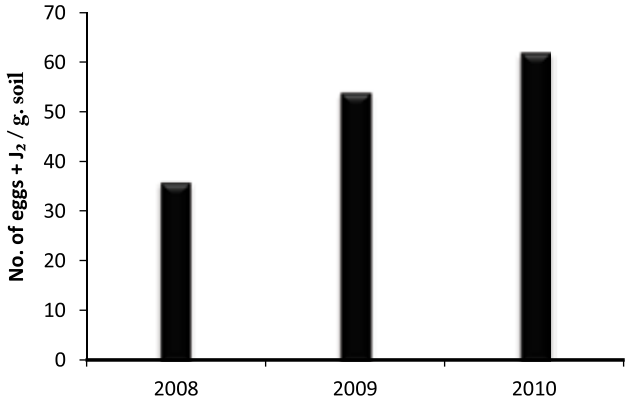


Figure 3. The effect of integrated management protocols on the number of *H. avenae* population (eggs + J_2/g soil) in the soil

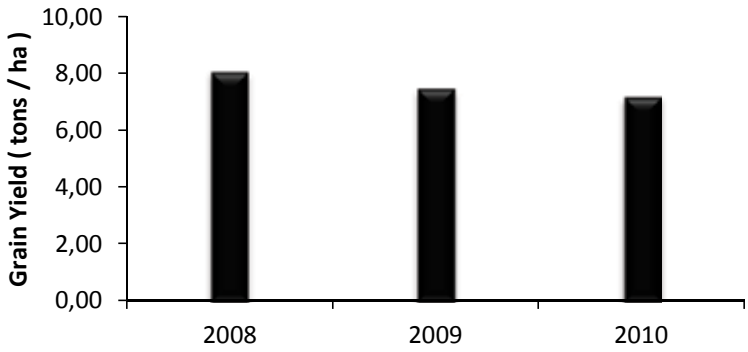


Figure 4. The effect of integrated management protocols on the grain yield of wheat cv. "Yecora Rojo"

the nematode population in the soil and increased the wheat grain yield (Figs. 1 & 2). However crop rotation with alfalfa was the most effective among all the studied treatments in reducing *H. avenae* population in soil and increasing the wheat grain yield. It is well-known tha t crop rotation with non-cereal crops is a very effective method in reducing *H. avenae* populations in the soil (Brown 1984, Al-Hazmi and Dawabah 2009, Dababat *et al.* 2015). Besides, alfalfa like all the leguminous crops has the ability to increase the soil nitrogen content which enhances the vegetative growth and grain yield of wheat plants. Bare fallowing has also a good potential to reduce *H. avenae* populations in soil (Smiley *et al.* 1994, Barker *et al.* 1998, Singh *et al.* 2009).

Under the management protocols in this study, the nematode population densities (eggs + J_2/g of soil) have greatly declined after the two-year crop rotation with alfalfa in 2006 and 2007 (Fig. 3), confirming the findings of the first experiments and also the findings of some previous work (Brown 1984, Al-Hazmi and Dawabah 2009, Dababat *et al.* 2015). But, these populations, again, gradually increased from 2008-2010, even though they didn't reach the same levels before crop rotation. However, wheat grain yield gradually decreased from 8.1 to 7.2 tons/ha during the 2008-2010 growing seasons (Fig. 4). Thus, we suggested that the fields be returned to the crop rotation or bare fallowing in 2011.

In conclusion, and due to the desire of the Saudi wheat growers who do not prefer to leave their fields under the bare fallowing, we suggested the following management protocols to manage the *H. avenae* problem in the Saudi fields:

1. Crop rotation with alfalfa for two years.
2. Growing a susceptible wheat cultivar + sanitation procedures + herbicide + NPK in the third year.

3. Growing a susceptible wheat cultivar + sanitation procedures + nematicide (liquid or granules) + herbicide + NPK in the fourth and fifth years.
4. Repeating the previous protocols, taking in consideration the chemical rotation of the nematicides, so that the nematicides used remain always effective.

REFERENCES

- Al-Hazmi AS, Dawabah AAM (2009) Present status of the cereal cyst nematode (*Heterodera avenae*) in Saudi Arabia. In: 'Cereal Cyst Nematodes: Status, Research and Outlook.' (Eds. IT Riley, JM Nicol, AA Dababat) pp. 56-60. (CIMMYT, Ankara, Turkey).
- Al-Hazmi AS, Dawabah AAM (2014) Effect of urea and certain NPK fertilizers on the cereal cyst nematode (*Heterodera avenae*) on wheat. *Saudi Journal of Biological Sciences*, 21, 191-196.
- Al-Hazmi AS, Ibrahim AAM, Abdul-Razig AT (1994) Occurrence, morphology and reproduction of *Heterodera avenae* on wheat and barley in Saudi Arabia. *Pakistan Journal of Nematology*, 12, 117-129.
- Al-Hazmi AS, Cook R, Ibrahim AAM (2001) Pathotype characterization of the cereal cyst nematode, *Heterodera avenae*, in Saudi Arabia. *Nematology*, 4, 379-382.
- Al-Rehiayani S (2007) Genetic differences among cereal cyst nematode (*Heterodera avenae*) populations in central Saudi Arabia using ITS-rDNA sequences. *International Journal of Nematology*, 17, 124-129.
- Barker KR, Pederson GA, Windham GL (1998) Plant and nematode interactions. *Agronomy Monograph* 36. American Society of Agronomy, Madison.
- Brown RH (1984) Ecology and control of cereal cyst nematode (*Heterodera avenae*) in southern Australia. *Journal of Nematology*, 16, 216-222.
- Dababat AA, Imren M, Erginbas-Orakci G, Ashrafi S, Yavuzaslanoglu E, Toktay H, Pariyar SR, Elekcioglu H, Morgounov A, Mekete M (2015) The importance and management strategies of cereal cyst nematodes, *Heterodera* spp., in Turkey. *Euphytica*, 202, 173-188.
- Dawabah AAM, Al-Hazmi AS (2007) Spreading of cereal cyst nematode with potato seed tubers in Saudi Arabia. *Pakistan Journal of Nematololgy*, 25, 339-340.
- Dawabah AAM, Al-Hazmi AS, Al-Rehiayani SM, Abdel-Mawgood AL, Motawei MI, Al-Otayk S, Sadder MT, Elgorban AM, Migdadi HM, Moustafa KA, Al-Doss AA (2012) Morphological and molecular characterization of cereal cyst nematode (*Heterodera avenae*) populations from arid environments. *Australian Journal of Crop Science*, 6, 970-979.

- Handoo ZA (2002) A key and compendium to species of *Heterodera avenae* group (Nematoda: Heteroderidae). *Journal of Nematology*, 34, 250-262.
- Hague NGM, Gowen SR (1987) Chemical control of nematodes. In: 'Principles and practice of nematode control in crops.' (Eds. RH Brown, BR Kerry) pp. 131-178. (Academic Press, Sydney, Australia).
- Ibrahim AAM, Al-Hazmi AS, Al-Yahya FA, Alderfasi A (1999) Damage potential and reproduction of *Heterodera avenae* on wheat and barley under Saudi field conditions. *Nematology*, 1, 625-630.
- Rivoal R, Cook R (1993) Nematode pests of cereals. In: 'The Cyst Nematodes.' (Ed. SB Sharma) (Kluwer Academic Publishers, Dordrecht, The Netherlands).
- Shepherd AM (1986) Extraction and estimation of cyst nematodes. In: 'Laboratory Methods for Work with Plant and Soil Nematodes. 6th edition.' (Ed. JF Southey). (Ministry of Agriculture, Fisheries and Food. Her Majesty's Stationary Office. London).
- Singh AK, Sharma AK, Shoran J (2009) *Heterodera avenae* and its management on wheat in India. In. 'Cereal Cyst Nematodes: Status, Research and Outlook.' (Eds. IT Riley, JM Nicol, AA Dababat), pp. 17-22. (CIMMYT, Ankara, Turkey).
- Smiley RW (2005) Plant parasitic nematodes affecting wheat yield in the Pacific Northwest. Extension Bulletin EM 8887. Oregon State University Extension Service, Corvallis, Oregon.
- Smiley RW, Yan G (2010) Cereal Cyst Nematodes: Biology and Management in Pacific Northwest Wheat, Barley, and Oat Crops. Pacific Northwest Extension Bulletin PNW 620. Oregon State University Extension Service, Corvallis, Oregon.
- Smiley RW, Nicol JM (2009) Nematodes which challenge global wheat production. In. 'Wheat Science and Trade.' (Ed. BF Carver) pp. 171-187. (Wiley-Blackwell, Ames, Iowa).
- Smiley RW, Ingham RE, Uddin W, Cook GH (1994) Crop sequences for managing cereal cyst nematode and fungal pathogens of winter wheat. *Plant Disease*, 78, 1142-1149.
- Youssif GM (1987) Effect of *Heterodera avenae* populations in infested field patches of wheat cultivation on plant growth and yield. 10th Symposium on the Biological Aspects of Saudi Arabia, Saudi Biological Society, Jeddah, Saudi Arabia. 20-24 April 1987.

TOWARDS HARBOURING THE DIVERSITY OF MICROORGANISMS ASSOCIATED WITH EGGS AND CYSTS OF CEREAL CYST NEMATODES FOR BIOLOGICAL CONTROL

SAMAD ASHRAFI[1,2,3,*], ABDELFATTAH A. DABABAT[2], AND WOLFGANG MAIER[1]

[1]Julius Kühn-Institut, Institute for Epidemiology and Pathogen Diagnostics, Braunschweig, Germany; [2]CIMMYT-Turkey, Ankara; [3]University of Kassel, Faculty of Organic Agricultural Sciences, Ecological Plant Protection, Witzenhausen, Germany. *Corresponding author email: ashrafi.samad@gmail.com

SUMMARY

The internationally increasing awareness of the hazards of the use of pesticides has already resulted in improving non-chemical based management strategy. This development requires applying integration of several control methods with the general aim to keep nematode populations below a damage threshold. In integrated pest management (IPM) systems, biological control is considered to have a great role in pest and disease regulation. This approach relies on the presence of self-regulating processes in microbial communities in nature. These processes often might be facilitated by antagonistically interacting microorganisms. In biological control, these antagonistic microorganisms can be exploited for combating agricultural pests and diseases.

The most economically important cereal cyst nematode (CCNs) species, including *Heterodera avenae*, *H. filipjevi* and *H. latipons*, are distributed worldwide. CCNs attack all major cereals and can lead to significant yield reductions,

* Ashrafi S, Dababat AA, Maier W (2015) Towards harbouring the diversity of micro-organisms associated with eggs and cysts of cereal cyst nematodes for biological control. In 'Nematodes of Small Grain Cereals: current status and research.' (AA Dababat, H Muminjanov and RW Smiley) pp. 285-288. (FAO: Ankara, Turkey).

especially in semi-arid climates. Despite the fact that mostly mixed populations of CCNs are reported from intensive cereal culturing systems, certain species seem to be dominant regionally leading to biogeographical patterns. In Turkey for example, *H. filipjevi* is the dominant CCN species in the Central Anatolian Plateau region.

Field observations on the experimental fields of CIMMYT in Turkey revealed a sharp decline of final nematode population of *H. filipjevi*. This sharp reduction of nematode populations was observed in intensive wheat mono cultures. Microscopic observations of cysts of the final nematode populations frequently demonstrated the presence of fungal structures, mostly in young gravid females of *H. filipjevi.* We thus hypothesised that the observed fungal structures or other microorganisms, like bacteria, might have played an important role in the reduction of the nematode population. Therefore, the present study was conducted to 1) isolate and identify microorganisms that potentially lead to soil suppressiveness against *H. filipjevi* in the experimental wheat fields of CIMMYT in Turkey, 2) evaluate the potential biocontrol impacts of the nematode-associated fungal and bacterial isolates, and 3) establish a focused screening system for nematode-parasitic fungi in nematode-suppressive soils.

In the first screening, 100 fungal and 63 bacterial isolates were obtained from the *H. filipjevi* cysts or wheat roots. The first screening was considered as a 'general screening' during which the gravid females and cysts were randomly cultured to isolate nematode antagonistic fungi or bacteria. The roots of wheat were also sampled to isolate potentially present fungal and bacterial endophytes. The biocontrol potential of fungal and bacterial isolates was evaluated against *H. filipjevi* under controlled conditions in a growth chamber. All fungal isolates were identified using light microscopy and molecular phylogenetic analyses. The most frequently isolated fungi were *Fusarium* spp., *Pochonia chlamydosporia*, *Acremonium* spp. and *Paecilomyces* spp. Most of the fungal isolates (88%) obtained from wheat roots were identified as *Fusarium* species while *P. chlamydosporia* was the most frequently isolated fungal species (21%) from nematode cysts. Of the fungal isolates tested against the nematode, 10 isolates including *P. chlamydosporia* (4), *Paecilomyces fumosoroseus* (2), *A. persicinum* (3), *Gliomastix murorum* (1) and *Fusarium acuminatum* (1) showed the strongest biocontrol activity and reduced the nematode populations by a maximum of 50%. The first three species belong to the ascomycetous family of the Clavicipitaceae that are especially well-known as invertebrate pathogens. Of the bacterial isolates evaluated against *H. filipjevi*, the 10 isolates showing

highest biocontrol potential were selected and molecularly identified. Of these, nine isolates were identified as *Bacillus* spp., mostly belonging to the *B. cereus* group, and one species belonged to the genus *Enterobacter*. The antagonistic *in vivo* screening of bacterial isolates against *H. filipjevi* reduced the nematode population by a maximum of 30%.

In further screenings here coined as 'focused screening', eggs that seemed to be infected by fungi were scrutinised. Egg infection was determined by the presence of fungal structures colonising the eggs. Fifty fungal isolates were obtained from the infected eggs of field-collected cyst samples. Molecular characterisation of newly isolated fungi indicated a wide variety of ascomyceteous species including representatives of the genera *Embellisia*, *Ophiosphaerella*, *Pleospora*, *Periconia*, *Ilyonectria*, *Arthrobotrys*, *Lecanicillium*, *Pochonia* and a few potentially so-far undescribed fungal isolates belonging to the Hypocerales, Helotiales and Pleosporales. While some of these fungi have been previously reported as endophytes, some others are known for insecticidal and nematicidal activities. The presence of some of these isolates in single infected eggs was repeatedly demonstrated using culture-dependent and independent (DNA-based) methods. The results showed a high diversity of fungal species that are associated with *H. filipjevi* and suggest that the nematode cysts and eggs constitute a rich source of nematode-parasitic fungi that could potentially be assessed for biocontrol of cereal cyst nematodes.

THE CEREAL CYST NEMATODES, *HETERODERA AVENAE* WOLLENWEBER IN TURKEY: LONG TERM RESEARCH WITH REGIONAL IMPLICATIONS

İ. HALIL ELEKÇIOĞLU[1], MUSTAFA İMREN[2,*], HALIL TOKTAY[3], REFIK BOZBUĞA[4], ECE B. KASAPOĞLU[1], ATILLA ÖCAL[5], AND ABDELFATTAH A. DABABAT[6]

[1]Cukurova University, Faculty of Agriculture, Department of Plant Protection, Adana, Turkey. [2]University of Abant Izzet Baysal, Faculty of Agriculture and Natural Sciences, Department of Plant Protection, Bolu, Turkey. [3]University of Niğde, Faculty of Agricultural Sciences and Technologies, Department of Plant Production and Technology, Niğde, Turkey. [4]Biological Research Institute, Adana, Turkey. [5]Batı Akdeniz Agricultural Research Institute, Muratpaşa, Antalya- Turkey. [6]CIMMYT Global Wheat Program, Ankara-Turkey. *Corresponding author email: mustafaimren@ibu.edu.tr

SUMMARY

Cereal cyst nematodes (CCNs), the *Heterodera avenae* group, are the most common pathogens to limit wheat production in many countries and cause serious economic damage in cereal crops worldwide, especially in temperate regions. This paper aimed to study CCNs to determine occurrence, distribution and frequency of CCNs in cereal fields of the South East and Eastern Mediterranean Regions, to investigate some biological features (hatching, inoculation time and rate) of *H. avenae* under laboratory condition, to identify the pathotype of *H. avenae* in Eastern Mediterranean Region by using the international host differential test materials, to screen some wheat varieties (international,

* Elekçioğlu İH, İmren M, Toktay H, Bozbuğa R, Kasapoğlu EB, Öcal A, Dababat AA (2015) The cereal cyst nematodes, *Heterodera avenae* Wollenweber in Turkey: Long term research with regional implications. In 'Nematodes of Small Grain Cereals: current status and research.' (AA Dababat, H Muminjanov and RW Smiley) pp. 289-305. (FAO: Ankara, Turkey).

national wheat and wild wheat relatives) against *H. avenae* and to estimate yield loss from *H. avenae* under field conditions. Results showed that 52% of the investigated wheat fields of the Eastern Mediterranean and South-Eastern Anatolia Regions were infested by CCNs. Three important species of this genus, *H. avenae* (Wollenweber), *H. latipons* (Franklin) and *H. filipjevi* Madzhidov (Stone) were identified and the infestation rates were found to be 51%, 40% and 9%, respectively. The pathotype of *H. avenae* was adequately characterized as pathotype Ha21 by indexing plants of the current International Test Assortment. It was also determined that sandy soil was the most proper soil type for *H. avenae* reproduction. The most appropriate inoculum number was 2.5 J2/ g of soil. The best inoculation time was the day seed was planted. The most suitable hatching condition of J2 was by pre-incubating cysts for two months at +4°C and then incubating them for 191 to 221 days at 10°C. Additionally, the resistance gene, *Cre1*, did not show complete effectiveness against *H. avenae*. The local varieties, Adana-99, Sorgül, Şırnak, Sogol Acırlı, and some wild wheat forms, were determined as moderately resistant. It was determined that the average yield losses caused by *H. avenae* varied between 4.36% and 25.7% depending on wheat varieties and field conditions.

Keywords: Cereal Cyst nematodes, identification, pathotype, resistance, yield loss

INTRODUCTION

Cereal cyst nematode (CCN), the *Heterodera avenae* group, have a global distribution and cause significant economic yield losses in many countries of the world, particularly where cereals are produced under rainfed conditions (Nicol 2002). The *H. avenae* group contains at least 12 species that mostly invade cereals and grasses. *H. avenae*, *H. filipjevi* and *H. latipons* are recognized as the most economically important species in West Asia, North Africa and Mediterranean countries (Nicol 2002, Tanha Maafi *et al.* 2003). CCN has several species and pathotypes and is found predominately on temperate cereals (Nicol & Rivoal 2008). Documented yield loss of CCN species have been reviewed by Nicol (2002), ranging from 15 to 20% on wheat in Pakistan, 40 to 92% on wheat and 17 to 77% on barley in Saudi Arabia, and 20% on barley and 23 to 50% on wheat in Australia.

Turkey is the one of the biggest wheat producers in the world, with 20 million tons produced in an area of 9.4 million ha (Turkish Statistical Institute 2012).

The CCN is the most important pathogen of wheat and other cereals in Turkey and has adverse effect on the quality and production of wheat. Three species of cyst-forming nematodes belonging to the *H. avenae* group were identified from cereal fields in Turkey, with *H. filipjevi*, *H. avenae* and *H. latipons* decreasing in prevalence in that order (Sahin 2010, İmren *et al.* 2012a, Dababat *et al.* 2014, 2015).

In this review, the summarised results are given from studies conducted on: i) the identification and distribution of *Heterodera* species in the Eastern Mediterranean and South-Eastern Anatolia Regions; ii) economic importance and improving our understanding of the some biological parameters such as hatching of *H avenae*; iii) identification of *H. avenae* pathotypes using the International Test Assortment; iv) estimating yield losses caused by *H. avenae*; and v) screening known sources of resistance against *H. avenae*.

SURVEY STUDIES

Seven hundred eleven soil samples were collected from ten provinces of the Eastern Mediterranean and South-Eastern Anatolia Regions during the four-year sampling period; 2009 – 2012. Soil samples were taken every year during May and June. *Heterodera* cysts were found in 52% of soil samples (Table 1).

Cereal cyst nematode species were identified on the basis of the vulval sections and second-stage juvenile morphometrics and morphological characters (Subbotin *et al.* 1999, Handoo 2002) and were identified as *H. avenae, H. latipons* and *H. filipjevi* (Fig. 1) (İmren *et al.* 2012a).

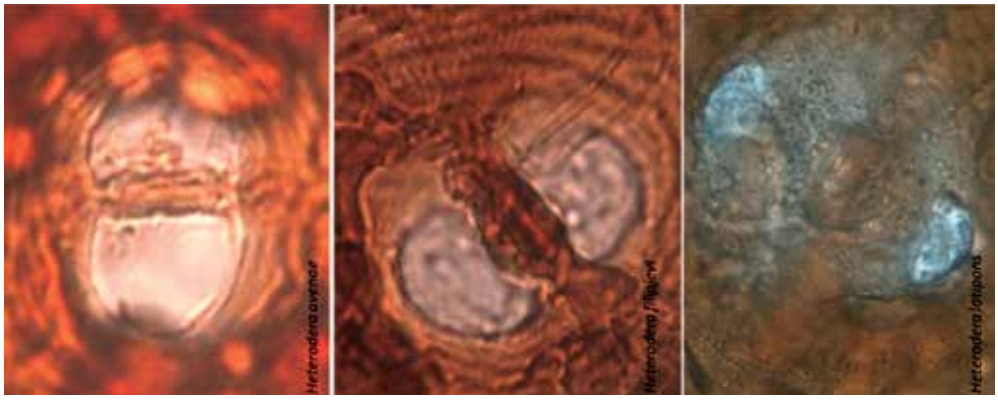


Figure 1. Cyst vulval cone patterns of three *Heterodera* species in the *H. avenae* group; *H. avenae* (left), *H. filipjevi* (center), *H. latipons* (right).

Table 1. Infestation rate of cereal cyst nematodes in wheat fields in the Eastern Mediterranean and South-Eastern Anatolia regions (İmren 2013)

Province	Total samples	Infested samples	Infestation rate (%)
Gaziantep	127	79	62
Kilis	124	71	57
Şanlıurfa	86	55	63
Diyarbakır	46	19	41
Mardin	85	41	48
Hatay	113	42	37
Adana	40	21	52,5
Osmaniye	22	9	40,9
K.Maraş	48	25	52
Şırnak	20	9	45
Total	711	371	52

Morphological and molecular results revealed that the frequencies of detection for *H. avenae*, *H. latipons* and *H. filipjevi* were 51%, 40% and 9% of all soil samples infested by CCN, respectively (İmren *et al.* 2012a).

PATHOYPE DETERMINATION OF *H. AVENAE*

The *H. avenae* group is highly heterogeneous with respect to virulence to specific host genotypes (Cook & Noel 2002, McDonald & Nicol 2005). This diversity was described and categorized by using test cultivars in what is called "The International Test Assortment" for defining CCN pathotypes. The pathotypes are broadly classified within one of three groups; 1, 2, and 3. Pathotype groups 1 and 2 are widely distributed in Europe, North Africa and Asia (Al-Hazmi *et al.* 2001, Cook and Noel 2002, Mokabli *et al.* 2002, McDonald and Nicol 2005) and group 3 is mostly found in Australia, Europe and North Africa (Rivoal and Cook 1993, Mokabli *et al.* 2002).

'The International Test Assortment' consists of 12 barley, six oat (*Avena sativa* L.) and six wheat differential cultivars to define selected pathotypes of *H. avenae*. It was also used to investigate pathotypes of three *H. avenae* populations; Karlık (Adana-Sarıçam), Imece (Hatay-Kırıkhan) and Besaslan (Hatay-Reyhanlı) in the Eastern Mediterranean Region of Turkey. Based on the scheme of Subbotin *et al.* (2010) and its subsequent revisions, three primary groups of pathotype are distinguished by the reactions of barley, oats and wheat differ-

entials. The similar responses of the differentials indicated that Turkish populations of *H. avenae* are predominately of the same pathotype. As a result, all populations demonstrated similar reactions and the three nematode populations were consistent with reactions for the Ha21 pathotype of the Ha1 group (İmren *et al.* 2013a). This pathotype was also found in Saudi Arabia (Al Hazmi *et al.* 2001). Responses for the tested differentials were compared, mainly on the basis of Subbotin *et al.* (2010), and were also compared to the findings of Romero *et al.* (1996), Al-Hazmi *et al.* (2001), McDonald and Nicol (2005), and Turner and Rowe (2006) to characterize the pathotype.

The single-dominant *Cre1* gene effectively prevented reproduction of three *H. avenae* populations collected from Imece, Karlik and Basaslan towns in the Eastern Mediterranean Region of Turkey, as was illustrated by results of a recent test with selected entries of the International Test Assortment. Wheat lines carrying the *Rha*E, *Rha*1 and *Rha*3 genes are resistant to populations of *H. avenae* from the Eastern Mediterranean Region of Turkey. Siri, having the *Rha*2 gene, exhibited resistance to each of the three soils in these tests, while KVL 191, also having *Rha*2 gene, is susceptible (İmren *et al.* 2013a). Resistances to Eastern Mediterranean Region of Turkey populations of *H. avenae* were also detected in barley lines carrying the *Rha*E gene in Emir, *Rha*1 gene Ortolan, and the *Rha*3 gene in Morocco. Likewise, resistances to the Eastern Mediterranean Region of Turkey populations were detected in the barley accessions La Estanzuela, Harlan 43, in the oat accession Sun II, and in the wheat accession Capa. These potential donor parents could prove useful where these crops have been damaged by *H. avenae*, such as oats in the Eastern Mediterranean Region of Turkey.

HATCHING STUDIES

The biology of the obligate parasite *H. avenae* coincides with the cereal growing period and produces one generation per year (Rivoal & Cook 1993) but there is no published information on the biological characteristic of *H. avenae* under *in-vitro* conditions in our country. Imren *et al.* (2012b) studied for the first time in Turkey, hatching of *H. avenae* under *in-vitro* conditions. In this study, five temperatures of 5, 10, 15, 20 and 25 °C were tested in laboratory experiments. The hatching was greater at lower temperatures (15 ,10 ,5°C) compared to higher temperatures of (20, 25°C), ranging between 46.7, 82.3 and 45.9% vs 30.7 and 19.0%, respectively. The highest cumulative hatching (82.3%) was obtained at a constant 10 °C for 252 days, and the lowest cumulative hatching (19.0%) was obtained to 25 °C for 252 days.

EFFECT OF *H. AVENAE* ON WHEAT YIELD

In Turkey, *H. avenae* is predominantly found in spring wheat growing areas in Adana Province in the Eastern Mediterranean Region of Turkey (Subbotin *et al.* 2003, İmren *et al.* 2012a). However, the impact of *H. avenae* on wheat yield had not been studied or quantified. The relationship between population density of *H. avenae* and grain yield of wheat is important in determining the economic impact of the nematode on this crop (Ibrahim *et al.* 1999). Such information is very important for the control of *H. avenae* on wheat in the Eastern Mediterranean Region of Turkey.

Studies were carried out in naturally-infested wheat fields in Adana (Karlık-Sarıçam) Province in the Eastern Mediterranean Region of Turkey during 2011-2012 and 2012-2013 wheat growing seasons (İmren *et al.* 2012a). Experiments were established using six varieties. Four local spring wheat varieties (Adana-99, Ceyhan-99, Karatopak and Osmaniyem) and two control lines, Seri-82 (susceptible) and Silverstar (tolerant), were tested in nematicide-treated and non-treated plots. *H. avenae* in the non-treated plots caused reductions in grain yield of all varieties, which were significantly different from each other. Also, grain yield reductions of Seri-82, Osmaniyem and Karatopak appeared to be greater than that of other varieties, especially Silverstar, Adana99 and Ceyhan99. The percentage reduction in yield of the varieties ranged between 4.36 and 25.7% when averaged over the two growing seasons (İmren and Elekcioğlu 2014).

RESISTANCE OF WHEAT CULTIVARS AGAINST *H. AVENAE*

We screened 139 wild wheat relatives, national and international (CIMMYT and CIMMYT/ICARDA/Turkey) germplasm for resistance against *H. avenae* (Ha21 pathotype) using local Turkish isolates as controls (Imren at al. 2013a). Results showed that four national wheat varieties, 17 wheat wild genotypes, and 23 international wheat genotypes were moderately resistant against the *H. avenae* population from the Eastern Mediterranean Region of Turkey (İmren *et al.* 2013b). Among these genotypes, the national bread wheat variety, Adana-99 (PFAU/SERI82//BOG"S") and some wild genotypes and international genotypes can be used in national wheat breeding programmes. However, *Cre1* was not shown to be completely resistant against *H. avenae*.

DISCUSSION

The cereal cyst nematodes occurred in 52% of infested cereal fields studied in this survey, and are more widespread than previously thought. Three important species, *H. avenae*, *H. latipons* and *H. filipjevi*, were identified in wheat fields of the Eastern Mediterranean and South-Eastern Anatolia Regions. CCN has been found in many countries and has caused significant economic damage to wheat, especially under rainfed wheat production systems (Nicol *et al.* 2002). *H. avenae* is economically important in temperate wheat-producing regions throughout the world, including North and South Africa, East and West Asia, Australia, Europe, Indian, the Middle East, and North America (Kort 1972, Sharma and Swarup 1984, Sikora 1988, Ibrahim *et al.* 1999, Rivoal and Cook 1993, Mokabli *et al.* 2002, Peng *et al.* 2007). *H. latipons* occurs mostly throughout the Mediterranean region but also in Asia and Europe (Kort 1972, Sikora and Oostendorp 1986, Sikora 1988, Mor *et al.* 1992, Scholz 2001, Peng *et al.* 2007). *H. filipjevi* was recently detected in North America (Smiley *et al.* 2008), Norway (Holgado *et al.* 2004), Germany (Grosse and Kohlmüller 2004), Iran (Tanha Maafi *et al.* 2003), Sweden (Cook and Noel 2002), India (Bishnoi and Bajaj 2002), Turkey (Rumpenhorst *et al.* 1996), Bulgaria, England, Poland, Estonia, Spain (Subbotin *et al.* 2003) and Russia (Balakhnina 1989).

The pathotype of *H. avenae* in Eastern Mediterranean Region was characterized as pathotype Ha21 (İmren *et al.* 2013c). Also, the pathotype of *H. filipjevi* collected from Kahramanmaraş (Elbistan and Afşin) and Ankara (Haymana) provinces were characterized as Ha33 pathotype (Toktay *et al.* 2013). Furthermore, Özarslandan (2008) reported that the reaction of *H. filipjevi* Yozgat population on the differential lines indicated they were different than the other five known *H. filipjevi* pathotypes. The different patterns of resistance are most probably due to presence of different species *(H. filipjevi* and *H. avenae*) and pathotypes (Hf31, Hf41 and Ha21, and a further subgroup within Ha21). Since breeding for resistance using conventional techniques is time and resources consuming, it is imperative to know beforehand the target species and their pathotypes. Further, since most of the resistance used is based on an oligogeneous system that often targets a single species, for even a single pathotype (Cook and Rivoal 1998), field use of this type of genetic system may create a selective pressure that will break the resistance. Also, the development of secondary species/pathotypes not targeted by the resistance genes involved may result in unbalanced biocenosis (Rivoal *et al.* 1986). Therefore, the best proposition should be to develop total resistance, which implies the presence

of genes having major effects on the plant-nematode relationship, and nematode populations that are homogenous for resistance. However, there may be practical difficulties in this approach as certain genes responsible for resistance in different germplasm may have the same or closely-linked loci. Males were produced on all the cultivars tested which indicate that second-stage juveniles do penetrate and develop further in these cultivars.

The hatching of *H. avenae* was greater at lower temperatures (5, 10, 15ºC) compared to higher temperatures of (20, 25ºC), ranging between 46.7, 82.3 and 45.9% vs 30.7 and 19.0%, respectively (İmren *et al.* 2012b). Similarly, Sahin *et al.* (2008) reported that highest hatching percentages of *H. filipjevi* were obtained with 15, 10 and 5ºC treatments; 94.1, 91.9 and 75.2%, respectively. Hatching of *H. filipjevi* at 20 and 25ºC was low; being 21.9 and 18.6%, respectively. Hatching significantly increased with the change of temperature from 5ºC to 20ºC and from 10ºC to 20ºC, at rates of 48.5% and 42.4%, respectively. The percentage reductions in wheat yield of varieties due to *H. avenae* ranged between 4.36 and 25.7% when averaged over the two growing seasons (İmren and Elekcioğlu 2014). The yield reduction on wheat caused by *H. avenae* range was 15-20% in Pakistan (Maqbool 1988), 40-90% in Saudi Arabia (Ibrahim *et al.* 1999), and 23 to 50% in Australia (Nicol 2002).

The use of resistant varieties offers the most effective and economic option to control damage from CCNs. Cultivars containing the *Cre1* gene, such as AUS10894, Loros (63/1.7.15.12) and Adana-99, have consistently been very effective against these populations in screenings performed in the glasshouse and outdoor nursery. Adana-99 is a Turkish spring wheat cultivar with good agronomic traits and was used as the *Cre1* gene-donor parent for crossings with spring and winter wheat cultivars adapted to the Eastern Mediterranean Region of Turkey. Many of these crosses have been shown to be resistant to *H. avenae* in preliminary tests. Additionally, effective resistance genes against the *H. filipjevi* population at Haymana are being identified using the International Test Assortment composed of barley, wheat and oat lines containing defined genes for resistance to members of *H. filipjevi* (Nicol *et al.* 2009). Also, the *Cre1* gene has been incorporated into Turkey-adapted wheat varieties (İmren *et al.* 2013c). The best possible outcome would be a demonstration that the *Cre1* gene is as effective against *H. filipjevi* as it is against *H. avenae* in Turkey. If the Turkey population of *H. filipjevi* is not controlled by *Cre1*, another gene identified from our tests will be introduced into the Turkish wheat varieties already carrying the *Cre1* gene, pyramiding these sources of resistance to both nema-

todes. This will be important in fields where *H. filipjevi* already coexists with *H. avenae*, and in *H. avenae* infested fields likely to also become infested with *H. filipjevi* due to the efficient spread of these species in soil transferred by wind, water, and contamination by soil of agricultural products, animals, equipment and humans (dusty or muddy boots or clothing). Moreover, resistance sources in Turkish national wheat and wild genotypes needs to be determined. These results indicate the potential to identify nematode resistant germplasm for use in both international and national breeding programs.

ACKNOWLEDGMENTS

This study was funded by the number of 110 O 632 of TUBITAK project (Turkey Scientific and Technical Research Council) and CIMMYT.

REFERENCES

- Abidou H, El-Ahmed A, Nicol JM, Bolat N, Rivoal R, Yahyaoui A (2005) Occurrence and distribution of species of the *Heterodera avenae* group in Syria and Turkey. *Nematologica Mediterranea*, 33, 1-18.
- Al–Hazmi AS, Cook R, Ibrahim AAM (2001) Pathotype characterisation of the cereal cyst nematode, *Heterodera avenae*, in Saudi Arabia. *Nematology*, 3 (4), 379–382.
- Balakhnina VP (1989) Resistance of varieties of *Triticum durum* Desf. and *Triticum aestivum* L. to the oat cyst nematode, In: Gelmintologiya Segonya: Problemy Perspectivy. Tezisy Dokladov Nauchnoi Konferentsii, Moskova, 4–6 April, Tom 2, Moscow, USSR: 36–37.
- Bishnoi SP, Bajaj H (2002) Response of resistant barley cultivars to the Indian populations of *Heterodera avenae* complex. *Indian Journal of Nematology*, 32, 125–128.
- Cook R, Rivoal R (1998) Genetics of resistance and parasitism. In: 'The Cyst Nematodes.' (Ed. SB Sharma). pp. 322-368. (Kluwer Academic, Dordrecht, Netherlands).
- Cook R, Noel GR (2002) Cyst nematodes: Globodera and *Heterodera* species. In: 'Plant Resistance to Parasitic Nematodes' (Eds. JL Starr JL, R Cook, J Bridge) pp. 71–105. (CABI Publishing, UK).
- Dababat AA, Orakçı GE, Toktay H, İmren M, Akın B, Braun HJ, Dreisigacker S, Elekcioğlu İH, Morgounov A (2014) Resistance of winter wheat to *Heterodera filipjevi* in Turkey. *Turkish Journal of Agriculture and Forestry*, 38, 180-186.

- Dababat AA, Imren M, Erginbas-Orakci G, Ashrafi S, Yavuzaslanoglu E, Toktay H, Pariyar SR, Elekcioglu H, Morgounov A, Mekete M (2015) The importance and management strategies of cereal cyst nematodes, *Heterodera* spp., in Turkey. *Euphytica*, 202, 173-188.
- Grosse E, Kohlmüller S (2004) Untersuch – ungen zur Verbreitung von Getreidezystennematoden nach einer neuen Differentialmethode. Mitt. Biol. Bundesanst. L and – Forstwitrsch. Berlin – Dahlem. Heft, 396, 563–564.
- Handoo ZA (2002) Key and compendium to species of the *Heterodera avenae* group (Nematode: Heteroderidae). *Journal of Nematology*, 34, 250-262.
- Holgado R, Andersson S, Rowe JA, Magnusson C (2004) First record of *Heterodera filipjevi* in Norway. *Nematologia Mediterranea*, 32, 205-211.
- Hooper DJ (1986) Handling, fixing, staining and mounting nematodes. In: 'Laboratory Methods for Work with Plant and Soil Nematodes.' (Ed. JF Southey) pp. 59–80. (CAB International, London, UK).
- Ibrahim AAM, Al–Hazmi AS, Al–Yahya FA, Alderfasi AA (1999) Damage potential and reproduction of *Heterodera avenae* on wheat and barley under Saudi field conditions. *Nematology*, 1 (6), 625-630.
- İmren M, Elekcioğlu İH (2014) Effect on yield of cereal cyst nematode: *Heterodera avenae* (Tylenchida: Heteroderidae) in some spring wheat varieties in Adana Province, Turkey. *Turkish Journal of Agriculture and Forestry*, 38, 820-823.
- İmren M (2013) Identification cereal cyst nematodes (*Heterodera* spp.) in cereal fields of Eastern Mediterranean Region and investigation of the resistance *Heterodera avenae* (Wollenweber, 1924) some wheat genotypes. Ph.D Thesis. University of Çukurova, Turkey.
- İmren M, Toktay H, Bozbuga R, Dababat AA, Elekcioğlu İH (2013a) Pathotype determination of the cereal cyst nematode, *Heterodera avenae* (Wollenweber, 1924) in the Eastern Mediterranean Region in Turkey. *Turkish Journal of Entomology*, 37 (1), 13-19.
- İmren M, Toktay H, Bozbuga R, Dababat AA, Özkan H, Elekcioğlu İH (2013b) Determination of resistance against to cereal cyst nematode, *Heterodera avenae* (Wollenweber, 1924) in some wheat germplasm. *Turkish Journal of Entomology*, 37 (2), 229-238.
- İmren M, Toktay H, Bozbuga R, Orakçı GE, Dababat AA, Elekcioğlu İH (2013c) Identification of genetic resistance to cereal cyst nematodes; *Heterodera avenae* (Wollenweber, 1924), *H. filipjevi* (Madzhidov, 1981) Stelter and *H. latipons* (Franklin, 1969) in some international bread wheat germplasms. *Turkish Journal of Entomology*, 37 (3), 277-282.

- İmren M, Toktay H, Özarslandan A, Dababat AA, Elekcioğlu IH (2012b) Effect of temperatures on incubation duration of *Heterodera avenae* cysts and optimization of some materials and methods. *Turkish Journal of Entomology*, 36 (4), 585-593.
- İmren M, Waeyenberge L, Viaene N, Toktay H, Dababat AA, Elekcioğlu İH (2012a) Molecular characterization of cereal cyst nematodes from South Anatolian Region in Turkey using ITS-rDNA sequences. *Turkish Journal of Entomology*, 36 (4), 491-499.
- Kort J (1972) Nematode diseases of cereals of temperate climates. In: 'Economic Nematology.' (Ed. JM Webster). pp. 97-126. (Academic Press, New York, NY, USA).
- Madani M, Vovlas N, Castillo P, Subbotin SA, Moens M (2004) Molecular characterization of cyst nematode species (*Heterodera* spp.) from the Mediterranean basin using RFLPs and sequences of ITS-rDNA. *Journal of Phytopathology*, 15, 229-234.
- McDonald A, Nicol JM (2005) Nematode parasites of cereals In: 'Plant Parasitic Nematodes in Tropical and Subtropical Agriculture.' (Eds. J Bridge, R Sikora, M Luc) pp. 131-191. (CABI Publishing, Wallingford, Oxon, UK).
- Mokabli A, Valette S, Gauthier J-P, Rivoal R (2002) Variation in virulence of cereal cyst nematode populations from North Africa and Asia. *Nematology*, 4(4), 521- 525.
- Mor M, Cohn E, Spiegel Y (1992) Phenology, pathogenicity and pathotypes of cereal cyst nematodes, *Heterodera avenae* Woll. and *H. latipons* (Nematoda: Heteroderidae) in Israel. *Nematologica*, 38, 444-501.
- Nicol JM *et al.* (2002) The frequency and diversity of the cyst and lesion nematode on wheat in the Turkish Central Anatolian Plateau. *Nematology*, 4(2), 272.
- Nicol JM (2002) Important nematode pests. In: 'Bread Wheat Improvement and Production.' (Eds. BC Curtis, S Rajaram, H Gómez Macpherson) pp. 345-366. (FAO Plant Production and Protection Series, FAO, Rome, Italy).
- Nicol JM, Rivoal R (2008) Global knowledge and its application for the integrated control and management of nematodes on wheat. In: 'Integrated Management and Biocontrol of Vegetable and Grain Crops Nematodes.' (Eds. A Ciancio, KG Mukerji) pp. 243–287. (Springer, Dordrecht, Rhe Netherlands).
- Özarslandan M (2008) Buğday Genotiplerinin Tahıl Kist Nematodu, *Heterodera filipjevi* (Madzhidov) Stelter Popülasyonlarına Karşı Dayanıklılıklarının Araştırılması. Çukurova Üniversitesi Fen Bilimleri Enstitüsü. Yüksek Lisans Tezi, Adana.

- Peng D, Zhang D, Nicol JM, Chen S, Waeyenberge L, Moens M, Li H, Tang W, Riley IT (2007) Occurrence, distribution and research situation of cereal cyst nematode in China. p. 350-351. In Proc. Int. Plant Protection Conference, 16th, Glascow, Scotland, UK. 15-18 Oct. 2007. Br. Crop Production Council, Alton, Hampshire, UK.
- Rivoal R, Cook R (1993) Nematode pests of cereals. In: 'Plant Parasitic Nematodes in Temperate Agriculture.' (Eds. K Evans, DL Trudgill, JM Webster) pp. 259-303. (CAB International, Wallingford, UK).
- Rivoal R (1986) Biology of *Heterodera avenae* Wollenweber in France. IV. Comparative study of the hatching cycles of two ecotypes after their transfer to different climatic conditions. *Revue de Nématologie*, 9, 405-410.
- Romero MD, Andres MF, Lopez-Brana I, Delibes A (1996) A pathogenic and biochemical comparison of two Spanish populations of the cereal cyst nematode. *Nematologia Mediterranea*, 24, 235-244.
- Rumpenhorst HJ, Elekçioğlu IH, Sturhan D, Öztürk G, Enneli S (1996) The cereal cyst nematode *Heterodera filipjevi* (Madzhidov) in Turkey. *Nematologia Mediterranea*, 24, 135-138.
- Şahin E (2010) Distribution of the Important Plant Parasitic Nematodes in Wheat Production Area in Central Anatolian Plateau and Studies on the Biology and control of Cereal Cyst Nematode *Heterodera filipjevi*. PhD. University of Cukurova, Turkey.
- Şahin E, Nicol JM, Yorgancılar A, Elekcioğlu İH, Tülek A, Yıldırım AF, Bolat N (2008) Seasonal variation of field populations of *Heterodera filipjevi, Pratylenchus thornei* and *P. neglectus* on winter wheat in Turkey. *Nematologica Mediteranea*, 36, 51-56.
- Scholz U (2001) Biology, pathogenity and control of cereal cyst nematode *Heterodera latipons* Franklin on wheat and barley under semiarid conditions and interactions with common root rot *Bipolaris sorokiniana* (Sacc.) Shoemaker [teleomorph: *Cochliobolus sativus* (Ito et Kurib.) Drechs. ex Dastur., Ph.D Thesis. University of Bonn, Germany.
- Sharma SB, Swarup G (1984) Cyst Forming Nematodes of India, India Cosmo Publication, New Delhi, India.
- Sikora RA, Oostendorp M (1986) Occurrence of plant parasitic nematodes in ICARDA experimental fields. ICARDA, Aleppo, Syria.
- Sikora RA (1998) Plant parasitic nematodes of wheat and barley in temperate and temperate semi-arid regions –a comporative analysis. In: 'Nematodes Parasitic to Cereals and Legumes in Temperate Semi-arid Regions.' (Eds. MC Saxena, RA Sikora, JP Srivastava) pp. 46-68. (ICARDA: Alepo, Syria).

- Smiley RW, Yan GP, Handoo ZA (2008) First record of the cyst nematode *Heterodera filipjevi* on wheat in Oregon. *Plant Disease*, 92, 1136.
- Subbotin S, Mundo-Ocampo A, Baldwin JG (2010) Systematics of Cyst Nematodes (Nematode: Heteroderinae). *Nematology Monographs and Perspectives*, 8A, 68. (Brill, Leiden, The Netherlands).
- Subbotin SA, Sturhan D, Rumpenhorst HJ Moens M (2003) Molecular and morphological characterisation of the *Heterodera avenae* species complex (Tylenchida: Heteroderidae). *Nematology*, 5, 515-538.
- Subbotin SA, Waeyenberge L, Molokanova IA, Moens M (1999) Identification of *Heterodera avenae* group species by morphometrics and rDNA-RFLPs. *Nematology*, 1, 195-207.
- Tanha Maafi Z, Subbotin SA, Moens M (2003) Molecular identification of cyst forming nematodes (Heteroderidae) from Iran and a phylogeny based on ITS – rDNA sequences. *Nematology*, 5, 99–111.
- Toktay H, İmren M, Bozbuga R, Orakçı GE, Dababat A, Elekcioğlu IH (2013) Pathotype characterization of the cereal cyst nematode *Heterodera filipjevi* (Madzhidov, 1981) Stelter in Turkey. *Turkish Journal of Entomology*, 37, 213-219.
- Turkish Statistical Institute (2012) (http://www.tuik.gov.tr), (Erişim tarihi: Aralık 2012).
- Turner SJ Rowe JA (2006) Cyst nematodes. In: 'Parasitic Flatworms: Molecular Biology, Biochemistry, Immunology and Physiology.' (Eds. AG Maule, NJ Marks) pp. 93-122. (CAB International, Wallingford, England).

MULTIPLICATION OF *PRATYLENCHUS NEGLECTUS* AND *P. THORNEI* ON PLANTS OTHER THAN WHEAT AND BARLEY

RICHARD W. SMILEY*

Oregon State University, Columbia Basin Agricultural Research Center, Pendleton, Oregon 97801, USA. *Corresponding author email: richard.smiley@oregonstate.edu

SUMMARY

Multiplication rates of the root-lesion nematodes *Pratylenchus neglectus* and *P. thornei* were quantified in the glasshouse for species and cultivars of 30 crops other than wheat or barley, of 18 rangeland grasses and forbs, and of 16 weeds. Hosting ability ratings were compared to that of a susceptible wheat control. Crops included chickpea, pea, lentil, oat, canola, mustard, camelina, Sudangrass, sunflower, flax, safflower and others. Rangeland plants included bluegrasses, fescues, smooth brome, wheatgrasses, hairy vetches, and alfalfa (lucerne). Weeds included broad-leaved and grass species found in agricultural fields in the Pacific Northwest USA. Replicated pot tests were performed in a glasshouse and the experiment was repeated. Plants were categorized as important multipliers of both *Pratylenchus* species, of one but not the other species, or of neither species. Results are providing guidance for improving the efficiencies of crop rotations and cultivar selections in fields where root-lesion nematodes are present, and for transitioning rangelands into crop production in a manner that minimizes the yield-limiting impacts of *Pratylenchus* species present in some rangeland soils. Results also assist in understanding the roles of weeds on nematode densities in cropping systems, and particularly the potential for increasing the density of *Pratylenchus* species in roots of ima-

* Smiley RW (2015) Multiplication of *Pratylenchus neglectus* and *P. thornei* on plants other than wheat and barley. In 'Nematodes of Small Grain Cereals: current status and research.' (AA Dababat, H Muminjanov and RW Smiley) pp. 303-314. (FAO: Ankara, Turkey).

zamox-resistant wheat cultivars that are treated with the herbicide to selectively eliminate infestations of jointed goatgrass or other weeds that are good hosts of *Pratylenchus* species.

INTRODUCTION

Root-lesion nematodes (*Pratylenchus* spp.) are widely distributed in rainfed fields of the low-precipitation regions (< 400 mm annually) of the Pacific Northwest (PNW) states of Idaho, Oregon and Washington (Kandel *et al.* 2013, Smiley *et al.* 2004, Strausbaugh *et al.* 2004). The most prevalent species are *P. neglectus* (Rensch) Filipjev Schuurmanns & Stekhoven and *P. thornei* Sher & Allen, which often occur individually but are also found as mixtures. Rainfed wheat yields in the PNW are often inversely correlated with the preplant density of *Pratylenchus* spp. (Smiley 2009, Smiley and Machado 2009, Smiley *et al.* 2004, 2005a, 2005b, 2013, 2014a). Yields of intolerant cultivars have been reduced by as much as 60%. These nematodes reduce wheat production by an estimated $51 million annually in the PNW (Smiley 2009). All commercial wheat cultivars appear to be susceptible.

Pratylenchus neglectus and *P. thornei* invade roots of many monocot and dicot plants (Castillo and Vovlas 2007, Smiley and Nicol, 2009). Wheat is by far the dominant economically-viable rainfed crop in the low-precipitation zone of the PNW. Field studies with potential rotation crops have provided insights of *Pratylenchus* spp. dynamics in dryland cropping systems. For instance, barley is generally more resistant than wheat (Smiley 2009, Smiley *et al.* 2004), and *Pratylenchus* spp. densities were significantly suppressed by barley but not by canola or wheat (Smiley and Machado 2009). These observations are important in view of increasing interest in soil-conserving farming systems that include annual spring crops, including no-till production of food legumes and of oilseed and biofuel crops. However, the hosting ability of North American cultivars of non-cereals such as canola, mustard, safflower, field pea, and lentil is still unknown. Research overseas indicated that some crops promoted as rotational options for wheat in the PNW could be important hosts of *P. neglectus* and/or *P. thornei*, and could be capable of increasing the risk to a subsequent intolerant cultivar of wheat (Hollaway *et al.* 2000, Taylor *et al.* 2000, Thompson *et al.* 2008, Vanstone *et al.* 2008). High densities of *P. neglectus* are detected following some canola and mustard crops in the PNW but information on the hosting abilities of these crops is lacking.

More than one million hectares of former wheat-producing land in the PNW is currently maintained under contracts to a Federal Conservation Reserve Program (CRP). Contracts are for 10-year intervals and require the planting of mixtures of grasses and forbs adapted to local environments and well suited for wildlife. When contracts expire the producer may negotiate another contract, may continue to maintain the rangeland for grazing animals without Federal funding, or may return the land to crop production. Land with expired CRP contracts in the PNW are typically returned immediately to wheat production. High densities of *P. neglectus* have been detected in wheat planted into former CRP land but risks associated with individual grasses in the CRP are unknown. Many rangeland grasses and legumes have been evaluated for their hosting efficiency for these nematodes but information on current cultivars planted in the PNW was generally lacking. These plants have been screened to determine potential differences in hosting abilities for *P. neglectus* and *P. thornei*.

Densities of *P. neglectus* and/or *P. thornei* are also found to be high where volunteer cereals or weeds are allowed to grow at any time within or between planted wheat crops. The role of weeds as bridging hosts for *Pratylenchus* spp. in the PNW is unknown. Assays of grass and broad-leaved weeds have been developed to determine their abilities to serve as hosts.

The goal of this research was to determine the hosting abilities to *P. neglectus* and *P. thornei* by several crop species and cultivars, rangeland grasses and weeds.

METHODS

Details of these experiments were published in Smiley *et al.* (2014b, 2014c). We evaluated 30 crop species and cultivars, 18 rangeland species and 16 weed species to quantify hosting abilities for *P. neglectus* and *P. thornei*. Assays were performed twice in a glasshouse.

Crop plants (Table 1) included alfalfa (lucerne), canola, camelina, chickpea, flax, hairy vetch, lentil, mustard, oat, pea, safflower, Sudangrass, Sudangrass/sorghum hybrid, sunflower, eastern gamagrass, and switchgrass. Rangeland species (Table 2) included wheatgrasses, fescues, bluegrasses, and bromes. Weeds (Table 2) included 15 species in six plant families; Amaranthaceae, Asteraceae, Brassicaceae, Chenopodiaceae, Euphorbiaceae, and Poaceae.

Controls included inoculated unplanted (bare) soil, non-inoculated unplanted soil, two *Pratylenchus*-susceptible wheat cultivars (Louise and Otis), and a moderately-resistant landrace wheat cultivar AUS28451.

Assays were conducted by planting one seed of each plant species into each of eight replicate pots of a fine sandy loam that had been partially sterilized in an oven to kill active nematodes. Each plant species was assayed against each *Pratylenchus* spp. in two repetitions of the experiment and data were averaged for the repetitions. The pots were arranged in the glasshouse using a randomized complete block design. Pots were watered as needed and plant nutrients were supplied by slow-release fertilizer cylinders placed into the soil. The glasshouse was maintained at about 22°C, with daylight supplemented by a 12-hour period using 1000-watt high-pressure sodium horticultural lamps.

Inoculum of *P. neglectus* was a composite of cultures collected from five locations. However, a single isolate of *P. thornei* was used. Pure cultures were maintained on carrot disks in an incubator. Each culture was identified to assure purity of species (Yan *et al.* 2008). Inoculum for the assays was extracted by slicing carrot disks, suspending them in water, recovering them using a 20 µm sieve, and storing them in water at 8°C. Soil was inoculated soon after seedlings emerged. The suspension of nematodes (2,000 to 3,000 nematodes/kg of soil) was placed into small diameter holes made on two sides of the seedling. Assays were terminated 16 and 12 weeks after inoculation during 2011 and 2012, respectively.

Nematodes were extracted from finely-chopped roots and soil using the Whitehead tray method. The reproductive factor (*Rf*) was calculated by dividing the final nematode density (*Pf*) by the initial nematode inoculum density (*Pi*). Hosting ability groups were designated as non-hosts (*Rf* <0.1), poor hosts (*Rf* = 0.1-0.9), minor hosts (*Rf* = 1.0-4.9), good hosts (*Rf* = 5.0-9.9), and very good hosts (*Rf* ≥10).

RESULTS

Crops: Only four cultivars were classified as a good or a very good host to both *Pratylenchus* spp.; Monida oat, Myles chickpea, and Athena and Morton lentil (Table 1). Thirteen cultivars were good hosts of *P. neglectus* but not of *P. thornei*, including all 10 brassica crops (canola, mustard and camelina). In contrast, good hosts of *P. thornei* but not of *P. neglectus* included four cultivars of pea and lentil. Eleven cultivars were poor to minor hosts to both *Pratylen-*

chus spp., including cultivars of alfalfa, pea, chickpea, safflower, sunflower, flax, Eastern gamagrass, and switchgrass.

Table 1. Hosting abilities[1] of crops other than wheat and barley

Crop	Cultivar	Pn[2]	Pt[2]	Crop	Cultivar	Pn[2]	Pt[2]
Canola (spring)	Hyola 401	VG	M	Lentil (winter)	Morton	G	G
	Goldrush	VG	P	Lentil (spring)	Athena	G	VG
Canola (winter)	Amanda	VG	M		Skyline	M	VG
	Dwarf Essex	VG	P	Safflower (winter)	KN 144	M	M
	Salut	VG	M	Safflower (spring)	Gila	M	P
Mustard (brown)	Pacific Gold	G	P		Girard	M	P
Mustard (yellow)	IdaGold	VG	M	Sunflower	2PD08	M	M
Camelina	Yellowstone	VG	P	Flax	Pembina	P	P
	Blaine Creek	VG	P	Oats	Monida	G	VG
	Calena	G	M	Alfalfa (Lucerne)	Don	M	M
Chickpea	Sierra	G	M		Ladak-65	P	P
	Myles	G	VG	Hairy vetch	Purple Bounty	G	M
	Dwelley	M	M		Purple Prosperity	G	M
Pea (green)	Journey	M	VG	Eastern gamagrass	Pete	P	P
Pea (yellow)	Universal	P	G	Switchgrass	Blackwell	P	P
	Badminton	P	M	Sudangrass	Piper	VG	M
Pea (Austrian winter)	Granger	M	VG	Sorghum/ Sudangrass cross	Greentreat Plus	G	M

[1] Hosting ability ratings are based upon the reproductive factor (*Rf*) of the nematode as determined by replicated testing of each plant entry in glasshouse assays. The *Rf* is calculated as the final nematode density (*Pf*, nematodes/pound of soil) divided by the density of *Pratylenchus* initially added into soil (*Pi*, nematodes/pound of soil). Ranges of *Rf* values were grouped into five hosting ability ratings; N = nonhost (*Rf* < 0.1), P = poor host (*Rf* = 0.1 to 0.9), M = minor host (*Rf* = 1.0 to 4.9), G = good host (*Rf* = 5.0 to 9.9), VG = very good host (*Rf* ≥ 10.0).

[2] Pn = *Pratylenchus neglectus*, Pt = *Pratylenchus thornei*

Rangeland grasses: Nine of the 14 rangeland grasses were good to very good hosts of both *Pratylenchus* spp. (Table 2). Three additional rangeland grasses were good to very good hosts of only *P. neglectus*, and two fescues were good hosts of *P. thornei* but not of *P. neglectus*.

Weed species: All of the nine broad-leaved weeds were poor hosts of *P. thornei* and only four were good to very good hosts of *P. neglectus* (Table 2). In contrast, all of the six grass weeds were good to very good hosts of at least one *Pratylenchus* spp. Most notable was the ranking of jointed goatgrass as a very good host of both species.

DISCUSSION

Knowledge of hosting abilities by many crops other than small grain cereals will become increasingly important as production increases for biofuel and biomass-producing crops such as canola, mustard, camelina, switchgrass and eastern gamagrass in the PNW. Our results are similar to reports from Australia which state that densities of *P. neglectus* but not *P. thornei* are likely to be increased by canola (as discussed in detail in Smiley *et al.* 2014c). Even some bio-fumigant green-manure crops such as mustard and sorghum are capable of increasing the density of *Pratylenchus* spp. in roots and soil before the green foliage is macerated and incorporated into the soil. When incorporated into soil, the disrupted plant cells release toxic products that expedite a decline in the number of nematodes remaining in soil.

Table 2. Hosting abilities[1] of weeds and of rangeland grasses

Cropland weeds	Pn[2]	Pt[2]	Range grasses	Variety	Pn[2]	Pt[2]
Broad-leaved			Bluegrass			
Common lambsquarters	M	P	Big	Sherman	VG	M
Dandelion	P	P	Thickspike	Critana	VG	VG
Horseweed	P	P	Brome			
Kochia	VG	P	Smooth	Manchar	G	G
Palmer amaranth	G	P	Fescue			
Prostrate spurge	P	P	Hard	Durar	M	G
Redroot pigweed	G	P	Sheep	Blacksheep	M	G
Russian thistle	M	P	Wheatgrass			
Tumble mustard	G	P	Beardless	Whitmar	G	G
Grass			Crested	Fairway	VG	G
Downy brome	M	G	Crested	Hycrest	VG	VG
Green foxtail	VG	M	Intermediate	Greenar	G	G
Jointed goatgrass	VG	VG	Siberian	Vavilov	G	VG
Large crabgrass	G	M	Snake river	Secar	VG	VG
Rattail fescue	M	VG	Standard crested	Nordan	G	VG
Wild oat	G	M	Tall	Alkar	G	M
			Western	Rosana	VG	M

[1] Hosting ability ratings are as shown on Table 1.
[2] Pn = *Pratylenchus neglectus*, Pt = *Pratylenchus thornei*

If those same crops are grown to maturity either to cut as hay crops or to extract oil from seed, as almost always occurs in rainfed low-precipitation areas

of the PNW, the *Pratylenchus* spp. density may remain elevated even though those same crops can be effective as a *Pratylenchus*-suppressing bio-fumigant if green vegetation of those hosts is incorporated into soil. In rainfed systems of the PNW, it appears that these crops could have variable effects on the density of *Pratylenchus* spp. when they are grown for seed production.

All 10 cruciferous oilseed crops within the Brassicaceae family were good to very good hosts of *P. neglectus* and poor to minor hosts of *P. thornei*. Canola cv. Amanda was the most susceptible host of *P. neglectus* encountered during our assays, including the susceptible wheat controls. These findings are important because canola production in the PNW is capable of expanding by up to 500,000 ha in response to the impending startup of the region's first oilseed processing plant. While cruciferous oilseed crops sometimes increase wheat yields compared to wheat monocultures, they are also generically proclaimed by many advisory personnel to break the 'disease cycle' of pathogens that invade wheat roots. This is clearly not true for *P. neglectus*, the most widely distributed species of *Pratylenchus* in the rainfed, low-precipitation cropping systems of the PNW. We previously reported that some of the highest densities of *P. neglectus* encountered in our field trials have occurred in fields recently cropped with canola or yellow mustard (Smiley 2009, Smiley and Machado 2009, Smiley *et al.* 2008, 2005b, 2014a). Relationships between *Pratylenchus* spp. and cruciferous oilseed crops are clearly complex, depending upon whether the crop is grown to maturity or incorporated as a green manure, the identity and molar quantities of various glucosinolate compounds in roots, leaves or seed of these crops, the degree to which foliar tissues are macerated when incorporated as a green manure, the water content of soil, and other factors (as discussed in detail in Smiley *et al.* 2014c).

Chickpea is a well-known host of both *P. thornei* and *P. neglectus* (Castillo and Vovlas 2007). However, reports of pea and lentil cultivars as hosts or non-hosts of *P. neglectus* and *P. thornei* have been varied throughout the world. As reported elsewhere, cultivars of interest in the PNW also varied in their hosting ability for these species. Additional cultivars need to be screened to help identify the effects of specific cultivars on *Pratylenchus* spp. in rotations of wheat and food legume crops in the PNW.

Cultivars of safflower, sunflower, and flax in our assays were classified as poor or minor hosts of both species of *Pratylenchus*. These findings are generally similar to those reported elsewhere in the world, as discussed by Smiley *et al.* (2014c).

Alfalfa and hairy vetch were evaluated because they are components of conservation grassland plantings in the PNW. However, in areas of higher precipitation or where supplemental irrigation is available, each of these species are also planted as a forage, hay or green manure crop. In our assays, both alfalfa cultivars were a poor to minor hosts of both *Pratylenchus* spp. In contrast, the hairy vetch cultivars were good hosts of *P. neglectus* and minor hosts of *P. thornei*.

It was clear that many of the rangeland grasses are capable of producing elevated densities of *P. neglectus* and/or *P. thornei* before conservation grasses are removed so the land can be converted into production of arable crops such as wheat. Our results with some rangeland grasses confirmed results of earlier research, as is discussed in Smiley *et al.* (2014b).

Jointed goatgrass was a very good host for both *P. neglectus* and *P. thornei*. The eight weeds that were most likely to amplify densities of *P. neglectus*, in order of decreasing hosting ability (based on *Rf* values), were kochia (13), jointed goatgrass (12), green foxtail (12), redroot pigweed (9), large crabgrass (9), tumble mustard (9), wild oat (8), and palmer amaranth (6). The three weeds most likely to amplify densities of *P. thornei*, in decreasing order, were jointed goatgrass (16), rattail fescue (11), and downy brome (5). Jointed goatgrass is of particular concern because it is a very good host of both *P. neglectus* and *P. thornei* and because it is widespread through winter wheat production areas of the PNW. This weed and downy brome are largely responsible for the fact that 'Clearfield' wheat cultivars are currently the dominant cultivars planted in the PNW because they are tolerant of the herbicide imazamox. These wheat cultivars are sprayed with imazamox to selectively remove sensitive weeds such as downy brome and jointed goatgrass from the crop. It appears likely that when jointed goatgrass is selectively eliminated from imazamox-tolerant winter wheat cultivars the nematodes could be expected to migrate from the dying goatgrass roots and thereby increase the inoculum density available for invading the wheat roots. This phenomenon, known as the 'green bridge', is well known for insect pests and fungal pathogens, and should also be investigated for plant-parasitic nematodes such as *Pratylenchus* spp.

Another common practice in PNW agriculture is to allow winter-annual grass weeds and volunteer wheat plants to persist overwinter after seeds germinate during the autumn. The weeds and volunteers often form a lawn that may persist as long as six months (November – April) during the 'sanitizing' phase of the 14 month fallow period between planted winter wheat crops (10 month

growing season; September – July). Common winter annual weeds such as jointed goatgrass and rattail fescue were shown in this study to be hosts of one or both *Pratylenchus* spp. These weeds are potential contributors to the elevated numbers of *Pratylenchus* spp. observed during the fallow phase of the winter wheat-fallow rotation (Smiley and Machado 2009). Our finding that rattail fescue is a very good host for *P. thornei* is also important because this weed is particularly well adapted to no-till cropping systems that are becoming increasingly practiced in the PNW.

Results of these assays provide insights that will be included among considerations for improving the efficiency of rainfed crop production systems in the PNW. It is likely that reduced wheat yields are caused in part by rotations that include other crops that are also important hosts of *Pratylenchus* spp. This appears to be very likely for some rotations of wheat with food legumes or brassica species. It is also likely that reduced production efficiency during a conversion of range land into wheat production is due in part to elevation of *Pratylenchus* spp. densities in the range. Where nematode densities have become elevated in grass lands it is likely be more efficient to plant a poor host or a non-host crop such as barley, safflower, flax, brown mustard, or selected cultivars of pea or chickpea before planting wheat. It is also likely that occurrences of specific weeds, such as jointed goatgrass, at any time in the cropping system can elevate densities of *Pratylenchus* spp. This must become recognized as an important attribute of weed management programs.

ACKNOWLEDGMENTS

This research was funded by a grant from a USDA Pacific Northwest Regional Research Program named Solutions to Economic and Environmental Problems (STEEP). Supplemental funding was from the Oregon Agricultural Experiment Station and the USDA-Agricultural Research Service. Seed was provided by J Chatelain, H Collins, D Johnson, S Machado, S Petrie, and D Wysocki. Technical assistance was provided by D Ball, M Baxter, C Dumont, J Gourlie, A Hitzman, S Isbell, M Peel, D Thill, C Sawyer, P Thorgersen, N Webster, and G Yan. The author thanks J Barroso for reviewing this paper.

REFERENCES

- Castillo P, Vovlas N (2007) *Pratylenchus*, Nematoda, Pratylenchidae: Diagnosis, biology, pathogenicity and management. *Nematological Monographs and Perspectives*, 6, 1-530.

- Hollaway GJ, Taylor SP, Eastwood RF, Hunt CH (2000) Effect of field crops on density of *Pratylenchus* in southeastern Australia: *P. thornei*. *Journal of Nematology*, 32(4S), 600-608.
- Kandel SL, Smiley RW, Garland-Campbell K, Elling AA, Abatzoglou J, Huggins D, Rupp R, Paulitz TC (2013) A survey of root lesion nematodes (*Pratylenchus* spp.) and their relationship with climatic factors in dryland wheat production areas of eastern Washington. *Plant Disease*, 97, 1448-1456.
- Smiley RW (2009) Root-lesion nematodes reduce yield of intolerant wheat and barley. *Agronomy Journal*, 101, 1322-1335.
- Smiley RW, Gourlie JA, Yan GP, Rhinhart KEL (2014a) Resistance and tolerance of landrace wheat in fields infested with *Pratylenchus neglectus* and *P. thornei*. *Plant Disease*, 98, 797-805.
- Smiley RW, Machado S (2009) *Pratylenchus neglectus* reduces yield of winter wheat in dryland cropping systems. *Plant Disease*, 93, 263-271.
- Smiley RW, Machado S, Gourlie JA, Pritchett LC, Yan GP, Jacobsen EE (2013) Effects of crop rotation and tillage on *Pratylenchus* spp. in the semiarid Pacific Northwest United States. *Plant Disease*, 97, 537-546.
- Smiley RW, Merrifield K, Patterson L-M, Whittaker RG, Gourlie JA, Easley SA (2004) Nematodes in dryland field crops in the semiarid Pacific Northwest USA. *Journal of Nematology*, 36, 54-68.
- Smiley RW, Nicol JM (2009) Nematodes which challenge global wheat production. In: 'Wheat: Science and Trade.' (Ed. BF Carver) pp. 171-187. (Wiley-Blackwell: Ames, Iowa).
- Smiley RW, Sheedy JG, Easley SA (2008) Vertical distribution of *Pratylenchus* spp. in silt loam soil of Pacific Northwest dryland crops. *Plant Disease*, 92, 1662-1668.
- Smiley RW, Whittaker, RG, Gourlie JA, Easley SA (2005a) *Pratylenchus thornei* associated with reduced wheat yield in Oregon. *Journal of Nematology*, 37, 45-54.
- Smiley RW, Whittaker RG, Gourlie JA, Easley SA (2005b) Suppression of wheat growth and yield by *Pratylenchus neglectus* in the Pacific Northwest. *Plant Disease*, 89, 958-968.
- Smiley RW, Yan GP, Gourlie JA (2014b) Selected Pacific Northwest rangeland and weed plants as hosts of *Pratylenchus neglectus* and *P. thornei*. *Plant Disease*, 98, 1333-1340.
- Smiley RW, Yan GP, Gourlie JA (2014c) Selected Pacific Northwest crops as hosts of *Pratylenchus neglectus* and *P. thornei*. *Plant Disease*, 98, 1341-1348.

- Strausbaugh CA, Bradley CA, Koehn AC, Forster RL (2004) Survey of root diseases of wheat and barley in southeastern Idaho. *Canadian Journal of Plant Pathology*, 26, 167-176.
- Taylor SP, Hollaway GJ, Hunt CH (2000) Effect of field crops on population densities of *Pratylenchus neglectus* and *P. thornei* in southeastern Australia: *P. neglectus*. *Journal of Nematology*, 32, 591-599.
- Thompson JP, Owen KJ, Stirling GR, Bell MJ (2008) Root-lesion nematodes (*Pratylenchus thornei* and *P. neglectus*): a review of recent progress in managing a significant pest of grain crops in northern Australia. *Australasian Plant Pathology*, 37, 235-242.
- Vanstone VA, Hollaway GJ, Stirling GR (2008) Managing nematode pests in the southern and western regions of the Australian cereal industry: continuing progress in a challenging environment. *Australasian Plant Pathology*, 37, 220-234.
- Yan GP, Smiley RW, Okubara PA, Skantar A, Easley SA, Sheedy JG, Thompson AL (2008) Detection and discrimination of *Pratylenchus neglectus* and *P. thornei* in DNA extracts from soil. *Plant Disease*, 92, 1480-1487.

EFFECTS OF CROPPING SYSTEMS ON DOMINANCE OF INDIVIDUAL SPECIES OF *PRATYLENCHUS*

RICHARD W. SMILEY*

Oregon State University, Columbia Basin Agricultural Research Center, Pendleton, Oregon 97801. *Corresponding author email: richard.smiley@oregonstate.edu

SUMMARY

Soils from cropping systems experiments in 280-mm and 400-mm precipitation zones of Oregon were evaluated by quantitative PCR (qPCR) to determine effects of rotations on inoculum density of *Pratylenchus neglectus* and *P. thornei*. Inoculum was determined as picograms of DNA/gram of soil and converted to nematodes/kg of soil by the Root Disease Testing Service in Adelaide, Australia. *Pratylenchus* densities reported by qPCR were two- to four-times greater but otherwise well correlated with densities detected by traditional extractions in the USA, presumably because qPCR also detects eggs. The qPCR revealed that winter wheat in both precipitation zones selected for a dominance of *P. neglectus* and that spring wheat and spring barley selected for *P. thornei*. A similar shift in species dominance was detected in a single field at another location during a three-year interval following conversion of a winter wheat field to spring cropping. Also, two neighboring fields used to produce either winter wheat or spring cereals in Idaho were reported to be dominated by either *P. neglectus* or *P. thornei* in the manner consistent with our findings within treatments of long-term experiments in Oregon. It remains unknown as to whether species-specific selections of *Pratylenchus* were a response to differences in cultivar genetics, to seasonal growth habit,

* Smiley RW (2015) Effects of cropping systems on dominance of individual species of *Pratylenchus*. In 'Nematodes of Small Grain Cereals: current status and research.' (AA Dababat, H Muminjanov and RW Smiley) pp. 315-326. (FAO: Ankara, Turkey).

or to some other selection pressure. Nevertheless, it is important to recognize that management strategies that are specific to a single *Pratylenchus* species may lead to a shifting dominance to another of these species in habitats where both species are present.

INTRODUCTION

Most information on effects of crop and soil management practices on diseases of wheat and other rainfed field crops has been developed from short-term (<5 yr) experiments. Few studies have defined effects of long-term (>30 yr) management treatments in which multiple pathogens are assumed to have attained equilibrium within that locality and cropping system. Long-term experiments (LTEs) can provide valuable insights into agronomic practices for sustainable crop production. These insights are not possible with short term experiments. Three LTEs near Pendleton, OR have been operated continuously for 84 years and are among the oldest active long-term agronomic studies in North America (Mitchell *et al.* 1991). These rainfed experiments occur in a semi-arid region (<400 mm precipitation annually, most of which occurs during autumn through spring) of the Pacific Northwest (PNW) and have provided unique insights into agricultural sustainability, soil health and productivity and economics of crop production; for an introduction to reports see Duff *et al.* (1995), Machado (2011), and Rasmussen and Smiley (1996). Findings have included relationships between diseases and characteristics of soil chemical, physical, and microbiological parameters. The only survey of diseases in LTEs at Pendleton (Smiley *et al.* 1996) was based upon assessment of disease symptoms and pathogen signs before it became known that *Pratylenchus* spp. are important components of cereal root disease complexes in the PNW (Smiley, 2009; Smiley and Machado 2009, Smiley *et al.* , 2004, 2005a, 2005b) and before real-time polymerase chain reaction (qPCR) procedures became readily available for quantifying *Pratylenchus* spp. inoculum in soil (Yan *et al.* 2008, 2012, 2013). The objective of this research was to evaluate effects of cropping systems on the population dynamics of *Pratylenchus* spp. and other soil-borne pathogens in regions where both *P. neglectus* and *P. thornei* were present.

METHODS

Experiments were established at three locations in Oregon; Moro, Mission and Pendleton. The experiment at Moro was a cropping systems experiment conducted from 2003 to 2012. The experiment at Mission was a series of three

single-year experiments on the same field. The experiments at Pendleton were long-term cropping systems studies maintained continuously over a range of periods from 11 to 84 years.

Moro: Details of this experiment were published in Smiley *et al.* (2013). This paper addresses results for only two of the 14 treatments, and sampling conducted only during the last year. Briefly, the experimental site receives 282 mm mean annual precipitation, nearly all of which occurs from late autumn (October) through spring (May). Mean daily air temperature is -1 °C during January and 19 °C during July and August. Soil is a moderately deep silt loam.

The experimental area consisted of 14 treatments (15 × 105 m plots) randomized within each of three blocks. Each phase of each rotation was present each year to allow data for each treatment to be collected every year. Crops were harvested in late July. The two treatments of interest for this report were winter wheat and spring wheat planted annually without tillage. Soil samples were collected from the three replicates of both treatments on 10 May 2012. Forty soil cores (2.5-cm diam × 30-cm deep) were composited for each of the six plots. Samples were dried in an oven at 40°C for 48 hours and 500 g of dry soil for each plot was sent to the Root Disease Testing Service, South Australia Research and Development Institute, Adelaide, Australia (Ophel-Keller *et al.* 2008). The lab extracted DNA from the entire 500 g of soil using the PreDicta B real-time PCR analysis system (http://www.sardi.sa.gov.au/products_and_services/entomology/diagnostic_service/predicta_b). Inoculum concentrations were reported for nine species or groups of soilborne plant-pathogenic fungi (as picograms DNA/g of soil) and for five plant-parasitic nematode species, including *Pratylenchus neglectus* and *P. thornei* (as nematodes/g of soil). Another sub-sample of each plot was extracted using the Whitehead tray method and *Pratylenchus* spp. were identified by morphological and molecular methods (Yan *et al.* 2008, 2012, 2013).

Mission: Details of this experiment were published in Smiley *et al.* (2014a). The focus of this report is on the pre-plant densities of *Pratylenchus* spp. in a field used for three experiments with spring wheat during 2011, 2012 and 2013. None of the specific variables in each experiment are discussed in this report.

Trials each year were located on nearby (< 200 m distances) sites within a single field located 10 km southeast of Pendleton, Oregon. The field receives

an average of 330 mm annual precipitation. The soil is a deep silt loam. The field was maintained without tillage and was planted regularly to winter wheat before and during 2008, was maintained as chemical fallow during 2009, was planted to canola during 2010, and was planted to spring wheat during 2011. Our spring wheat experiments were planted after canola during 2011 and following spring wheat during 2012 and 2013. Pre-plant density of plant-parasitic nematodes in each experimental area (800 m^2) was determined by collecting two composite soil samples (25 cores of 2.5-cm diam × 30-cm depth) from each experimental area at or before the time of planting. Samples were sent to Western Laboratories (Parma, Idaho) for extraction and enumeration of nematodes. The lab uses a modified Oosterbrink elutriator and centrifugal flotation extraction method. Densities of plant-parasitic nematodes were reported and nematodes were identified to the genus level. Nematode suspensions from Western Labs were returned to our laboratory for identification of *Pratylenchus* spp. using morphological and molecular methods (Yan *et al.* 2008, 2013, 2014).

Pendleton: Details of this experiment are being prepared for publication in the journal *Plant Disease* during 2016. The objective of this two-year survey was to assess inoculum densities of pathogens in five long-term experiments to provide focus for future research on disease complexes in wheat-based cropping systems. We also sampled three shorter-term (10-13 yr) experiments to expand the value of this research. All experiments are at Oregon State University's Columbia Basin Agricultural Research Center, near Pendleton. The climate is temperate with warm, dry summers (21°C) and cool, wet winters (1°C). About 75% of the 397 mm mean annual precipitation occurs from November to May. The soil is a deep silt loam. Only two of the experiments at Pendleton are discussed in this paper; i) blocks of winter wheat, spring wheat and spring barley planted annually, and ii) blocks of cultivated or non-cultivated soils planted to 3-year rotations of winter wheat, spring wheat and fallow. One sample (20 soil cores of 2.5-cm diam × 30-cm deep) was collected from each half of each cereal sub-block during two separate years; 2013 and 2015. Samples (400 g) were sent to Australia for DNA extraction and analysis, as described previously.

Annual cereals. A field was planted annually to winter wheat from 1931 to 1982 using a conventional tillage system consisting of a moldboard plow followed by disking. In 1982 the block was divided into three sub-blocks (20 x 100 m) that have been planted annually to either winter wheat (84 yr), spring wheat

(33 yr) or spring barley (33 yr) on the same sub-block each year. Soil in each block continues to be cultivated as it was from 1931 to 1982, except that tillage occurs during the autumn in the winter wheat sub-block and during the spring in the sub-blocks planted to spring cereals. An identical series of the three cereal sub-blocks was established during 1997 on an adjacent field that is not tilled between crops (18 years). Prior to 1997 the block had been used for a winter wheat-cultivated fallow rotation.

Three-year rotations. Fields were managed as 3-yr rotations of winter wheat, spring wheat and fallow, all without cultivation (no-till). Two sub-blocks were present and were sampled for each of the three rotational phases during each year (2013 and 2015). Sampling was as described for the annual cereals experiment.

RESULTS

Moro: During the final year of the experiment, soil samples were uniformly mixed and a subsample was sent to the Root Disease Testing Service (RDTS). Another subsample was extracted in our laboratory by using the Whitehead tray method. Both methods indicated that winter wheat had a greater density of *P. neglectus* and that spring wheat had a greater density of *P. thornei* (Table 1). DNA data showed that these trends were relatively consistent in the annual rotations (Table 1), in the 2-year rotations of winter wheat with fallow or winter pea (data not shown), and in 3-year rotations that include winter wheat as one of the two crops (data not shown). It was notable that in the winter wheat-winter pea rotation, there was a greater inoculum density of *P. neglectus* when the current crop was winter wheat and a greater inoculum density of *P. thornei* when the current crop was winter pea, each reflecting the influence of the crop during the previous year, yet the density of generic '*Pratylenchus* sp.' was equivalent in each phase of the rotation.

Mission: Initial densities of *Pratylenchus* spp. in the field were 1,316, 12,144 and 26,400 *Pratylenchus* spp./kg of soil during 2011, 2012 and 2013, respectively. During 2011 the population included a much greater proportion of *P. neglectus* than *P. thornei* but specific proportions were not determined. During 2012, there was a 2:1 ratio of *P. thornei* to *P. neglectus*, and during 2013 the pre-plant density was distributed as a 9:1 ratio of *P. thornei* to *P. neglectus*. The proportion of *Pratylenchus* spp. clearly shifted within a single field during the 3-year course of our investigation, apparently during a transition from a winter

wheat-based cropping system to several successive plantings of spring wheat on the field during the course of our investigation.

Pendleton: *Annual cereals experiment*: Treatment means are shown in Table 2. The treatment effect for year was significant for *P. thornei*, with inoculum density being greater during the wetter (2013) than the drier (2015) year (12,157 vs. 8,618 nematodes/kg). The treatment effect for tillage was not significant for either *P. neglectus* or *P. thornei*, however, the treatment effect of crop was significant for both species. A far higher inoculum density of *P. neglectus* occurred in winter wheat than in spring wheat or spring barley; 22,121, 3,835 and 2,129 nematodes/kg, respectively ($HSD_{0.05}$ = 4,072). For *P. thornei*, inoculum densities were higher in spring barley than in spring wheat and winter wheat; 17,682, 12,002 and 1,478 nematodes/kg, respectively ($HSD_{0.05}$ = 3,595). The year × tillage interaction was significant for *P. thornei*; inoculum density was unaffected by the year in cultivated soil (10,653 and 10,928 nematodes/kg) but was greater during the wetter than drier year (13,386 vs. 6,582 nematodes/kg) in the no-till block. All interactions among main treatments

Table 1. Relationship between two methods for quantifying *Pratylenchus neglectus* and *P. thornei* in soils collected from annual winter wheat and annual spring wheat plantings in a long-term experiment at Moro, Oregon (from Smiley *et al.* 2013)

Nematode and extraction method[1]	Winter wheat	Spring wheat	WW:SW ratio
P. neglectus			
Whitehead	6,631 a	1,747 b	3.8
DNA	10,913 a	1,110 b	9.8
P. thornei			
Whitehead	1,691 a	7,189 a	0.2
DNA	1,874 b	7,959 a	0.2

[1] Subsamples of well-mixed soil from each of three replicates for each crop were used to extract nematodes from soil; Whitehead = Whitehead tray method, or DNA = counts from qPCR analyses and standard curves. *Pratylenchus* sp. are reported as nematodes/kg of soil.

were significant for *P. thornei* but only one interaction was significant for *P. neglectus*. Tillage × crop interactions were significant both *P. neglectus* and *P. thornei*. Inoculum density of *P. neglectus* was greater in cultivated than in no-till blocks of winter wheat (26,595 vs. 17,648 nematodes/kg) and the opposite occurred for spring wheat (1,285 vs. 6,384 nematodes/kg) and spring barley (48 vs. 4,211 nematode/kg). In contrast, inoculum of *P. thornei* was greater in cultivated than in no-till blocks for spring barley (27,155 vs. 8,209 nematodes/kg) and the opposite occurred for spring wheat (4,773 vs. 19,230 nematodes/

kg) and winter wheat 443 vs. 2,514 nematodes/kg of soil). The year × crop interaction was significant for *P. thornei*. The interaction reflected a lack of differences between years for spring barley (18,428 vs. 16,936 nematodes/kg during 2013 and 2015) and winter wheat (2,173 vs. 784 nematodes/kg) and an important difference between years for spring wheat (5,252 vs. 18,751 nematodes/kg during 2013 and 2015). The year × tillage × crop interaction was significant for *P. thornei*. The density of *P. thornei* was particularly high in the cultivated spring barley during both 2013 and 2015, and was also high in the no-till spring wheat during 2015. Other trends were not apparent for explaining this 3-way interaction.

Table 2. Influence of cereals planted annually into cultivated or non-cultivated soils on inoculum densities of *Pratylenchus neglectus* and *P. thornei*.

Tillage[1]	Crop[2]	*P. neglectus*	*P. thornei*	*Pn:Pt ratio*
		(nematodes/kg of soil)		
Plow	SB	48 c[3]	27,155 a	0.0 b
Plow	SW	1,285 c	4,723 c	0.3 b
Plow	WW	26,595 a	443 c	60.0 a
No-till	SB	421 c	8,209 ab	0.1 b
No-till	SW	6,384 c	1,923 ab	3.3 b
No-till	WW	17,648 b	2,514 c	7.0 ab

[1] Blocks were managed either with inversion tillage using a moldboard plow (Plow) since 1931 or without tillage (No-till) since 1997. Each block was divided into three sub-blocks that were planted annually to the same crop since the inception date indicated. [2] Crops were spring barley (SB), spring wheat (SW) and winter wheat (WW). [3] Data are means of samples collected during two years. Numbers followed by the same letter within a column are not significantly different at $P = 0.05$ according to Tukey's honestly significant different test (HSD).

Table 3. Influence of cereals planted into a non-cultivated 3-year rotation (winter wheat-spring wheat-fallow) on inoculum densities of *Pratylenchus neglectus* and *P. thornei*.

Crop phase[1]	*P. neglectus*	*P. thornei*	*Pn:Pt ratio*
	(nematodes/kg of soil)		
WW	11,992 a[2]	9,172 ab	1.3 a
SW	3,219 b	19,382 a	0.2 b
Fallow	449 c	7,373 b	0.1 b

[1] Crop phase indicates the influence of the management of the block during the year prior to sampling; winter wheat (WW), spring wheat (SW) or fallow (Fallow). [2] Data are means of samples collected during two years. Numbers followed by the same letter within a column are not significantly different at $P = 0.05$ according to Tukey's honestly significant different test (HSD).

Three-year rotation experiment: Significant treatment differences were detected for both *P. neglectus* and *P. thornei*. Treatment means are shown in Table 3. There was no significant treatment effect of year. The effect of the previous rotational phase was significant for both pathogens. For *P. neglectus*, the inoculum density was greatest following winter wheat, intermediate after spring wheat, and lowest following fallow (Table 3). For *P. thornei*, the inoculum density was greatest following spring wheat, intermediate after winter wheat, and lowest following fallow.

DISCUSSION

In the experiment at Moro we determined that annually planted winter wheat had selected for a greater density of *P. neglectus* and annually planted spring wheat had selected for a greater density of *P. thornei* (Smiley *et al.* 2013). In successive experiments on a single field at Mission we observed a shift from a dominance of *P. neglectus* to a dominance of *P. thornei* over a 3-year period at a time when the cropping system was changed from producing winter wheat to planting spring wheat annually (Smiley *et al.* 2014a). Samplings from the well-stabilized LTEs at Pendleton supported our previous findings regarding the dynamics of *Pratylenchus* species on winter versus spring cereals. This occurred in the 83-year-old monoculture blocks planted annually to winter wheat, spring wheat or spring barley, and also in the 16-year-old fields of 3-year rotations of winter wheat, spring wheat and chemical fallow. Additionally, a similar phenomenon was reported by wheat producers on two neighboring farms in Idaho that produce either winter wheat only or spring wheat only.

In the semi-arid PNW winter wheat is planted on more than 95% of the rainfed fields. However, the steady increase in crop grown without tillage has caused a commensurate increase in plantings of spring cereals and other field crops. It remains unknown as to whether the differential selection of *Pratylenchus* spp. we observed was associated with a difference in hosting abilities among cultivars of winter and spring wheat, or whether it was associated with the different growth habits of these crops. However, we clearly established that different wheat crops differ in their ability to host reproduction of these species. For instance, we reported that some locally-popular cultivars of winter wheat are poor hosts of *P. neglectus* but very good hosts of *P. thornei* (references are in Smiley *et al.* 2013). We also reported from glasshouse assays that a popular spring wheat cultivar is a much better host of *P. thornei* than of *P. neglectus*

(Smiley *et al.* 2014b). It appears that the choice of wheat cultivar could have an impact on the dominance of these two species of *Pratylenchus* in locations where both are known to be present. Regardless of the mechanism causing the shift in *Pratylenchus* species dynamics, these observations will influence the development of recommendations for controlling these nematodes. For instance, we clearly need to amplify our emphasis on developing cultivars that carry dual-species resistance. Toward that goal, we recently registered a mapping population that includes lines with resistance to both *P. neglectus* and *P. thornei* (Smiley *et al.* 2014a; Thompson *et al.* 2015). Until commercial cultivars with dual-resistance are released, it remains very important for growers to have access to diagnostic services that identify *Pratylenchus* to the species level. Toward that goal, DNA-based testing methods for distinguishing *P. neglectus* and *P. thornei* (Yan *et al.* 2008, 2012, 2013) are now offered as a commercial service by at least one diagnostic laboratory in our region.

ACKNOWLEDGMENTS

Funding was from the Idaho Wheat Commission, Oregon Wheat Commission, Washington Wheat Commission, Idaho Agricultural Experiment Station, Oregon Agricultural Experiment Station, and USDA-Agricultural Research Service. Technical assistance was primarily provided by J Gourlie, E. Jacobsen, S Machado, L Pritchett, K Rhinhart, P Thorgersen and G Yan. The author thanks H Peter-Contesse and A Boettcher for reviewing this paper.

REFERENCES

- Duff B, Rasmussen PE, Smiley RW (1995) Wheat/fallow systems in the semi-arid regions of Pacific NW America. In. Agricultural Sustainability: Economic, Environmental and Statistical Considerations. (Eds. V Barnett, R Payne, R Steiner) pp. 85-111. (John Wiley & Sons, London)
- Machado S (2011) Soil organic carbon dynamics in the Pendleton long-term experiments: Implications for biofuel production in Pacific Northwest. *Agronomy Journal*, 103, 253-260.
- Mitchell CC, Westerman RL, Brown JR, Peck TR (1991) Overview of long-term agronomic research. *Agronomy Journal*, 83, 24-29.
- Ophel-Keller K, Hartley D, McKay A, Herdina, Curran J (2008) Development of a routine DNA-based testing service for soilborne diseases in Australia. *Australasian Plant Pathology*, 37, 243-253.

- Rasmussen PE, Smiley RW (1996) Soil carbon and nitrogen change in long-term agricultural experiments at Pendleton, Oregon. In 'Soil Organic Matter in Temperate Agroecosystems.' (Eds. EA Paul, K Paustian, ET Elliot, CV Cole) pp. 353-360. (CRC Press, Boca Raton, FL)
- Smiley RW (2009) Root-lesion nematodes reduce yield of intolerant wheat and barley. *Agronomy Journal*, 101, 1322-1335.
- Smiley RW, Collins HP, Rasmussen PE (1996) Diseases of wheat in long-term agronomic experiments at Pendleton, Oregon. *Plant Disease*, 80, 813-820.
- Smiley RW, Gourlie JA, Yan GP, Rhinhart KEL (2014a) Resistance and tolerance of landrace wheat in fields infested with *Pratylenchus neglectus* and *P. thornei*. *Plant Disease*, 98, 797-805.
- Smiley RW, Machado S (2009) *Pratylenchus neglectus* reduces yield of winter wheat in dryland cropping systems. *Plant Disease*, 93, 263-271.
- Smiley RW, Machado S, Gourlie JA, Pritchett LC, Yan GP, Jacobsen EE (2013) Effects of crop rotation and tillage on *Pratylenchus* spp. in the semiarid Pacific Northwest United States. *Plant Disease*, 97, 537-546.
- Smiley RW, Merrifield K, Patterson L-M, Whittaker RG, Gourlie JA, Easley SA (2004) Nematodes in dryland field crops in the semiarid Pacific Northwest USA. *Journal of Nematology*, 36, 54-68.
- Smiley RW, Whittaker, RG, Gourlie JA, Easley SA (2005a) *Pratylenchus thornei* associated with reduced wheat yield in Oregon. *Journal of Nematology*, 37, 45-54.
- Smiley RW, Whittaker RG, Gourlie JA, Easley SA (2005b) Suppression of wheat growth and yield by *Pratylenchus neglectus* in the Pacific Northwest. *Plant Disease*, 89, 958-968.
- Smiley RW, Yan GP, Gourlie JA (2014b) Selected Pacific Northwest crops as hosts of *Pratylenchus neglectus* and *P. thornei*. *Plant Disease*, 98, 1341-1348.
- Thompson AL, Smiley RW, Garland-Campbell K (2015) Registration of the LouAu (Louise/IWA8608077) wheat recombinant inbred line mapping population. *Journal of Plant Registrations*, 9, doi:10.3198/jpr2015.01.0002crmp.
- Yan GP, Smiley RW, Okubara PA (2012) Detection and quantification of *Pratylenchus thornei* in DNA extracted from soil using real-time PCR. *Phytopathology*, 102, 14-22.
- Yan GP, Smiley RW, Okubara PA, Skantar A, Easley SA, Sheedy JG, Thompson AL (2008) Detection and discrimination of *Pratylenchus neglectus* and *P. thornei* in DNA extracts from soil. *Plant Disease*, 93, 1480-1487.

- Yan GP, Smiley RW, Okubara PA, Skantar AM, Reardon CL (2013) Developing a real-time PCR assay for detection and quantification of *Pratylenchus neglectus* in soil. *Plant Disease*, 97, 757-764.

PART 5

MOLECULAR TECHNOLOGIES





MOLECULAR IDENTIFICATION OF CEREAL CYST NEMATODES: STATUS, PROSPECTS AND RECOMMENDATIONS

L. WAEYENBERGE[1,*] AND N. VIAENE[1,2]

[1]ILVO, Institute for Agricultural and Fisheries Research, Burg. Van Gansberghelaan 96, 9820 Merelbeke, Belgium. [2]Laboratory for Agrozoology, Ghent University, Coupure links 653, 9000 Ghent, Belgium, *Corresponding author email: lieven.waeyenberge@ilvo.vlaanderen.be

SUMMARY

Nematode identification using morphological and morphometrical data is not only time-consuming, it is also problematic due to the high phenotypic plasticity among populations and the absence of clear diagnostic characteristics for cryptic species. Therefore, the traditional way of microscopic identification has been increasingly complemented with molecular techniques. At first, PCR-RFLP profiling and sequencing of parts of the rDNA cistron were applied. However, other molecular techniques were needed to speed up acquisition of results and reduce costs. This has led to the development of species-specific primers for end-point PCR and DNA-probes used in real-time PCR. During the last 15 years, several species-specific assays have been developed for detection of *Heterodera* species, of which only few cereal cyst nematode species (CCN). However, more assays for CCN are desirable, preferably those allowing multiplexing and on-the-spot sample examination. To achieve this, sequencing DNA regions different from the traditional rDNA cistron, and screening for DNA-polymorphisms within species, is recommended. By facilitating fast and accurate detection, in combination with low costs and reduced labour and

* Waeyenberge L, Viaene N (2015) Molecular identification of cereal cyst nematodes: status, prospects and recommendations. In 'Nematodes of Small Grain Cereals: current status and research.' (AA Dababat, H Muminjanov and RW Smiley) pp. 329-334. (FAO: Ankara, Turkey).

time, molecular techniques will become the most important tools for detection and identification. They can be applied by national and regional plant protection agencies, but also by farmers and industry. These tools are expected to almost completely replace the traditional way of identification as the number of specialists in this field is decreasing spectacularly. New technologies, including digital PCR (dPCR), Loop-Mediated Isothermal Amplification (LAMP) and Next Generation Sequencing (NGS) will likely lead to even more promising molecular diagnostic tools.

INTRODUCTION

The genus *Heterodera* contains more than 60 species. Amongst them, the cereal cyst nematodes are the most economically important. Especially *Heterodera avenae*, *H. filipjevi* and *H. latipons* can cause serious yield reduction in wheat-producing regions throughout the world. Less prevalent species of cyst nematode species associated with wheat include *H. arenaria*, *H. bifenestra*, *H. hordecalis*, *H. mani*, *H. pakistanensis*, *H. pratensis*, *H. zeae*, and *Punctodera punctata*.

Rapid and reliable identification of cereal cyst nematodes is important for monitoring their movement or introduction. Accurate identification of the cyst nematode up to species level is required for the development of control measures relying on rotation and resistance. Morphological identification posed particular problems, with many isolates not reliably identifiable to species level. However, molecular techniques were introduced to offer a solution for this.

HISTORY

In 1993, Ferris *et al.* published the first sequences of the internal transcribed spacers of ribosomal RNA genes (ITS-rDNA) from several isolates of cyst nematodes belonging to the genus *Heterodera*. A few years later, Szalanski *et al.* (1997) used nucleotide sequencing and polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP) profiling to assess variation between and/or within a few Heteroderid species. From 2000 onwards, a number of articles contributed to the expansion of ITS-RFLP profiles and sequences of more than 40 different species of cyst nematodes belonging to the genus *Heterodera*. It was demonstrated that these molecular techniques were very useful for *Heterodera* species identification. However, some limitations could be noted: (i) a combination of several RFLP profiles is needed to clearly separate

cereal cyst nematode species from each other and from other *Heterodera* species, increasing costs considerably, (ii) identical ITS sequences can be found in morphologically clearly distinct *Heterodera* species making it unable to separate them, (iii) heterogeneity is present in several *Heterodera* species, resulting in composite RFLP profiles which are difficult to interpret. Due to additional technical limitations, RFLP profiling is still rarely applied and sequencing (DNA-barcoding) became more popular. However, DNA-regions other than ITS-rDNA should be investigated and the technique is quite labour-intensive.

In the last decade, emphasis has been placed on the creation of species-specific primers and DNA probes to be used in conventional and (semi-) quantitative PCR (Real-Time PCR). These techniques make diagnostic procedures more effective and accessible, even to scientists not specialised in taxonomy. However, species-specific primers or probes remained absent for cereal cyst nematodes until very recently.

PRESENT STATUS

In 2001, Amiri *et al.* designed a primer specific for species from the *H. schachtii sensu stricto* group, using the available ITS-rDNA sequence information. In the same year, Subbotin *et al.* (2001) published a method to rapidly identify juveniles and cysts of the soybean cyst nematode *H. glycines*, based on PCR with species-specific primers. In 2002, Amiri supplemented his research with a species-specific primer to detect *H. schachtii*. In 2005, Madani *et al.* used this *H. schachtii* specific primer in combination with SYBR green I dye to detect and quantify *H. schachtii* nematodes in soil extracts using real-time PCR. All the above mentioned authors performed their research at the Institute of Agricultural and Fisheries Research (ILVO), Belgium. The expertise developed in ILVO has been applied from 2010 onwards, to resolve the need for similar diagnostic tools for cereal cyst nematodes.

In 2013, the first species-specific end-point PCR assay for the detection of the cereal cyst nematode species, *Heterodera latipons*, was published (Toumi *et al.* 2013a, fig. 1). This paper was followed by another in which two species-specific primer sets are described to detect the cereal cyst nematodes *H. avenae* and *H. filipjevi* (Toumi *et al.* 2013b). Considering the above mentioned limitations of ITS-rDNA sequences, other DNA regions were explored. The *H. latipons* species-specific primer set amplifies part of the actin gene, while the species-specific primer sets for *H. avenae* and *H. filipjevi* target part of the COI

(cytochrome oxidase subunit I) gene of the mtDNA (mitochondrial DNA). All three assays were able to detect the respective target species amongst 14 other *Heterodera* species and a *Punctodera punctata* population. The assays were also able to detect 1 infective juvenile of the respective target species mixed with 100 infective juveniles of a non-target *Heterodera* species.

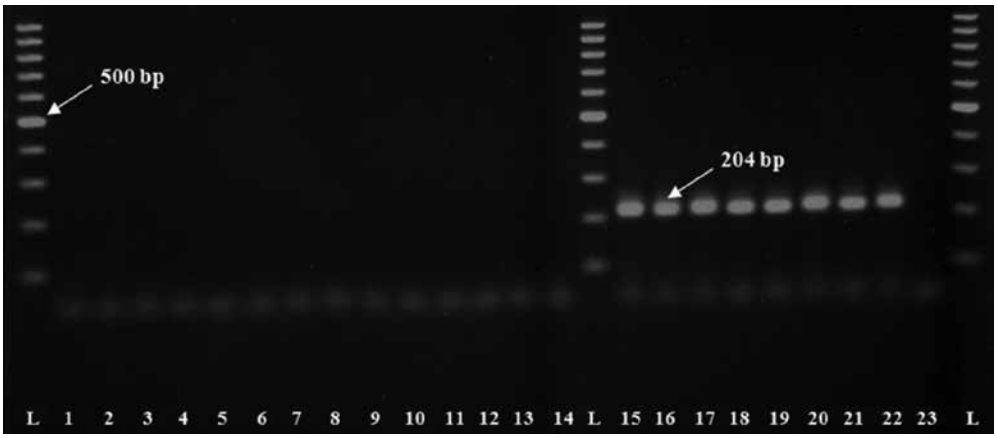


Figure 1. *Heterodera latipons* species-specific PCR. L: 100 bp DNA ladder; 1: *Heterodera pratensis*, 2: *Punctodera punctata*, 3: *H. avenae*, 4: *H. hordecalis*, 5: *H. glycines*, 6: *H. schachtii*, 7: *H. betae*, 8: *H. filipjevi*, 9: *H. goettingiana*, 10: *H. humuli*, 11: *H. ciceri*, 12: *H. trifolii*, 13: *H. carotae*, 14: *H. daverti*, 15-22: different populations of *H. latipons*, 23: negative control (Toumi *et al.* 2013a).

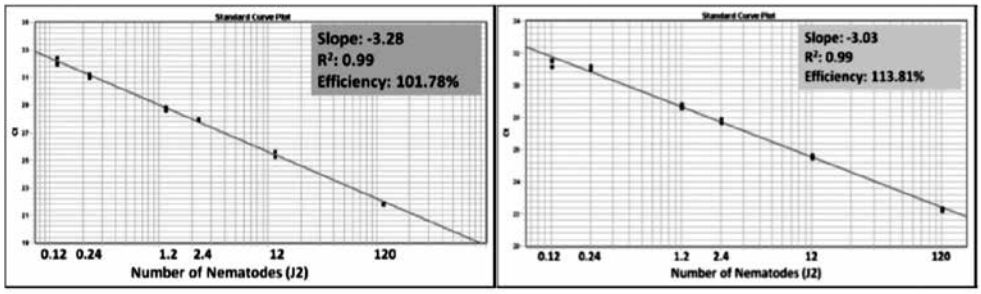


Figure 2. Standard curve of the qPCR assays (SensiFast Probe Hi-Rox) for *Heterodera latipons* (left panel) and *H. avenae* (right panel). Threshold cycle number (Ct) plotted against the dilution series (1/10, 1/50, 1/100, 1/500 and 1/1000) of DNA extracted from 120 infective juveniles (Toumi *et al.* , 2015).

Real-time PCR or qPCR is more sensitive than end-point PCR due to the alternative way of signal detection. It is also faster since it eliminates the time-consuming post-PCR agarose gel electrophoresis. A qPCR assay for the quantitative detection of *H. avenae* and *H. latipons* was published in 2015 by Toumi *et al.* The species-specific qPCR primer sets were based on sequences within the mitochondrial COI gene. Their specificity was confirmed by the lack of amplification of DNA extracted from infective juveniles from 14 other *Het-*

erodera species. The assays were demonstrated to be efficient and sensitive as well as they showed a highly significant linearity between their Ct-values and the tested dilution rates (Fig 2), and were able to detect DNA of a single infective juvenile mixed with DNA from 100 infective juveniles from a non-taget *Heterodera* species.

PROSPECTS AND RECOMMENDATIONS

In the last decade, several surveys showed that mixtures of 2 and sometimes even 3 cereal cyst nematode species coexist in the same field. The species-specific (q)PCR assays can be applied for accurate detection of the three most economically important cereal cyst nematodes, but at this stage the assays cannot be combined in a multiplex performance. Neither do species-specific assays exist for the detection of other cereal cyst nematode species. More species-specific primers or probes with comparable properties should be developed so that they can be used in a multiplex PCR to detect simultaneously different cereal cyst nematode species.

The (q)PCR assays are designed and optimised to be used on DNA extracted from infective juveniles released from cysts and not on DNA extracted directly from soil samples. It is rather easy to extract cysts from soil and by doing so avoiding the presence of potential soil-borne amplification inhibitors when performing DNA-extraction directly on soil. However, inspection services can benefit from on-the-spot sample processing in which DNA is extracted directly from soil. The so-called LAMP (Loop-Mediated Isothermal Amplification) technique is particular interesting for on-site diagnosis as the amplification runs at a single temperature avoiding the need for a thermo-cycler.

One major challenge remains the presence of virulence groups. Several pathotypes occur within *H. avenae*, and the same is anticipated for *H. filipjevi* and *H. latipons*. New technologies have opened the door to high-throughput sequencing (Next Generation Sequencing methods). The screening of more DNA-regions simultaneously (amplicon sequencing) or of complete genomes (whole genome sequencing) offers opportunities for in-depth species diagnosis.

It is clear from the work performed over the last 20 years that molecular techniques are powerful tools for nematode diagnostics. Using these tools, scientists have solved a number of problems, but still a large number remain a challenge.

REFERENCES

- Amiri S, Subbotin SA, Moens M (2001) An efficient method for identification of the *Heterodera schachtii* sensu strict group using PCR with specific primers. *Nematologia Mediterranea*, 29, 241-246.
- Amiri S, Subbotin SA, Moens M (2002) Identification of the beet cyst nematode *Heterodera schachtii* by PCR *European Journal of Plant Pathology*, 108, 497-506.
- Ferris VR, Ferris JM, Faghihi J (1993) Variation in spacer ribosomal DNA in some cyst-forming species of plant parasitic nematodes. *Fundamental and Applied Nematolo*gy, 16, 177-184.
- Madani M, Subbotin SA, Moens M (2005) Quantitative detection of the potato cyst nematode, Globodera pallid, and the beet cyst nematode, *Heterodera schachtii*, using Real-Time PCR with SYBR green I dye. Molecular and Cellular Probes, 19, 81-86.
- Szalanski AL, Sui DD, Harris TS, Powers TO (1997) Identification of cyst nematodes of agronomic and regulatory concern with PCR-RFLP of ITS1. *Journal of Nematology*, 29, 255-267.
- Subbotin SA, Deliang P, Moens M (2001) A rapid method for the identification of the soybean cyst nematode *Heterodera glycines* using duplex PCR. *Nematology*, 3, 365-371.
- Toumi F, Waeyenberge L, Viaene N, Dababat A, Nicol JM, Ogbannaya F, Moens M (2013a) Development of a species-specific PCR to detect the cereal cyst nematode, *Heterodera latipons*. *Nematology*, 15,709-717.
- Toumi F, Waeyenberge L, Viaene N, Dababat A, Nicol JM, Ogbannaya F, Moens M (2013b) Development of two species-specific primer sets to detect the cereal cyst nematodes *Heterodera avenae* and *Heterodera filipjevi*. *European Journal of Plant Pathology*, 136,613-624.
- Toumi F, Waeyenberge L, Viaene N, Dababat A, Nicol JM, Ogbannaya F, Moens M (2015) Development of qPCR assays for quantitative detection of *Heterodera avenae* and *H. latipons*. *European Journal of Plant Pathology* DOI 10.1007/s10658-015-0681-0.

MOLECULAR CHARACTERIZATION OF EXPANSIN GENE FROM THE CEREAL CYST NEMATODE *HETERODERA AVENAE*

LIU JING, HUAN PENG, AND DE-LIANG PENG*

State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193;
*Corresponding author email: dlpeng@ippcaas.cn

INTRODUCTION

The cereal cyst nematode *Heterodera avenae* is one of the most economically important plant-parasitic nematodes in the world. Its life cycle from egg to adult passes through four juvenile stages. The second-stage juvenile (J2) of *H. avenae* is also the infective stage that penetrates the root tip and migrates intracellularly through the cortex to the vascular cylinder, where it inserts its stylet into a selected parenchyma cell and induces its transformation into a feeding site (Jones 1981). The plant primary cell wall is a complex and dynamic association of different high molecular weight polysaccharides and structural, enzymatic and catalytic proteins (Somerville *et al.* 2004). Nematode secreted effectors to the host cell, including cellulose and expansin, which modify and degrade the host cell wall to facilitate parasitism, such as b-1,4-endoglucanases (Ledger *et al.* 2006), Pectate lyase (de Boer *et al.* 2002), Cellulase (Smant *et al.* 1998), Expansin (Qin *et al.* 2004, Long *et al.* 2012). Expansins are proteins that can rapidly induce extension of plant cell walls by weakening the non-covalent interactions during cell growth (Cosgrove 1998, 2000). *Globodera rostochiensis* can also secret a functional expansin and contains two domains: domain I at the N-terminal end shows significant similarity to the carbohydrate-binding module family II (CBM II) of nematode endoglucanases, and domain II at the

* Liu J, Peng H, Peng DL (2015) Molecular characterization of expansin gene from the cereal cyst nematode *Heterodera avenae*. In 'Nematodes of Small Grain Cereals: current status and research.' (AA Dababat, H Muminjanov and RW Smiley) pp. 335-338. (FAO: Ankara, Turkey).

C-terminal shows similarity to plantβ-expansin. Cloning and function analysis of expansin gene (*HaEXPB2*) from the cereal cyst nematode *H. avenae* will help to increase our understanding, on a molecular basis, of pathogenesis as well as plant cellular processes particular in plant-nematode interactions.

MATERIALS AND METHODS

Nematodes: *Heterodera avenae* was cultured on glasshouse-grown wheat *Triticum aestivum* cv. Wenmai 19. The temperature of seedling cultures was maintained at 16°C for the first week and then 22°C for the remaining growth period. Wheat roots infected with *H. avenae* J2s were harvested 5, 10, 20 and 30 days post-inoculation (dpi), and different parasitic stages of *H. avenae* were collected by root blending and sieving (De Boer *et al.* 1999). Adult white females were directly hand-picked from root surfaces under a dissecting microscope at 40 dpi.

Cloning of *H. avenae* genes encoding putative secreted proteins: About 46 putative secreted protein genes with signal peptide and without transmembrane domain were first selected from the transcript libraries for cloning. The genes were amplified by PCR using gene-specific primers from J2 cDNA and cloned into the plant expression vector pGDG. The putative secreted protein genes were PCR amplified following the protocol provided in the platinum Ex-Taq DNA polymerase kit (Takara). DNA fragments were amplified using platinum ExTaq DNA polymerase and gene-specific pairs of primers corresponding to the ORF regions that exclude the predicted signal peptide sequence. PCR was performed for 35 cycles using the following parameters; initial denaturation at 94°C for 30 s followed by 35 cycles of 94°C for 30 s; 55 °C for 30 seconds; 72°C for 1 min per Kb and a final extension at 72°C for 5 min. PCR products were purified with Gel Extraction Kit (Promega) before cloning in the pGDG vector.

Gene expression analysis by real-time quantitative PCR: Total RNA was isolated from pre-parasitic J_2, parasitic J_2, J_3, J_4 or adult females with TRIzol (Invitrogen) according to the manufacturer's instructions. All total RNA samples were treated with RNase-free DNase1 to remove DNA contamination. First-strand cDNA synthesis was conducted by SuperScript™ III First-Strand Synthesis System for RT-PCR kit (Invitrogen), according to the manufacturer's instructions. Transcriptions of *HaEXPB2* in different developmental stages of *H. avenae* were determined by real-time quantitative PCR. Actin genes of *H.*

avenae were amplified from each sample as a positive control using the primers ActinF and ActinR.

RESULTS AND DISCUSSION

We cloned putative secreted protein genes from the transcript libraries, and obtained 46 genes with signal peptide and without transmembrane domain. The genes were cloned to the plant expression vector pGDG and trans-expression in *Nicotiana benthamiana*. Ha-EXPB2 is one of the putative secreted proteins. The full length cDNA of *HaEXPB2* was cloned from *H. avenae,* with 870 bp ORF encoding a 289 amino acid protein. The deduced protein Ha-EXPB2 had 89% identity to Ha-EXPB1, containing a signal peptide, a CBM II and an expansin domain. *HaEXPB2* transcript specifically accumulated in the subventral gland cells of the pre-parasitic J2s by situ hybridization. *HaEXPB2* was relatively more highly expressed in the parasitic J2s by quantitative real-time RT-PCR analysis. The results suggested a role in the early parasitic-stage process, most likely in modifying host cell wall to facilitate migration within the plant.

ACKNOWLEDGMENTS

This study was supported by the National Natural Science Foundation of China (No. 30921140411) and the National Key Basic Research Program of China (973 Program, 2013CB127502), the Special Fund for Agro-scientific Research in the Public Interest (No. 201503114, 200903040).

REFERENCES

- De Boer JM, Davis EL, Hussey RS, Popeijus H, Smant G, Baum TJ (2002) Cloning of a putative pectate lyase gene expressed in the subventral esophageal glands of *Heterodera glycines*. *Journal of Nematology*, 34, 9-11.
- Cosgrove DJ (1998) Cell wall loosening by expansins. *Plant Physiology*, 118, 333-339.
- Cosgrove DJ (2000) Loosening of plant cell walls by expansins. *Nature*, 407, 321-326.
- Long H, Peng D, Huang W, Liu Y, Peng H (2012) Identification of a putative expansin gene expressed in the subventral glands of the cereal cyst nematode *Heterodera avenae*. *Nematology*, 2012, 571-577.
- Jones M (1981) Host cell responses to endoparasitic nematode attack: structure and function of giant cells and syncytia. *Annals of Applied Biology*, 97, 353-72.

- Ledger TN, Jaubert S, Bosselut N, Abad P, Rosso MN (2006) Characterization of a new beta-1,4-endoglucanase gene from the root-knot nematode *Meloidogyne incognita* and evolutionary scheme for phytonematode family 5 glycosyl hydrolases. *Gene*, 382, 121-128.
- Smant G, Stokkermans JP, Yan Y, de Boer JM, Baum TJ, Wang X, Hussey RS, Gommers FJ, Henrissat B, Davis EL, Helder J, Schots A, Bakker J (1998) Endogenous cellulases in animals: isolation of ß-1,4-endoglucanase genes from two species of plant-parasitic cyst nematodes. *Proceedings of the National Academy of Sciences of the United States of America*, 95, 4906-4911.
- Qin L, Kudla U, Roze EHA, Goverse A, Popeijus H, Nieuwland J, Overmars H, Jones JT, Schots A, Smant G, Bakker J, Helder J (2004) Plant degradation: a nematode expansion acting on plants. *Nature*, 427, 30.
- Somerville C, Bauer S, Brininstool G, Facette M, Hamann T, Milne J, Osborne E, Paredez A, Persson S, Raab T, Vorwerk S, Youngs H (2004) Toward a system approach to understanding plant cell walls. *Science*, 306, 2206-2211.

MOLECULAR DIAGNOSIS AND DETECTION OF RICE ROOT-KNOT NEMATODE (*MELOIDOGYNE GRAMINICOLA*)

CHO CHO HTAY, HUAN PENG, AND DELIANG PENG*

State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, China. *Corresponding author email: pengdeliang@caas.cn

SUMMARY

Meloidogyne graminicola is one of the major constraints to rice production because of its unique biology and life cycle due to its adaptation to flooded conditions. The genetic variation among 20 populations of *M. graminicola* (16 from Myanmar and four from China) was investigated using the rDNA-ITS sequences data alignments. The result showed that all the populations were clearly separated from other species and it was also observed that there was low genetic variation among the tested isolates. A set of species-specific primers was designed based on the resulting sequences to develop a species-specific molecular tool for accurate identification of *M. graminicola*. The primers reliability, specificity and sensitivity test proved that the primer set (Mg-F3 and Mg-R2) was able to produce the expected fragment sizes, 369 bp from DNA template of target nematode populations but not from non-target organisms. A duplex PCR test will allow saving the diagnostic time and costs by amplifying species of interest from a nematode mixture. Thus, this species-specific primer set may be a powerful tool to improve the taxonomic identification for non-specialists and also for the design of successful management practices.

* Htay CC, Peng H, Peng DL (2015) Molecular diagnosis and detection of rice root-knot nematode (*Meloidogyne graminicola*). In 'Nematodes of Small Grain Cereals: current status and research.' (AA Dababat, H Muminjanov and RW Smiley) pp. 339-346. (FAO: Ankara, Turkey).

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most widely consumed staple foods for a large part of the world's human population, especially in Asia. The rice root-knot nematode *Meloidogyne graminicola* (Golden and Birchfield) is the most damaging root-knot nematode of rice, and is widely distributed in all rice growing ecosystems of the world (Le *et al.* 2009). *M. graminicola* has short life cycle, wide host range and is well adapted to flooded conditions, which makes this species difficult to control (De Waele & Elsen 2007). To select effective management schemes, accurate and rapid identification of root-knot nematodes up to the species level is the most essential work.

The morphological characteristics of adult females (perineal pattern), males and juveniles (J2) were traditionally used to identify *Meloidogyne* spp. (Eisenback *et al.* 1981). However, the diagnosticians have to face the challenges to identify this species correctly due to extremely similar morphological features between species, wide host ranges, life stages in different habitats, indistinct species boundaries, sexual dimorphism, species with a potential hybrid origin and polyploidy (Blok & Powers 2009). Moreover, species and race identification of *Meloidogyne* based on phenotypic characterization is time-consuming because adult forms are hardly accessible since males are usually rare and females are malformed (Besnard *et al.* 2014). Morphological identification also requires a lot of skill (Hooper 1990).

Therefore, the use of biochemical and molecular tools has been introduced to help nematode taxonomists, to identify the nematode species as well as to investigate the phylogeography and population dynamics of nematodes (Blok & Powers 2009, Gilabert & Wasmuth 2013). In the present study, phylogenetic analysis was carried out based on ITS-rDNA region of individual juveniles (J2) to investigate the genetic diversity of rice root-knot isolates originating from different locations in Myanmar and China. In addition, a set of species-specific primers designed for fast and reliable diagnosis of *M. graminicola* according to the ITS-rDNA sequences obtained from this study will be allowed to identify specifically to the target nematode species from a mixture of species in a single step PCR.

METHODS

Soil and plant root samples infested with rice root-knot nematode were collected from rice fields of different locations in Myanmar and China. Nema-

todes were extracted from soil and root samples by using the Whitehead tray method. A single nematode (J2) was hand-picked and placed into a sterile PCR tube to extract genomic DNA, followed by PCR amplification with the general primer set TW81 and AB28. The amplified products were electrophoretically separated on 1.5% agrose gel and stained with ethidium bromide, and then the gel was visualized under a UV trans-illuminator and photographed.

The ITS amplified fragments for three individual nematodes from each population were purified using the TIANgel Purification Kit, followed by ligation and transformation into competent cells *E. coli*, DH5α. The positive colonies with the expected insert size were sent for sequencing. The resulted ITS sequences were deposited at NCBI and analyzed for intraspecific differences among the specimens. All the resulting sequences were manually edited with EditSeq and aligned with Clustal (W), and then analyzed by Maximum likelihood (ML) based on 1000 bootstrap replicates with MEGA 5 software. Through sequence alignments, a pair of species-specific primers was developed based on the regions unique that could be used as a molecular tool for the diagnosis of *M. graminicola* by using the Primer Premier 5 software. The selected potential species-specific primers were compared with Nucleotide (Nt) dataset using primer-blast to examine the specificity. The PCR conditions were optimized for specificity by performing a temperature gradient PCR to determine the optimum annealing temperature.

To test the reliability of the primers, extracted DNA from 20 populations of *M. graminicola* that provided template DNA for sequencing was amplified with the optimized conditions. Additional primer reliability was tested with different life stages of nematodes; including eggs, J2, J3, J4, female and male. Investigation on the potential of specific primers set was done with duplex PCR containing two sets of primers in a single test. The first primers set D2D3 which amplify D2-D3 expansion regions of the 28S rRNA gene (Nunn 1992) was used as an internal control. The second set included a species-specific primer set which targeted to nematode DNA sequences of interest. To investigate the primers sensitivity, different numbers of J2 DNA (n = 1, 2, 3, 4 and 5) were used to determine the minimum amount of J2 detectable in the PCR assays. Sensitivity was also performed in a PCR tube containing *M. graminicola* genomic DNA alone or in a combination of DNA from non-target root-knot nematode species; *M. enterolobii* and *M. incognita* with the ratio of 1:1.

RESULTS

Sequence and phylogenetic analysis: In the present study, PCR amplification of the ITS containing region yielded a single fragment with a length of approximately 579 bp in all populations tested by using the general primer set. The blast result showed that all nucleotide sequences of ITS sequences were 98-100% similar to those of a *M. graminicola* isolate from Genbank. Phylogenetic analysis indicated that all the isolates and other species were clearly separated into independent clusters with a high bootstrap value of 100%. All the Chinese isolates, five Myanmar isolates, and JN241866.1 clustered together with a bootstrap value of 76%, and 65% bootstrap with the rest of the Myanmar isolates.

Primer design: The species-specific forward (MgF3) and reverse (MgR2) primers were designed. The length of the expected amplified fragment with the specific primers is 369 bp, which contained part of the ITS 1 and 5.8 S regions of ribosomal DNA.

Primer reliability, specificity and sensitivity test: In the primer reliability test, the expected band size of 369 bp was amplified by PCR with the specific primer set from all of the tested populations of *M. graminicola*. Additionally, the reliability test with different life stages of nematode (eggs, J2, J3, J4, female and male) showed that amplification of all developmental stages gave positive results as well. For the primer specificity test, duplex PCR was performed with two sets of primers. After optimizing PCR cycling conditions, every target nematode species of *M. graminicola* yielded two distinct fragments; ~766 bp for 28S regions and 369 bp for specific primer set. A single band of ~766 bp was observed from the 28S region of non target species. Primer sensitivity was investigated with different numbers of J2 in the PCR assays. The results showed that the minimum detection concentration required for the PCR assay is a single juvenile. It was also observed that the higher the juvenile numbers used in PCR, the stronger the bands. Moreover, specific bands were observed in PCR tubes containing target nematode species either alone or in a mixture with non-target nematode species. DNA mixtures of *M. graminicola* and *M. enterolobii* produced two fragments of 369 bp and ~230 bp. The mixture of *M. graminicola* and *M. incognita* generated two fragments of 369 bp and ~1000 bp. No fragments were generated in the no DNA-template.

DISCUSSION

In the present study, molecular identification based on ITS-rDNA of 20 populations was reported by using a general ITS primer set. Nucleotide variability was observed in a comparison of the ITS-rDNA sequences obtained from the same individual nematode as well as different nematodes of same sample. The observation of the differences in the ITS sequences between clones of the same population could be due to variation among copies of the ITS within an individual, or due to errors arising through PCR amplification or cloning or sequencing (Pokharel *et al.* 2007).

Furthermore, sequence analysis showed that 20 tested isolates clearly divided into two groups; 16 populations from Myanmar which were again separated into two subgroups leading to the conclusion that genetic variation was not corresponding to their geographical conditions. However, the second group included four populations from China that belonged to the same cluster. This may be due to the low population tested or to a lower degree of sequence dissimilarity among the Chinese isolates. In order to be strong data on the genetic diversity of *M. graminicola*, it would be necessary to add the isolates of these nematodes to those originating from different countries, climatic conditions, or geographic locations.

In addition, this current study also reports a species-specific primer set which can be used as a molecular tool in the diagnosis of the rice root-knot nematode, *M. graminicola*. In the primer reliability test, the newly developed primer set produced a unique specific band of 369 bp with genomic DNA from 20 *M. graminicola* isolates. Likewise, a specific PCR amplicon was produced with DNA from different developmental stages of the target nematode species. The primer specificity analyzed with other nematode species showed that a specific fragment was observed only in the PCR tests with DNA from *M. graminicola*, and no specific products were amplified when PCR was carried out with DNA templates from non-target nematode species. Thus, the newly developed species-specific primers might be able to be used as a molecular marker that satisfies the three criteria reported by Hübschen *et al.* (2004) to diagnose the rice root-knot nematode *M. graminicola*. Additionally, duplex PCR using two sets of primers was developed in a single-step amplification to save the amount of time, labor and costs which are required for identification of pathogens.

In conclusion, the high level of homogeneity among the isolates tested indicated that it might be important information for rice breeders to improve varieties

with higher yield and resistance to economically important nematodes in the future. Also, it suggested that similar control methods against this nematode could be used in the locations where the samples were collected. Furthermore, the species-specific primer set developed in the present report appear to be a useful molecular marker which is reliable, specific and sensitive for the rapid diagnosis of *M. graminicola*. However, further investigation on the validation of this newly-developed primer set will be required using larger populations of the same species as well as closely-related species.

ACKNOWLEDGMENTS

The present study was supported by the Organization for Women in Science for the Developing World (OWSD) and the Swedish International Development Cooperation Agency (SIDA). Research facilities were provided by the Special Fund for Agro-scientific Research in the Public Interest (No. 201103018).

REFERENCES

- Besnard G, Juhling F, Chapuis E, Zedane L Lhuillier É, Mateille T, Bellafiore S (2014) Fast assembly of the mitochondrial genome of a plant parasitic nematode (*Meloidogyne graminicola*) using next generation sequencing. *Comptes Rendus Biologies*, 337, 295-301.
- Blok VC, Powers TO (2009) Biochemical and molecular identification. In: 'Root-knot Nematodes.' (Eds. RN Perry, M Moens, J Starr) pp. 98-112 (*CABI, Wallingford UK).*
- De Waele D, Elsen A (2007) Challenges in tropical plant nematology. *Annual Review of Phytopathology*, 45, 457-85.
- Eisenback JD, Hirschmann H, Sasser JN, Triantaphyllou AC (1981) A more complete characterization of the four most common species of root-knot nematodes (*Meliodogyne* spp.) with pictorial key. *IMP Publication, Raleigh, North Carolina,USA*.
- Gilabert A, Wasmuth JD (2013) Unravelling parasitic nematode natural history using population genetics. *Trends in Parasitology*, 29, 438-448.
- Hübschen J, Kling L, Ipach U, Zinkernagel V, Bosselut N, Esmenjaud D, Brown DJF, Neilson R (2004) Validation of the specificity and sensitivity of species-specific primers that provide a reliable molecular diagnostic for *Xiphinema diversicaudatum*, *X. index* and *X. vuittenezi*. *European Journal of Plant Pathology*, 110, 779-788.

- Hooper DJ (1990) Extraction and processing of plant and soil nematodes. In: 'Plant Parasitic Nematodes in Subtropical and Tropical Agriculture.' (Eds. M Luc, RA Sikora, J Bridge) pp. 45-68. (CAB International, Wallingford, UK).
- Le HTT, Padgham JL, Sikora RA (2009) Biological control of the rice root-knot nematode *Meloidogyne graminicola* on rice, using endophytic and rhizosphere fungi. *International Journal of Pest Management*, 55, 31-36.
- Nunn GB (1992) Nematode molecular evolution. *Ph.D. dissertation*, *University of Nottingham, U.K.*
- Pokharel RR, Abawi GS, Zhang N, Duxbury JM, Smart CD (2007) Characterization of isolates of *Meloidogyne* from rice-wheat production fields in Nepal. *Journal of Nematology*, 39, 221-30.

A DRAFT GENOME SEQUENCE FOR THE CEREAL CYST NEMATODE, *HETERODERA AVENAE*

UMA RAO*, MUKESH KUMAR, PRAKASH BANAKAR, AND PRASOON KUMAR THAKUR

Division of Nematology, Indian Agricultural Research Institute, New Delhi, India. *Corresponding author email: umarao@iari.res.in

SUMMARY

The extent of crop losses caused by plant parasitic nematodes is substantial and contributes to significant reductions in crop yields resulting in annual yield losses of about US$ 173 billion (Elling *et al.* 2013). The cereal cyst nematode (CCN), *Heterodera avenae* (Wollenweber 1924), is one of the economically important cyst nematode species that attack wheat and barley crops in many cereal growing regions of the world (Smiley and Nicol 2009). Although agronomic management practices are often deployed to manage plant nematodes, control with chemical nematicides is also used for high value crops. Because chemical nematicides are toxic and persistent, they are a human health risk and most are being phased out (Dhawan and Pankaj 1998). There is an urgent need to find new gene targets which can be used to develop novel and environmentally friendly nematode control methods (Elling *et al.* 2013, Brown and Kerry 1987).

H. avenae is an obligate sedentary endoparasite that reproduces mainly by amphimixis. After infection of host plants, they develop a close interaction with and feed from a group of interconnected syncytial cells of their host plants. The life cycle starts with an egg present inside an encysted female.

* Rao U, Kumar M, Banakar P, Thakur PK (2015) A draft genome sequence for the cereal cyst nematode, *Heterodera avenae*. In 'Nematodes of Small Grain Cereals: current status and research.' (AA Dababat, H Muminjanov and RW Smiley) pp. 347-352. (FAO: Ankara, Turkey).

The first stage larva (J1) develops within the egg and second stage larva (J2) hatches in response to low soil temperatures (5–15°C) (Mokabli *et al.* 2001). J2s may invade main or lateral roots in the zone of elongation and migrate intracellularly towards the vascular cylinder (Gheysen and Fenoll 2002). Each J2 selects a single cell that becomes the initial feeding cell (IFC). In a susceptible host, the cells next to the IFC expand and become interconnected after local dissolution of cell walls at pit fields, resulting in the formation of a multinucleate syncytium with characteristics of transfer cells. J2s feed from associated syncytia and undergo three molts, during which they develop either into a mobile male or become a sedentary endoparasitic female that continues to feed until reproduction is completed. The female then dies and forms a cyst which protects the eggs inside it. As a survival strategy not all J2s hatch simultaneously in the same season, but hatching can occur over several years with some unhatched eggs retained within the cyst (Wyss and Grundler 1992).

We isolated a *H. avenae* Indian strain from wheat roots cultivated in Rajasthan, India. Preliminary analysis of *18S rRNA* sequence of this strain revealed 100% similarity with Genus *Heterodera.* The genomic DNA was isolated using the DNeasy kit (Real Biotech Corporation, Taiwan). Whole genome shotgun (WGS) sequencing was performed using the illumina gx 72bp short read sequencing along with Roche 454 to achieve 95x coverage. Hybrid assembly approach used for the assembly of genome using Velvet and SOAPdeNOVO software resulted in approximately 75.3 Mb (7,8957,772 bases) draft genome. The assembled genome contains 17,342 scaffolds (longest scaffolds 50,404 bp with a N50 of 6,687 bp) and with GC content 34.27% similar to *Globodera pallida* (36.7%). Repeat Masker with updated RepBase v20140131 (http://www.repeatmasker.org) generated a library using Repeat Masker. We have identified 27% repetitive elements in *H. avenae* genome. Among them, we found 89.74% interspersed repeats, small RNA (0.03%), Simple repeats (6.48%) and low complexity (2.98%) (Figure 1).

Gene prediction was done by maker pipeline with SNAP gene prediction software using scaffold greater than 1000 bp that resulted in 14,433 (Brandi *et al.* 2008) out of which 12,230 gene models with gene density 168.03/mb, 72,912 cds and 13,280 transcripts (1.08 transcripts per gene) were obtained. 66.4% transcripts showed significant hit with NCBI non-redundant database (nr). Blastp results showed top hits with *Ascaris suum* and other plant parasitic nematodes like *H. avenae, H. glycines*, *Globodera rostochiensis, Meloidogyne javanica, M. incognita and G. pallida.* We applied OrthoVenn clustering to

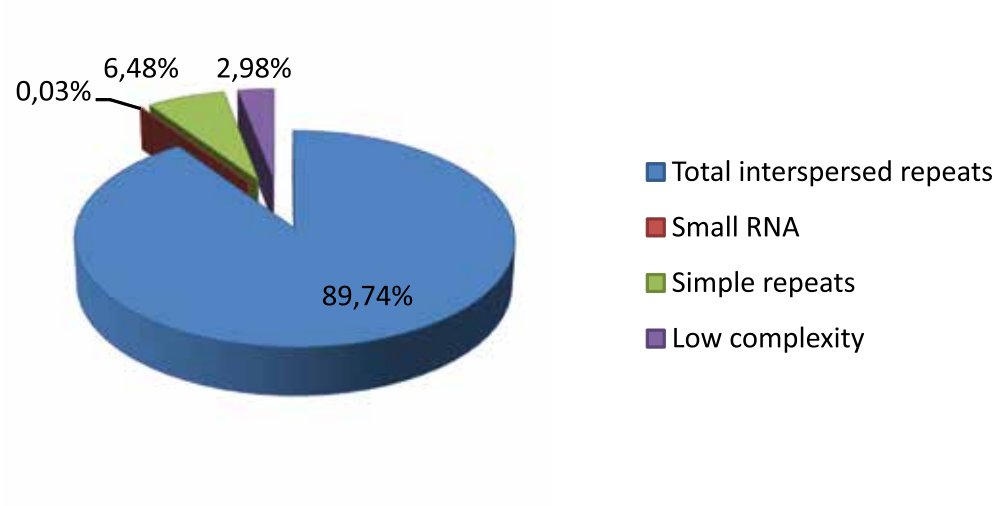


Figure 1. Repetitive elements in *H. avenae* genome consist mostly of interspersed repeats. Approximately 10% of the genome is classified as repeat elements such as small RNA, Simple repeats and low complexity

identify the gene clusters enriched in four nematode species (*M. incognita, G. pallida, C. elegans and H. avenae*). The species (*M. incognita, G. pallida, C. elegans* and *H. avenae*) form 12,847 clusters, 7,884 orthologous clusters (at least contains two species) and 580 single-copy gene clusters (Figure 2).

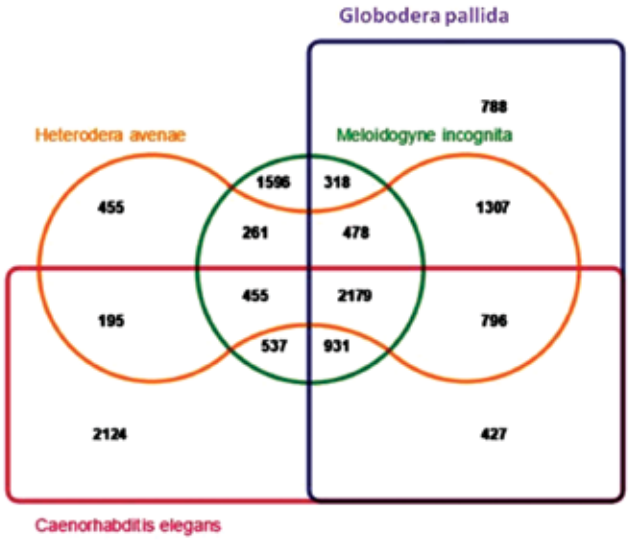


Figure 2. Result of OrthoVenn showing Venn diagram the distribution of shared gene families (orthologous clusters) among *M. incognita*, *G. pallida*, *C. elegens* and *H. avenae*

Gene functions were annotated by KEGG, pfam, and Clusters of Orthologous Groups (COG) databases. We used signalp to identify effector genes in *H. avenae*. Interestingly, secretory peptides were identified in approximately 25% of the transcripts. Several genes that are secretory in nature and could be involved in host pathogen interaction were found. All the three types of rRNA, namely, 5S, 18S, and 28S, have also been annotated.

Analysis of the parasitome, to decipher the genes required for successful parasitism, revealed the presence of genes which have already been described, and also some novel ones. Such genes, if vital for parasitism, provide new targets that can be studied further for developing new management strategies. Known parasitism genes, or genes having a 'parasitic signature' were identified computationally, and some were validated by providing transcriptional evidence. Some of these potential targets have been studied in more detail. A hydolase, which had only been reported previously in root knot nematodes, was also found in *H. avenae*. Analyzing the genome of this unique parasite may help us in understanding the ability of cyst nematode adaptation and plant nematode interaction.

ACKNOWLEDGMENT

We would like to acknowledge and extend our gratitude to Department of Biotechnology, Government of India for financial support under the Indo Australia Strategic Research fund (PR12678).

REFERENCES

- Elling AA (2013) Major emerging problems with minor *Meloidogyne* species. *Phytopathology*, 103:1092–1102.
- Brandi LC, Ian K, Sofia MCR, Genis P, Eric R, Barry M, Carson H, Alejandro SA, Mark Y (2008) MAKER: An easy-to-use annotation pipeline designed for emerging model organism genomes. *Genome Research*, 18(1), 188–196.
- Yin Y, Mao X, Yang J, Chen X, Mao F, Xu Y (2012) dbCAN: A web resource for automated carbohydrate-active enzyme annotation. *Nucleic Acids Research*, 40(web Server issue), W445-451.
- Brown RH, Kerry BR (1987) Principles and Practice of Nematode Control in Crops. Academic Press, Sydney New South Wales, Australia

- Estécio MR, Gallegos J, Vallot C, Castoro RJ, Chung W, Maegawa S, Oki Y, Kondo Y, Jelinek J, Shen L, Hartung H, Aplan PD, Czerniak BA, Liang S, Issa JP (2010) Genome architecture marked by retrotransposons modulates predisposition to DNA methylation in cancer. *Genome Research*, 20, 1369-1382.
- Mokabli, A, Valette S, Gauthier J-P, Rivoal R (2001) Influence of temperature on the hatch of *Heterodera avenae* Woll. populations from Algeria. *Nematology*, 3, 171-178.
- Dhawan SC, Pankaj (1998) Nematodes affecting wheat and rice and their management. In: 'Pathological Problems of Economic Crop Plants and their Management.' (Ed. SMP Khurana) pp. 95-117. (Scientific Publishers, Jodhpur, India).
- Smiley RW, Nicol JM (2009) Nematodes which challenge global wheat production. In. 'Wheat: Science and Trade.' (Ed. BF Carver) pp. 171-187. (Wiley-Blackwell: Ames, Iowa).
- Smit A, Hubley R, Green P (1996-2010) RepeatMasker Open-3.0. <www.repeatmasker.org>.
- Gheysen G, Fenoll C (2002) Gene expression in nematode feeding sites. *Annual Review of Phytopathology*, 40, 191-219.
- Wyss U, Grundler FMW (1992) Feeding-behavior of sedentary plant parasitic nematodes. *Netherlands Journal of Plant Pathology*, 98, 165-173.

FUNCTIONAL VALIDATION OF GENES IN CEREAL CYST NEMATODE, *HETERODERA AVENAE*, USING SIRNA GENE SILENCING

NAGAVARA PRASAD GANTASALA, MUKESH KUMAR, PRAKASH BANAKAR, PRASOON KUMAR THAKUR, AND UMA RAO*

Division of Nematology, Indian Agricultural Research Institute, New Delhi, India. *Corresponding author email: umarao@iari.res.in

SUMMARY

India is the second largest producer of wheat next to China, accounting for about 35% of the total food grain production in the country. *Heterodera avenae* (CCN, cereal cyst nematode) causes 10-15% loss of wheat yields in India and most probably equivalent losses in many other countries ensuring it as one of the most economically important of cyst nematodes. However, the present CCN management options are inadequate in many countries. Designing of modern novel approaches based on genomics is becoming quite popular globally. In view of this, we have attempted to explore the utility of some of the unique and novel genes reported by us in the CCN transcriptome (Kumar *et al.* 2014). For this, we have selected four unique vital genes for functional validation by *in vitro* RNAi; *nuclear hormone receptor, polyadenylate binding protein, intron binding protein* and *epsin*.

Nuclear hormone receptors are ligand-activated transcription factors that regulate gene expression by interacting with specific DNA sequences upstream of their target genes. In response, these receptors work with other proteins to regulate the expression of specific genes, there by controlling the development, homeostasis, and metabolism of the organism (Evans 1988, Olefsky 2001). A unique property of nuclear receptors that differentiates them from other class-

* Gantasala NP, Kumar M, Banakar P, Thakur PK, Rao U (2015) Functional validation of genes in cereal cyst nematode, *Heterodera avenae*, using siRNA gene silencing. In 'Nematodes of Small Grain Cereals: current status and research.' (AA Dababat, H Muminjanov and RW Smiley) pp. 353-356. (FAO: Ankara, Turkey).

es of receptors is their ability to directly interact with and control the expression of genomic DNA. As a consequence, nuclear receptors play key roles in both embryonic development and adult homeostasis (Mangelsdorf *et al.* 1995, Novac and Heinzel 2004). Nuclear receptors are specific to metazoans (animals) and are not found in protists, algae, fungi, or plants. There are 270 nuclear receptors in the nematode *Caenorhabditis elegans* alone (Sluder and Maina 2001)>pMM:¦jres°. The second gene selected is *Poly (A)-binding protein* (*PAB* or *PABP*) which is a RNA-binding protein that binds to the poly (A) tail of mRNA (Kahvejian *et al.* 2005). *PAB* may be involved in cytoplasmic regulatory processes of mRNA metabolism such as pre-mRNA splicing, translationally coupled mRNA turnover and other functions. The third gene is *intron-binding spliceosomal protein* required to link pre-mRNA splicing and snoRNP (small nucleolar ribo nucleoprotein) biogenesis.

It plays a key role in position-dependent assembly of intron-encoded box C/D small snoRNP, splicing being required for snoRNP assembly. It may act by helping the folding of the snoRNA sequence and binds to intron of pre-mRNAs in a sequence-independent manner, contacting the region between snoRNA and the branch point of introns (40 nucleotides upstream of the branch point) during the late stages of splicing (Beenken *et al.* 1991). Lastly, *epsins* are a family of highly conserved membrane proteins that are important in creating membrane curvature. *Epsins* contribute to membrane deformations like endocytosis, and block vesicle formation during mitosis (Sen *et al.* 2012). *Epsin* contains various protein domains that aid in function. Starting at the N-terminus is the ENTH domain. In general, most vertebrates contain at least two *epsin* paralogs. The two paralogs, *epsin-1* and *epsin-2* are members that contribute to the clathrin coated endocytotic machinery and are localized at the plasma membrane. In mammals, the two main classes of *epsin's* are expressed throughout tissues but has the highest expression in the brain, whereas the third *epsin* has higher expression in the epidermis and the stomach (Tessneer *et al.* 2013). In addition, *epsin* is thought to play a role in the notch signaling pathway, which is critical for normal embryonic development. The *epsin* homologue of *C.elegans* is EPN-1. The *epsin* homologue of *Drosophila melanogaster* is liquid facets and was first identified due to its role in eye patterning in flies. Knockout of *epsin-1* and *epsin-2* in mice showed embryonic death at day10. Further investigation showed vascular defects in the embryo proper, placenta and yolk sac which are characteristic of a loss in notch signaling (Musse *et al.* 2012).

We have successfully cloned and sequenced *nuclear hormone receptor, ployadenalyte binding protein, intron binding protein* and *epsin* genes from *H. avenae*. Gene sequences were submitted in NCBI database. Gene silencing through siRNA soaking was performed. Target gene transcripts were quantified using qRT-PCR to confirm the effect of gene silencing which revealed 6-fold down regulation in epsin and 1.5-, 3.3-, 2.1-fold up regulation in *nuclear hormone receptor, intron binding protein* and *ployadenalyte binding protein*, respectively. The effect of gene silencing was determined on the nematode infection, development and reproduction and it was found that there was a reduction of 71%, 26%, 60% in females and eggs due to the silencing of *epsin, intron binding protein* and *ployadenalyte binding protein* respectively. On the other hand, silencing of nuclear hormone receptor led to a 25% increase in females and eggs.

REFERENCES

- Beenken SW, Karsenty G, Raycroft L, Lozano G (1991) An intron binding protein is required for transformation ability of p53. *Nucleic Acids Research*, 19, 4747-4752.
- Evans RM (1988) The steroid and thyroid hormone receptor superfamily. *Science*, 240, 889-895.
- Kahvejian A, Svitkin YV, Sukarieh R, M'Boutchou MN, Sonenberg N (2005) Mammalian poly(A)-binding protein is a eukaryotic translation initiation factor, which acts via multiple mechanisms. *Genes & Development*, 19, 104-113.
- Kumar M, Gantasala NP, Roychowdhury T, Thakur PK, Banakar P, Shukla RN, Jones MG, Rao U (2014) De novo transcriptome sequencing and analysis of the cereal cyst nematode, *Heterodera avenae*. *PloS One*, 9, e96311.
- Mangelsdorf DJ, Thummel C, Beato M, Herrlich P, Schutz G, Umesono K, Blumberg B, Kastner P, Mark M, Chambon P, Evans RM (1995) The nuclear receptor superfamily: the second decade. *Cell, 83*, 835-839.
- Musse AA, Meloty-Kapella L, Weinmaster G (2012) Notch ligand endocytosis: mechanistic basis of signaling activity. *Seminars in Cell & Developmental Biology*, 23, 429-436.
- Novac N, Heinzel T (2004) Nuclear receptors: overview and classification. *Current Drug Targets. Inflammation and Allergy*, 3, 335-346.
- Olefsky JM (2001) Nuclear receptor minireview series. *The Journal of Biological Chemistry*, 276, 36863-36864.
- Sen A, Madhivanan K, Mukherjee D, Aguilar RC (2012) The epsin protein family: coordinators of endocytosis and signaling. *Biomolecular Concepts*, 3, 117-126.

- Sluder AE, Maina CV (2001) Nuclear receptors in nematodes: themes and variations. *Trends in Genetics*, 17, 206-213.
- Tessneer KL, Cai X, Pasula S, Dong Y, Liu X, Chang B, McManus J, Hahn S, Yu L, Chen H (2013) Epsin Family of Endocytic Adaptor Proteins as Oncogenic Regulators of Cancer Progression. *Journal of Cancer Research Updates*, 2, 144-150.
- Urwin PE, Lilley CJ, Atkinson HJ (2002) Ingestion of double-stranded RNA by preparasitic juvenile cyst nematodes leads to RNA interference. *Molecular Plant-Microbe Interactions*, 15, 747-752.

GENOME WIDE ASSOCIATION STUDIES FOR IDENTIFICATION OF DIFFERENT RESISTANCE GENES IN WHEAT OF FERTILE CRESCENT

FAHEEM SHEHZAD BALOCH[1,*], MUSTAFA İMREN[2], AHMAD ALSALEH[3], ABDELFATTAH A. DABABAT[4], MAHMOOD AYAZ[5], AND HALIL TOKTAY[5]

[1]Abant İzzet Baysal University, Faculty of Agriculture and Natural Science, Department of Field Crop, Gölköy Kampüs, 14280 Bolu, [2]Abant İzzet Baysal University, Faculty of Agriculture and Natural Science, Department of Plant Protection, Gölköy Kampüs, 14280 Bolu, [3]Department of Biotechnology, University of Cukurova, Adana, Turkey, [4]International Maize and Wheat Improvement Centre - Turkey office P.K. 39 Emek 06511 Ankara-Turkey, [5]Department of Plant production, Faculty of Agricultural sciences and Technologies, Nigde University, Nigde, Turkey. *Corresponding author email: balochfaheem13@gmail.com

SUMMARY

Cereal cyst nematodes (*Heterodera avenae* group) are crucial pest pests in most wheat growing areas and cause economic losses when above threshold levels. It is well known that developing resistant/tolerant wheat varieties is the most effective control method against cyst nematodes. Different mapping experiments have been done and diagnostic markers "*Cre1* and *Cre3*" resistance genes against the cereal cyst nematode have been developed. Huge amounts of germplasm from the Turkish National Breeding Program and the joint TURKEY CIMMYT International Winter Wheat Improvement Program have been tested by previous researchers and they found that the Iranian land race

* Baloch FS, İmren M, Alsaleh A, Dababat AA, Ayaz A, Toktay H (2015) Genome wide association studies for identification of different resistance genes in wheat of fertile crescent. In 'Nematodes of Small Grain Cereals: current status and research.' (AA Dababat, H Muminjanov and RW Smiley) pp. 357-368. (FAO: Ankara, Turkey).

"Sardari" was resistant against the *Cre1* gene. Many genes have been identified for the resistance mechanism in wheat. In our studies, we also found that some wheat varieties were resistant against cyst nematodes but those wheat cultivars did not show any evidence at the molecular level about the presence of resistant genes. This showed that either some other resistant genes were present or some other genetic mechanism occurs in those varieties. The Fertile Crescent is the primary center of domestication and diversity of cereals and of many other important crops. In spite of the importance of genetic resources from this area, there is a severe lack of information about the genetic structure of the wheat gene pool for resistance mechanisms against these pests from this region. Turkey contributes a critical role in the Fertile Crescent. Despite of the importance of gene pool from Turkey, no efforts had been made to get information about the genetic structure of the Anatolian wheat gene pool as it regards nematode genetic mechanisms. Therefore, we are planning to do genome-wide association mapping studies of the gene pool from the Fertile Crescent using 100-660K wheat high-throughput genotyping.

Keywords: Cereal, nematodes, next generation sequencing, association mapping

INTRODUCTION

Cereal cyst nematodes (CCN) are distributed in cereal growing areas throughout the world and cause significant economic yield losses in many countries, particularly where rain fed cereal growing systems are the predominant cropping system (Nicol *et al.* 2003). These nematodes infect a wide range of host plants, including wheat (*Triticum aestivum* L.) and grain crops that are grown in rotation with wheat. Cereal cyst nematodes together with biotic and biotic factors such as water stress and fungal pathogens results in synergistic negative effects. Researchers have reported high yield losses in various geographic regions of the world. Yield losses were 15–20% in Pakistan, 40–90% in Saudi Arabia, 23–50% in Australia, and 24% in the USA due to CCNs, as referenced by Nicolos *et al.* (2003). Similarly Whitehead (1998) reported that 10% of cereal production worldwide is lost due to plant-feeding nematodes. Economic and yield losses caused by CCN is approximately 78% around the world (Barker *et al.* 1998).

Twelve important species are included in the CCN group. *Heterodera avenae*, *H. filipjevi* and *H. latipons* are considered the most important species and con-

stitute a major limiting biotic factor to cereal production in temperate rain fed growing regions including China, India, Turkey, Australia, the United States, and many countries in Europe (Rivoal and Cook 1993, Dixon *et al.* 2009).

Cereal cyst nematodes have been recognized as a global problem and, as a consequence, in the last three decades various research efforts, in particular in Australia, Spain and France, have identified resistant accessions from various *Triticum*, *Aegilops* and *Secale* species. Several attempts were implemented to control CCNs around the world. Crop rotation, using resistant varieties and lines with different tillage techniques are recommended to control nematodes in wheat production. Crop rotation is generally ineffective in reducing the occurrence and severity of CCN. Chemical methods can be applied but they are not preferred by the producers because of the high cost per unit area in wheat fields.

Resistance is considered one of the most appropriate control methods as this is cost-effective, environmentally friendly and achievable through collaborations of research groups around the world. Resistant crops do not allow nematodes to reproduce and increase in number in the roots. The development of resistant wheat cultivars to nematodes has become increasingly important due to the prevalence and wide host range of the nematode, and the usage restrictions and inefficacy of nematicides.

Plants that reduce the nematode population in root systems and in the soil are considered to be resistant. Resistant cultivars have the capacity to reduce nematode reproduction and nematode densities in the soil (Rohde 1972). Tolerant wheat cultivars can survive and yield well within nematode-infested soils but allow reproduction, thus leaving nematodes within the soil to attack subsequent crops. Therefore, the use of resistant and tolerant cultivars is now considered the most efficient, economical and environmentally acceptable means for nematodes control (Castillo *et al.* 1998). Resistant crop species and cultivars are valuable in crop rotations because they reduce the threat to subsequent crops. Resistance sources around the world were obtained from wild wheat relatives through breeding programs (Ogbonnaya *et al.* 2001). It is reported that nine resistance genes were transferred to control the cereal cyst nematode *H. avenae* in bread wheat; *Cre1* gene from *Triticum aestivum*; *Cre2*, *Cre5* and *Cre6* genes from *Aegilops ventricosa*; *Cre3* and *Cre4* genes from *Triticum tauschii*, *Cre7* from *Aegilops* species; and *Cre8* and *CreR* from *Secale cereale* (Barloy *et al.* 2007).

Developing nematode-resistant cultivars has proven difficult mainly due to the complex genetic basis of nematode resistance, the interaction of nematode and plant with the environment, and imperfect screening methods. Nematode distributions are greatly influenced by environmental factors such as temperature, soil fertility, soil texture, rainfall, and planting date, which makes characterization and evaluation of cultivar performance extremely challenging. Evaluation of resistance against nematodes can be laborious and costly, requiring replicated inoculated trials involving counting of nematodes or the extraction of DNA from soil and root systems, followed by real-time polymerase chain reaction (PCR) to estimate the quantity of nematodes. Molecular markers for nematode resistance could therefore be very useful selection tools in wheat breeding, allowing phenotyping resources to be allocated only to progeny that had been pre-selected as likely to carry the resistance allele. Host-plant resistance (Cook and Evans 1987) is one of the most effective methods of managing CCNs as it is environmentally sustainable and requires no additional equipment or cost. However, farmers will only use resistant cultivars if they are comparable to other commonly cultivated wheat cultivars in terms of yield performance. The continuous cultivation of wheat varieties with tolerance to CCNs can increase the nematode population and have adverse effects on the successive crop, particularly if it is a susceptible variety. The use of host-plant resistance requires a sound knowledge of the virulence spectrum of the target species and pathotypes.

Plant breeding has successfully improved crop resistance to both biotic and abiotic stresses, including drought, through phenotypic selection (Araus *et al.* 2008, Cooper *et al.* 2009). Previously, molecular markers linked to genes for resistance to nematode resistance have been identified using bi-parental populations obtained by crossing resistant and susceptible wheat genotypes. Sources of resistance to *H. avenae* populations worldwide have been collated, reviewed, and their gene designation reported (Rivoal *et al.* 2001, Nicol 2002, Nicol *et al.* 2003, McDonald and Nicol 2005, Nicol and Rivoal 2007). Though quantitative trait loci (QTL) mapping has been successful, bi-parental QTL mapping generally requires years to develop a mapping population and gene discovery is limited to the genetic background of the two parents. Linsell *et al.* (2014) identified three QTL on chromosomes 2B and 6D co-located with genomic regions previously linked to *P. thornei* resistance in wheat, validating the robustness of these QTL as useful sources of resistance in different genetic backgrounds. Wheat cultivars resistant to *H. avenae* in one region may be fully susceptible in other regions, as demonstrated by Imren *et al.* (2013) for landrace and national cultivars evaluated in Turkey. QTL identified in one genetic

background and working in one environment may not be productive in the other. That is the reason QTL mapping is a long process and could be replaced with association mapping to identify markers that are strongly associated with traits of interest using historical linkage among diverse germplasm.

Association mapping (AM) is an alternative to bi-parental linkage mapping that uses natural populations, thereby eliminating the need for developing mapping populations. AM is credited for detecting QTL with great resolution from populations of diverse origins. AM uses linkage disequilibrium (LD) between alleles within diverse populations to identify markers associated with particular traits. Recently, AM has been used to identify marker-trait associations in higher plants including disease resistance in potato and wheat. With increasing numbers of single-nucleotide polymorphisms (SNPs) combined with declining costs in genotyping, AM has become an attractive approach for revealing the genetic basis of target traits in crop species. The effective utilization of resistance genes requires the phenotypic and genotypic characterization of the mapping population under study. This has been widely exploited in many genetic studies, either through the use of classical bi-parental crosses and linkage mapping to determine the number and chromosomal locations of stripe rust resistance genes (Yang *et al.* 2003), or the use of recent approaches such as genome-wide association mapping (GWAM) which involves a collection of adapted germplasm. A diagrammatic scheme of linkage disequilibrium mapping is shown in Figure 1.

The advantages of GWAM over a bi-parental mapping population include higher mapping resolution, increase in allele number, and time savings for establishing a marker-trait association and immediate application of its results in a breeding program. High-density SNPs data are widely used to detect marker–trait associations in QTL mapping experiments and genome-wide association studies (GWAS) (Jia *et al.* 2013, Tian *et al.* 2011, Zhao *et al.* 2011). Advances in next-generation sequencing have significantly facilitated the discovery of SNPs by whole genome (Berkman *et al.* 2012, Chia *et al.* 2012, Xu *et al.* 2012), transcriptome (Allen *et al.* 2011, Cavanagh *et al.* 2013), or reduced-representation sequencing in diverse populations of individuals (Elshire *et al.* 2011).

Lev-Yadun *et al.* (2000) proposed a "core area" for the origins of agriculture within the Fertile Crescent. This was based on the proposition that wild einkorn and wild emmer from this area are genetically more closely related to the domesticated wheat plants than elsewhere. Wheat landraces are an important potential source of new resistance genes since relatively few landraces have

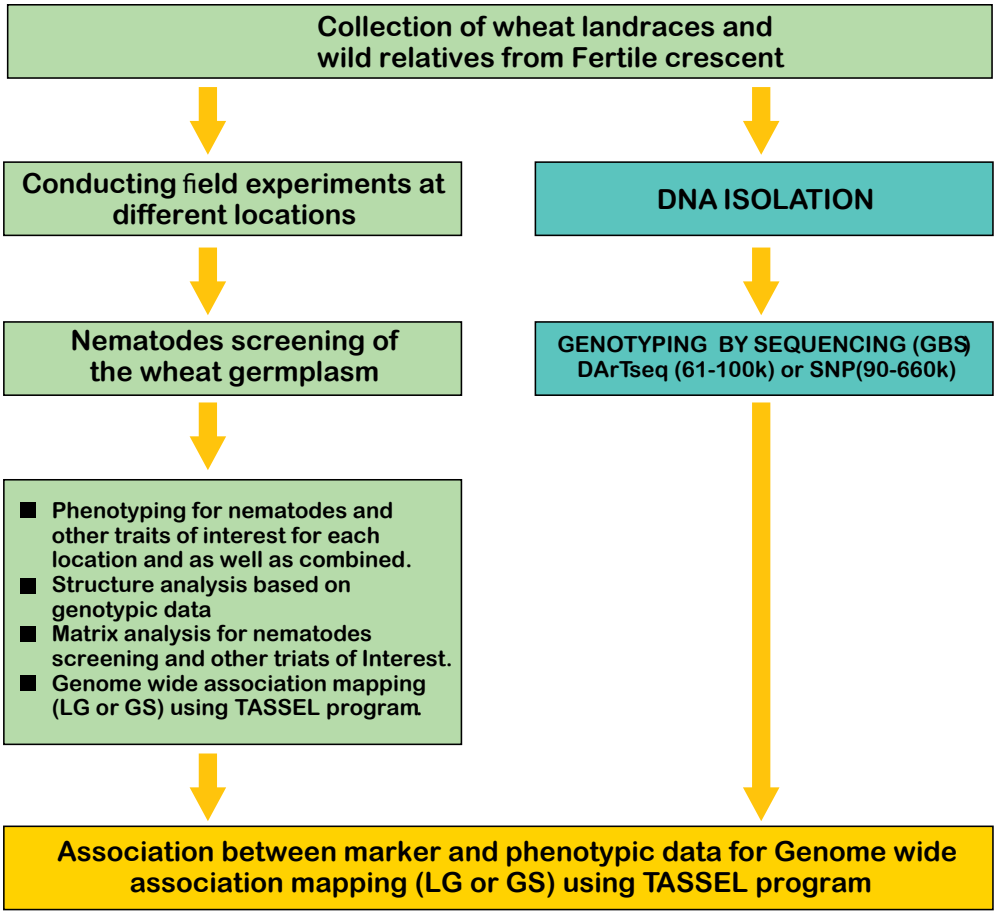


Figure 1. Scheme of genome-wide association mapping (GWAS) for nematode resistance in wheat

been used in modern plant breeding. Studies have demonstrated that wheat landraces can be an important source of nematode resistance. The Iranian bread wheat landrace, Sardari, was found to test positive with the *Cre1* primer, demonstrating the importance of landraces originating from the Fertile Crescent and its surroundings. Therefore, it is possible that some of the land races and early and advanced cultivars may contain these resistant genes.

Evaluation of the landraces from the areas of domestication and diversity of any species can provide useful information for understanding the pattern of evolution and how socio-economic and geo-ecological factors of those areas have influenced their genetic structure. These landraces serve great purposes such as: 1) they can serve as sources of new genes or new variations (alleles); 2) they can possess high variation at inter- and intra-population levels, and this intra-population diversity is very important because it can provide a buffering

capacity against increasing stochastic environmental variation; 3) their genetic structure could provide very useful insights towards a better understanding of the role of society, the agro-climatic conditions, and of their interaction to design their genetic structure. These reasons make it of utmost importance to investigate landraces (Karaköy *et al.* 2012). We therefore anticipate that new or underutilized genes for resistance to different nematode pathotypes may exist in winter wheat landraces.

Some researchers from various parts of the world screened wheat landraces and their exotic germplasm for presence of *Cre1* and *Cre3* genes by using the known DNA-based markers, and testing the effectiveness of these genes against *H. filipjevi* under controlled greenhouse conditions. They reported that *Cre1* or *Cre3* markers were found in the genetic background of a limited number of Turkish winter bread and durum wheat accessions investigated. However, the number of accessions used in that experiment was limited and requires further investigation in a larger subset. However some studies showed that some wheat lines harboring the *Cre* genes are susceptible to CCNs. Therefore some complex genetic mechanism appears to be serving as a background role in nematode resistance. Therefore more new QTL are indispensable to be identified and new molecular markers linked with resistance need to be developed for marker-assisted selection. For example, in Turkey, the *Cre1* gene appears effective against *H. filipjevi*, but *Cre3* is not (Akar *et al.* 2009, Nicol *et al.* 2009). The *Cre3* gene is effective against *H. avenae* in Australia (Vanstone *et al.* 2008), but not in Europe (Majnik *et al.* 2003). The *Cre2* and *Cre4* resistance genes from *Aegilops*, and an unidentified resistance gene from wheat line AUS4930, offer promising sources of resistance against an array of CCN species and pathotypes (Nicol *et al.* 2001). Several lines containing *Cre5* were tested by Dababat *et al.* (2014a) and did not successfully confer resistance to CCNs. Imren *et al.* (2013) used six *Cre* genes in international bread wheat germplasm to identify genetic resistance to *H. avenae*, *H. filipjevi*, and *H. latipons*. The results indicated that the resistant genes *Cre1*, *Cre3*, and *Cre7* provided resistance against both *H. avenae* and *H. latipons*. The other genes, *Cre8* and *CreR*, provided resistance against *H. filipjevi* only. None of the *Cre* genes studied provided complete resistance to the three CCN species; see more detail in Dababat *et al.* 2015.

Genetic improvement of cultivated wheat remains slow, because of narrow genetic base or limited genetic variation for nematodes resistance among cultivated or elite lines. Lack of requisite genetic variation for nematode resistance in cultivated wheat has necessitated systematic collection, documentation and

evaluation of wild wheat species for use in wheat improvement programs focused on nematode resistance. Due to the lack of genetic diversity for nematode resistance in cultivated modern bread wheat, new sources of resistance are being sought from wild wheat progenitors (Ogbonnaya 2008, Zwart *et al.* 2010). Hexaploid bread wheat originated in the Fertile Crescent in the Middle East through a few random crossings between wild wheat species (Nesbitt 2001). It has been suggested that in the original hybridization, only a limited number of nematode resistance genes from the diploid *Aegilops tauschii* and the tetraploid *Triticum turgidum* wild progenitors were involved, and thus hexaploid wheat lacks the diverse genetic sources of resistance genes that its ancestors possess (Breiman and Graur 1995). The wild relatives of wheat provide a good opportunity for increasing the genetic variation of cultivated wheat, since the variability for resistance to biotic and abiotic stresses in the modern wheat varieties is insufficient. Many improved cultivars of crops are vulnerable to insect pests as they lack the defense mechanisms of their wild progenitors. Similarly *Cre2*, *Cre5* and *Cre6* genes in *Aegilops ventricosa*; *Cre3* and *Cre4* genes in *Triticum tauschii*, and *Cre7* in *Aegilops* species have been derived from wild relatives of wheat. Initially, Thompson and Haak (1997) screened accessions of *Ae. tauschii* from Iran and found *Pratylenchus thornei* resistance in all taxonomic subgroups of this species. Resistance found in *Ae. tauschii* can be transferred to bread wheat by direct crossing (Gill and Raupp 1987) or by developing synthetic hexaploids through hybridization with a durum, *T. turgidum*, which can then be crossed to bread wheats (Lagudah *et al.* 1993, Mujeeb-Kazi 1995). In recent years, effective sources of resistance to *P. thornei* and *P. neglectus* have been identified and mapped in synthetic hexaploid wheat lines (Ogbonnaya 2008, Thompson *et al.* 2009, Toktay *et al.* 2006) and Middle Eastern landraces (Schmidt *et al.* 2005, Sheedy and Thompson 2009, Thompson *et al.* 2009). QTL for resistance to *P. thornei* have been identified on the B and D genomes on five different chromosomes in the investigated sources (Schmidt *et al.* 2005, Toktay *et al.* 2006). The mode of resistance to *P. thornei* is multigenic and additive (Zwart *et al.* 2004) and thus makes it a suitable trait for marker-assisted selection. Therefore it is of utmost importance to screen wild wheat germplasm from its area of diversity for nematode resistance, and to transfer nematode-resistance genes into cultivated bread wheat through marker-assisted selection.

REFERENCES

- Akar T, Caliskan M, Nicol JM, Uranbey S, Sahin E, Yazar S, William M, Braun HJ (2009) Molecular characterization of cereal cyst nematode di-

agnostic markers *Cre1* and *Cre3* in some winter wheat germplasm and their potential use against *Heterodera filipjevi*. *Field Crops Research*, 114, 320–323.

- Allen AM, Barker GLA, Berry ST, Coghill JA, Gwilliam R, Kirby S, Robinson P, Brenchley RC, D'Amore R, McKenzie N, Waite D, Hall A, Bevan M, Hall N, Edwards KJ (2011) Transcript-specific, single-nucleotide polymorphism discovery and linkage analysis in hexaploid bread wheat (*Triticum aestivum* L.) *Plant Biotechnology Journal*, 9, 1086–1099.
- Barloy D, Lemoine J, Abelard P, Tanguy AM, Rivoal R, Jahier J (2007) Marker-assisted pyramiding of two cereal cyst nematode resistance genes from *Aegilops variabilis* in wheat. *Molecular Breeding*, 20, 31-40.
- Berkman PJ, Lai K, Lorenc MT, Edwards D (2012) Next-generation sequencing applications for wheat crop improvement. *American Journal of Botany*, 99, 365–371.
- Breiman A, Graur D (1995) Wheat Evolution. *Israel Journal of Plant Sciences*, 43(2), 85-98.
- Castillo P, Vovlas N, Jiménez-Diaz RM (1998) Pathogenicity and histopathology of *Pratylenchus thornei* populations on selected chickpea genotypes. *Plant Pathology*, 47, 370-376.
- Cavanagh CR, Chao S, Wang S, Huang BE, Stephen S, Kiani S, Forrest K, Saintenac C, Brown-Guedira GL, Akhunova A, See D, Bai G, Pumphrey M, Tomar L, Wong D, Kong S, Reynolds M, da Silva ML, Bockelman H, Talbert L, Anderson JA, Dreisigacker S, Baenziger S, Carter A, Korzun V, Morrell PL, Dubcovsky J, Morell MK, Sorrells ME, Hayden MJ, Akhunov E (2013) Genome-wide comparative diversity uncovers multiple targets of selection for improvement in hexaploid wheat landraces and cultivars. *Proceedings of the National Academy of Sciences*, 110, 8057–8062.
- Chia J-M, Song C, Bradbury PJ, Costich D, de Leon N, Doebley J, Elshire RJ, Gaut B, Geller L, Glaubitz JC, Gore M, Guill KE, Holland J, Hufford MB, Lai J, Li M, Liu X, Lu Y, McCombie R, Nelson R, Poland J, Prasanna BM, Pyhäjärvi T, Rong T, Sekhon RS, Sun Q, Tenaillon MI, Tian F, Wang J, Xu X, Zhang Z, Kaeppler SM, Ross-Ibarra J, McMullen MD, Buckler ES, Zhang G, Xu Y, Ware D (2012) Maize HapMap2 identifies extant variation from a genome in flux. *Nature Genetics*, 44, 803–807.
- Elshire RJ, Glaubitz JC, Sun Q, Poland JA, Kawamoto K, Buckler ES, Mitchell SE (2011) A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. *PLoS ONE*, 6, e19379.
- Gill BS, Raupp WJ (1987) Direct genetic transfer from *Aegilops squarrosa* L. to hexaploid wheat. *Crop Science*, 27(3), 445-450.

- Imren M, Toktay H, Bozbuğa R, Dababat A, Elekçioğlu H (2013) Pathotype determination of the cereal cyst nematode, *Heterodera avenae* (Wollenweber, 1924) in the Eastern Mediterranean Region in Turkey. *Türkish Journal of Entomology* 37, (1), 13-19.
- Jia G, Huang X, Zhi H, Zhao Y, Zhao Q, Li W, Chai Y, Yang L, Liu K, Lu H, Zhu C, Lu Y, Zhou C, Fan D, Weng Q, Guo Y, Huang T, Zhang L, Lu T, Feng Q, Hao H, Liu H, Lu P, Zhang N, Li Y, Guo E, Wang S, Wang S, Liu J, Zhang W, Chen G, Zhang B, Li W, Wang Y, Li H, Zhao B, Li J, Diao X, Han B (2013) A haplotype map of genomic variations and genome-wide association studies of agronomic traits in foxtail millet (*Setaria italic*). *Nature Genetics*, 45, 957–961.
- Karaköy T, Erdem H, Baloch FS, Toklu F, Eker S, Kilian B, Özkan H (2012) Diversity of macro-and micronutrients in the seeds of lentil landraces. *The Scientific World Journal, 2012*.
- Lagudah ESR, Appels D, McNeil, Schachtman DP (1993) Exploiting the diploid 'D' genome chromatin for wheat improvement. In. Gene Conversion and Exploitation. (Eds JP Gustafson, R Appels, R Raven). (Plenum Press, New York).
- Lev-Yadun S, Gopher A, Abbo S (2000) The cradle of agriculture. *Science*, 288, 1602-1603.
- Malosetti M, van der Majnik J, Ogbonnaya FC, Moullet O, Lagudah ES (2003) The *Cre1* and *Cre3* nematode resistance genes are located at homologous loci in the wheat genome. *Molecular Plant Microbe Interactions*, 16, 1129–1134.
- Mujeeb-Kazi A (1995) Interspecific crosses-hybrid production and utilisation. In: 'Utilizing Wild Grass Biodiversity in Wheat Improvement, 15 Years of Wide Cross Research.' (Eds. A Maujeeb-Kazi, GP Hettel). CIMMYT, Mexico.
- Nesbitt M (2001) Wheat evolution: Integrating archaeological and biological evidence. In: 'Wheat Taxonomy: The Legacy of John Percival, The Linnean Special Issue 3.' (Ed. TLS London) pp 37-59. (Academic Press, London).
- Nicol JM (2002) Important nematode pests of cereals. In: 'Bread Wheat: Improvement and Production.' (Eds BC Curtis, S Rajaram, G Macpherson) pp. 345-366. FAO Plant Production and Protection Series, No. 30 (FAO, Rome, Italy).
- Nicol JM, Ogbonnaya F, Singh AK, Bishnoi SP, Kanwar RS, Li H, Chen S, Peng D, Bolat N, Sahin E, Elekcioglu IH (2009) Current global knowledge of the usability of cereal cyst nematode resistant bread wheat germplasm through international germplasm exchange and evaluation. In: 'Cereal

Cyst Nematodes: Status, Research and Outlook.' (Eds. IT Riley, JM Nicol, AA Dababat) pp. 149–153. (CIMMYT, Ankara, Turkey).

- Nicol JM, Rivoal R (2007) Global knowledge and its application for the integrated control and management of nematodes on wheat. In: 'Integrated Management and Biocontrol of Vegetable and Grain Crops Nematodes.' (Eds. A Ciancio, KG Mukerji) pp. 243-287. (Springer, Dordrecht, The Netherlands).
- Nicol JM, Rivoal R, Taylor S, Zaharieva M (2003) Global Importance of cyst (*Heterodera* spp.) and lesion nematodes (*Pratylenchus* spp.) on cereals: distribution, yield loss, use of host resistance and integration of molecular tools. *Nematology Monographs and Perspectives*, 2, 1–19.
- Nicol JM, Turner SJ, Coyne DL, Nijs L, Hockland S, TahnaMaafi Z (2011) Current nematode threats to world agriculture. In: 'Genomics and Molecular Genetics of Plant Nematode Interactions. (Eds. J Jones, G Gheysen, C Fenoll). (Springer, New York)
- Ogbonnaya FC, Imtiaz M, Bariana HS, McLean M, Shankar MM (2008) Mining synthetic hexaploids for multiple disease resistance to improve bread wheat. *Crop and Pasture Science*, 59, 421–431.
- Ogbonnaya FC, Seah S, Delibes A, Jahier J, López Braña I, Eastwood RF, Lagudah ES (2001) Molecular-genetic characterisation of a new nematode resistance gene in wheat. *Theoretical and Applied Genetics*, 102, 623- 629.
- Özkan H, Willcox G, Graner A, Salamini F, Kilian B (2010) Geographic distribution and domestication of wild emmer wheat (*Triticum dicoccoides*). *Genetic Resources and Crop Evolution*, 58(1), 11-53.
- Rivoal R, Cook R (1993) Nematode pests of cereals. In: 'Plant Parasitic Nematodes in Temperate Agriculture.' (Eds, K Evans, DL Trudgill, JM Webster) pp. 259–303. (CAB International, London).
- Rivoal R, Bekal S, Valette S, Gauthier J-P, Fradj MBH, Mokabli A, Jahier J, Nicol J, Yahyaoui A (2001) Variation in reproductive capacity and virulence on different genotypes and resistance genes of Triticeae, in the cereal cyst nematode species complex. *Nematology*, 3, 581-592.
- Rohde RA (1972) Expression of resistance in plants to nematodes. *Annual Review of Phytopathology*, 10, 233-251.
- Schmidt AL, McIntyre CL, Thompson J, Seymour NP, Liu CJ (2005) Quantitative trait loci for root lesion nematode (*Pratylenchus thornei*) resistance in Middle-Eastern landraces and their potential for introgression into Australian bread wheat. *Australian Journal of Agricultural Research*, 56, 1059-1068.
- Sheedy JG, Thompson JP (2009) Resistance to the root-lesion nematode (*Pratylenchus thornei*) in Iranian landrace wheat. *Australasian Plant Pathology*, 28, 478-489.

- Thompson JP, Haak MI (1997) Resistance to root-lesion nematode (*Pratylenchus thornei*) in *Aegilops tauschii* Coss, the D-genome donor to wheat. *Australian Journal of Agricultural Research*, 48(5), 553-559.
- Thompson JP (2008) Resistance to root-lesion nematodes (*Pratylenchus thornei* and *P. neglectus*) in synthetic hexaploid wheats and their durum and *Aegilops tauschii* parents. *Australian Journal of Agricultural Research*, 59(5), 432-446.
- Tian F, Bradbury PJ, Brown PJ, Hung H, Sun Q, Flint-Garcia S, Rocheford TR, McMullen MD, Holland JB, Buckler ES (2011) Genome-wide association study of leaf architecture in the maize nested association mapping population. *Nature Genetics*, 43, 159–162.
- Toktay H, McIntyre CL, Nicol JM, Ozkan H, Elekcioglu HI (2006) Identification of common root-lesion nematode (*Pratylenchus thornei* Sher et Allen) loci in bread wheat. *Genome*, 49(10), 1319-1323.
- Vanstone VA, Hollaway GJ, Stirling GR (2008) Managing nematode pests in the southern and western regions of the Australian cereal industry: continuing progress in a challenging environment. *Australasian Plant Pathology*, 37, 220–234.
- Xu X, Liu X, Ge S, Jensen JD, Hu F, Li X, Dong Y, Gutenkunst RN, Fang L, Huang L, Li J, He W, Zhang G, Zheng X, Zhang F, Li Y, Yu C, Kristiansen K, Zhang X, Wang J, Wright M, McCouch S, Nielsen R, Wang J, Wang W (2012) Resequencing 50 accessions of cultivated and wild rice yield markers for identifying agronomically important genes. *Nature Biotechnology*, 30, 105–111.
- Yang T, Xie C, Sun Q (2003) Situation of the sources of stripe rust resistance of wheat in the post-CYR32 era in China. *Acta Agronomica Sinica*, 29, 161–168.
- Zhao K, Tung C-W, Eizenga GC, Wright MH, Ali ML, Price AH, Norton GJ, Islam MR, Reynolds A, Mezey J, McClung AM, Bustamante CD, McCouch SR (2011) Genome-wide association mapping reveals a rich genetic architecture of complex traits in *Oryza sativa*. *Nature Communications*, 2, 467.
- Zwart RS, Thompson JP, Milgate AW, Bansal UK, Williamson PM, Raman H, Bariana HS (2010) QTL mapping of multiple foliar disease and root-lesion nematode resistances in wheat. *Molecular Breeding*, 26, 107-124.

MOLECULAR TOOLS AID IN DETECTING AND MANAGING CEREAL NEMATODES IN THE USA

RICHARD W. SMILEY[1,*] AND GUIPING YAN[1,2]

[1]Oregon State University, Columbia Basin Agricultural Research Center, Pendleton, Oregon 97801, and, currently, [2]Department of Plant Pathology, North Dakota State University, Fargo, North Dakota 58108, USA. *Corresponding author email: richard.smiley@oregonstate.edu

SUMMARY

Pratylenchus spp. (root-lesion nematodes, RLN) and *Heterodera* spp. (cereal cyst nematodes, CCN) are the most important nematodes that affect small grain production in rainfed fields of the Pacific Northwest (PNW). Each genus includes two species that are important in the region. Information on management strategies for these four species has been greatly amplified in the PNW during the past decade and accurate identification of the species is now as important as determining the overall number of nematodes. These nematodes were previously identified only at the genus level but we now provide management recommendations showing that crops and cultivars differ in resistance and/or tolerance to individual species. In this paper we report examples of research that illustrate the importance of identifying these species, and comparisons of applications of DNA-based tests to distinguish four locally important species of RLN and CCN. Some of these PCR tests are now available as a routine service by a commercial laboratory that previously identified these nematodes only to the genus or group level. A PCR-RFLP test is also used in research laboratories to distinguish the locally important species of *Heterodera*. Molecular diagnostics now provide a readily-accessible tool that complements management recommendations to minimize crop losses caused by *Pratylenchus* and *Heterodera*.

* Smiley RW, Yan GP (2015) Molecular tools aid in detecting and managing cereal nematodes in the USA. In 'Nematodes of Small Grain Cereals: current status and research.' (AA Dababat, H Muminjanov and RW Smiley) pp. 369-380. (FAO: Ankara, Turkey).

INTRODUCTION

Research in the Pacific Northwest (PNW) states of Idaho, Oregon and Washington has shown that cereals are far more affected by two species of *Pratylenchus* and two species of *Heterodera* compared to other plant-parasitic nematodes. Introductions to that literature are exemplified by Smiley (2009a, 2009b), Smiley and Yan (2015), Yan and Smiley (2010), and Yan *et al.* (2008, 2012, 2013a, 2013b). The research in the PNW shows that some wheat and barley cultivars differ in resistances and tolerances to the two species within each genus. For cereal producers to effectively utilize results of the research it became important for them to also have the ability to identify these nematodes to the species level in each field. Most commercial laboratories in the PNW only reported numbers of *Pratylenchus* and *Heterodera* at the genus level due to complexities and liability associated with identifying species in diagnostic environments in which high numbers of samples must be processed within very short periods. More precise and rapid procedures were required before management recommendations could be effectively used by cereal producers. End-point PCR, real-time PCR, and PCR-RFLP methods were therefore developed to address the practical needs of cereal producers and to also serve the needs of research laboratories.

The objective of this paper is to present examples of individual reports that addressed these issues. References to appropriate papers are cited for those who wish to examine these results in greater detail.

METHODS

Experiments are described to illustrate the need for species specific identification of *Pratylenchus* spp. and *Heterodera* spp. in the PNW, and development of molecular tools to increase the efficiency of providing species specific identifications.

Tolerance of spring cereals to *P. neglectus* and *P. thornei*. Field experiments were performed to screen spring wheat and spring barley during multiple years at locations where either *P. neglectus* or *P. thornei* were the only species or were the dominant species (details are in Smiley 2009). Experiments at each location were planted into non-tilled fields. Experiments included up to 34 spring wheat and six barley cultivars, each of which was planted into three replicates of 1.4 × 9.1 m plots that were either treated or not treated with aldicarb; Temik

15G, 4.2 kg ha^{-1} of active ingredient. This nematicide is not registered for use in commercial crops but serves as an excellent screening tool in research trials. Trials were of a strip-plot design with cultivars being planted side-by-side in drill rows that were treated or not treated with nematicide. Aldicarb was placed 10 cm below the seed in alternate drill strips. Grain was harvested from each plot using a small-plot combine. Cultivar tolerance was rated by determining the percentage yield increase due to reduced nematode stress in the nematicide-treated versus the control drill strips. Tolerance ratings were very tolerant (VT, <5% yield response), tolerant (T, 5-10%), moderately tolerant (MT, 10-15%), moderately intolerant (MI, 15-30%), intolerant (I, 30-50%), or very intolerant (VI, >50%).

Resistance of spring wheat to *P. thornei*. Wheat lines were assayed against *P. thornei* in a glasshouse to examine the resistances of F_3-generation lines and parental lines of a cross between partially-resistant cultivar Persia 20 and susceptible cultivar Alpowa (details are in Yan *et al.* 2012). Plants were grown in a silt loam. At the one-leaf stage each pot was inoculated with *P. thornei* derived from pure cultures maintained on wheat roots. The species identity was confirmed using the methods described by Yan *et al.* (2008). After 16 weeks incubation, roots were cut to fine pieces and mixed thoroughly with soil, and nematodes were extracted from the soil and plant roots using the Whitehead tray method. Nematodes were quantified using microscopic method and reported as nematodes/kg of soil plus roots. Nematode DNA was extracted from 0.5 g soil of each subsample in triplicate and quantified using real-time PCR (Yan *et al.* 2012). Multiplication rates (MR) of the nematode were calculated by dividing the final population of nematodes by the initial population in each pot. The lines were classified as resistant (R, MR≤ 1), intermediate (I, MR 1-9.9) and susceptible (S, MR ≥10).

Standard PCR for identifying *Pratylenchus* spp. A species-specific end-point PCR protocol developed in our lab was validated for detecting and discriminating *P. neglectus* and *P. thornei* in soil samples harboring a range of population densities of *P. neglectus* and *P. thornei* (details are in Yan *et al.* 2008). The nematodes were extracted from soil using the Whitehead tray method and identified using traditional microscopy. DNA was also extracted from 0.5 g of soil and DNA extractions were performed four times for each sample. PCR was performed as described in Yan *et al.* (2008) to compare results with morphological identifications.

Real-time PCR for identifying *Pratylenchus* spp. Fifteen soil samples were collected from rainfed wheat fields, divided, and submitted to three commercial labs and one research lab for identification and quantification of *Pratylenchus* spp. using traditional procedures (details are Yan *et al.* 2012 and 2013b). Lab 1 utilized the Whitehead tray method for nematode extraction and Labs 2-4 used the wet-sieving and sugar-density flotation method. Species identity in each soil sample was confirmed by conventional PCR method (Yan *et al.* 2008) and nematode DNA was extracted from 0.5 g of soil and analyzed using real-time PCR (qPCR). Three independent DNA extractions were conducted for each soil sample and each extract was assayed in duplicate qPCR reactions. Results from the qPCR and traditional methods were compared.

Resistance of spring wheat to *H. avenae* and *H. filipjevi*. Six spring wheat cultivars were assayed in a single silt loam soil series that was naturally infested by *H. avenae*, *H. filipjevi*, or by neither species (details are in Smiley and Yan 2015). Wheat cultivars Ouyen and Louise were known to be either resistant or susceptible, respectively, to *H. avenae*. Cultivars Sönmez and SY Steelhead were thought to be resistant to *H. filipjevi*. Roots were washed and numbers of swollen white females were counted nine weeks after plants emerged in small pots of naturally-infested soil. Resistance reactions were compared to the pre-assigned susceptible control; Louise. Resistance ratings were very resistant (VR, ≤1% of white females produced on Louise), resistant (R, 1.1-5%), moderately resistant (MR, 5.1-15%), moderately susceptible (MS, 15.1-30%), susceptible (S, 30.1-60%), or very susceptible (VS, >60%). The *Heterodera* species was identified by the presence (*H. filipjevi*) or absence (*H. avenae*) of an underbridge in the cyst. The experiment was repeated using soils collected during a second sampling.

End-point PCR and PCR-RFLP for identifying *Heterodera* spp. Soil samples were collected from commercial rainfed fields in a 20 km^2 area in eastern Washington where both *H. avenae* and *H. filipjevi* were known to occur (details are in Smiley and Yan 2015). We had no previous knowledge of infestations by either species in most fields sampled and there were no patches of stunted or uneven growth to focus the areas sampled. Samples from each field consisted of a composite of 10 shovel slices (2.5 × 10 × 15 cm) from a 2- to 4-ha area. Subsamples were sent to Western Laboratories in Parma, ID for extraction and

enumeration using a modified Oostenbrink elutriator extraction method. *Heterodera* was reported at the genus level and the lab returned suspensions to us for species identification using molecular and morphological procedures (Yan *et al.* 2010 and 2013a). Subsamples retained from the original sample were used to extract cysts using the sieving-decanting method and also to extract juveniles using the Whitehead tray method. These extractions provided specimens for species additional identifications in our laboratory. Nematode DNA was extracted from each sample and species-specific PCR was conducted. The species identity was also confirmed by PCR-RFLP restriction pattern analysis and by evaluating morphological features of cysts.

RESULTS

Tolerance of spring cereals to *P. neglectus* and *P. thornei*. Cultivars of barley and wheat differ in levels of tolerance to *P. neglectus* and *P. thornei* and there is no clear relationship between tolerance or intolerance of an individual cultivar to both species (Table 1). For example, Jefferson is moderately intolerant of *P. neglectus* and tolerant of *P. thornei*. Louise and Yecora Rojo wheat and Radiant barley have nearly equal reactions to both species. Growers must be able to learn which species is present on each field if they wish to plant a tolerant cultivar as part of their disease management program.

Resistance of spring wheat to *P. thornei*. Resistance reactions are more easily determined using glasshouse experiments than field trials. Wheat lines from a mapping population clearly demonstrated different levels of resistance, as quantified using either traditional methods or qPCR assays of DNA extracts from soil (Table 2). The table is abbreviated to illustrate differences that were experienced in these assays. Both methods resulted in identical reaction assignments for 17 of 20 lines shown in Yan *et al.* (2012). Three lines differed in reaction type, indicating that real-time PCR predicted the phenotypes in 85% of the tested lines. There, were no instances for which the two procedures provided contradictory assignments of susceptible versus resistant phenotypes. There was a significant positive correlation ($R^2 = 0.65$, $P < 0.001$) between the estimates of the numbers of *P. thornei* determined by the real-time PCR assay and by the Whitehead method.

Table 1. Yield increase and tolerance rankings when spring barley and wheat were planted into plots treated or not treated with a nematicide in fields infested by *Pratylenchus neglectus* or *P. thornei* [1]

Cultivar	*P. neglectus*		*P. thornei*	
	Yield[2]	Tolerance[3]	Yield	Tolerance
Barley				
Camas	7.1	T	3.9	VT
Bob	14.0	MT	4.3	VT
Radiant	19.5	MI	17.8	MI
Wheat				
Tara 2002	13.3	MT	5.5	T
Jefferson	15.8	MI	5.6	T
Louise	18.0	MI	17.0	MI
Otis	18.7	MI	8.8	T
Eden	26.6	MI	33.3	I
Yecora Rojo	26.9	MI	26.1	MI
Alpowa	31.7	I	10.6	MT
Alturas	33.7	I	24.8	MI
IDO377S	83.0	VI	27.6	MI

[1] Selected data from Smiley (2009a); means are from 2 (*P. neglectus*) or 3 trials (*P. thornei*).
[2] Percentage yield increase; 100×(aldicarb-treated yield – untreated control yield) / untreated yield.
[3] Tolerance ratings were very tolerant (VT, <5% yield increase), tolerant (T, 5-10%), moderately tolerant (MT, 10-15%), mod. intolerant (MI, 15-30%), intolerant (I, 30-50%), very intolerant (VI, >50%).

Table 2. Maximum range of responses[1] when identifying resistance to *Pratylenchus thornei* (Pt) in wheat using either a traditional method or a real-time PCR (qPCR) method

Wheat line	Microscopic estimate			qPCR estimate		
	Pt[2]	MR[3]	Reaction[4]	Pt[5]	MR	Reaction
1	343	0.2	R	2,007	1.0	R
2	375	0.2	R	4,776	2.4	I
3	1,468	0.7	R	1,626	0.8	R
4	1,533	0.8	R	423	0.2	R
5	4,865	2.4	I	13,514	6.8	I
6	4,934	2.5	I	3,297	1.6	I
7	5,301	2.7	I	1,239	0.6	R
8	14,245	7.1	I	6,100	3.1	I
9	24,526	12.3	S	8,957	4.5	I
10	35,510	17.8	S	45,017	22.5	S

[1] Maximum variability of data selected from Yan *et al.* (2012); the full data set showed a high correlation ($R^2 = 0.65$, $P < 0.001$) between methods and also that identical resistance assignments were made by both methods for 17 of 20 lines tested.
[2] *P. thornei*/kg of soil based on Whitehead tray extractions.
[3] Multiplication rate (MR) after 16 weeks of incubation. Initial inoculation rate was 2,000 *P. thornei*/kg.
[4] Resistance ratings based on MR; (R) ≤ 1; intermediate (I) 1-9.9; susceptible (S) ≥ 10.
[5] *P. thornei*/kg of soil based on means of two independent DNA extractions, each of which was analyzed in duplicate qPCR reactions.

End-point PCR for identifying *Pratylenchus* spp. There was good correlation between traditional and PCR identification procedures (Table 3). The limit of PCR detection for *P. neglectus* was 343 nematodes/kg of soil, as determined by the Whitehead tray method. The limit of PCR detection for *P. thornei* was 126 nematodes/kg of soil. The PCR method was more accurate and required less time than traditional methods.

Table 3. *Pratylenchus neglectus* (Pn) and *P. thornei* (Pt) extracted from naturally-infested soils using traditional procedures compared to PCR assays[1]

Soil	Nematodes/kg of soil[2]		PCR assay[3]	
	Pn	Pt	Pn	Pt
1	87	0	-	-
2	343	0	+	-
3	2,697	0	+	-
4	3,609	570	+	+
5	16,836	324	+	+
6	17,959	0	+	-
7	0	0	-	-
8	0	117	-	-
9	0	126	-	+
10	0	9,430	-	+

[1] Selected data from Yan *et al.* (2008).
[2] Numbers derived from Whitehead tray extractions.
[3] Presence (+) or absence (-) detected by PCR.

Real-time PCR for identifying *Pratylenchus* spp. Using well-mixed subsamples from naturally-infested fields, we compared nematode numbers detected by a qPCR assay and those reported by four labs that use traditional methods (Table 4). There was a strong ($R^2 = 0.76$; $P < 0.001$) correlation between numbers based on qPCR and those reported by Lab 1. The qPCR generally produced higher estimates than those from the Whitehead tray method. Few samples showed significant differences between methods (Yan *et al.* , 2012 and 2013b). Poor correlations existed between numbers based on qPCR and reports from Labs 2-4, each of which used wet-sieving and sugar-flotation extraction methods.

Resistance of spring wheat to *H. avenae* and *H. filipjevi*. Numbers of swollen white females were significantly fewer on Ouyen and WB Rockland than on other cultivars in soil infested by *H. avenae*, and were less on Sönmez and

SY Steelhead than on other cultivars in soil infested by *H. filipjevi* (Table 5). Ouyen and WB Rockland were characterized as resistant to *H. avenae* and Sönmez and SY Steelhead were resistant to moderately resistant to *H. filipjevi*. If disease management based on cultivar resistance is to be used successfully in commercial practice, the grower clearly must know which species is present on each field.

Table 4. *Pratylenchus neglectus* and *P. thornei* in naturally-infested field soils using real-time PCR (qPCR) compared to traditional methods at three commercial and one research lab[1]

Soil	*P. neglectus*[2]					*P. thornei*				
	qPCR	Lab 1	Lab 2	Lab 3	Lab 4	qPCR	Lab 1	Lab 2	Lab 3	Lab 4
1	0	53	220	70	0	0	0	0	0	54
2	0	0	0	0	36	0	0	0	0	0
3	1,155	974	442	20	0	3,672	812	898	120	2,846
4	2,945	553	1,240	120	0	0	0	0	0	1,254
5	8,571	629	5,520	10	6,226	6,515	2,691	0	110	692
6	10,076	2,175	4,480	140	5,186	0	0	0	0	0
7	15,254	1,733	4,522	140	8,366	0	0	1,938	0	0
8	17,374	5,964	6,413	70	0	184	1,361	5,247	60	7,162
9	25,669	4,016	3,460	100	5,400	88	0	0	0	0
10	27,847	2,156	3,948	100	5,234	90	0	1,692	0	0
11	28,503	3,700	8,444	160	1,936	7,774	1,871	5,176	40	484
12	42,246	5,385	5,566	120	0	0	0	4,554	0	1,036

[1] Selected data from Yan *et al.* (2012 and 2013b).
[2] Estimates using qPCR were based on three independent DNA extractions, each of which was analyzed in duplicate reactions. Lab 1 used the Whitehead tray extraction method. Labs 2, 3 and 4 used the wet-sieving and sugar-density flotation extraction methods.

End-point PCR and PCR-RFLP for identifying *Heterodera* spp. Using well-mixed subsamples from naturally-infested fields, we compared nematode identities detected by PCR and PCR-RFLP using suspensions that were quantified at the genus level and returned to our lab for identifications (Table 6). There was an absolute agreement for identities determined by the two molecular methods. This occurred for soils infested with only *H. avenae*, only *H. filipjevi*, or with both species.

Table 5. *Heterodera avenae* or *H. filipjevi* white females produced on roots of five unknown spring wheat cultivars compared to a susceptible control (Louise) in naturally-infested soils [1]

Species	Louise	Ouyen	WB 936	WB Rockland	Sönmez	SY Steelhead
(*number of white females/plant*)						
H. avenae	33.3	1.1	21.8	1.3	23.7	25.9
H. filipjevi	60.4	19.1	46.4	30.6	2.9	5.7
(*resistance rating*)[2]						
H. avenae		R	VS	R	VS	VS
H. filipjevi		S	VS	S	R	MR

[1] Selected data from Smiley and Yan (2015); data are means of two repetitions.
[2] Resistance reactions; VR = resistant (≤1% of white females produced on control), R = resistant (1.1-5%), MR = moderately resistant (5.1-15%), MS = moderately susceptible (15.1-30%), S = susceptible (30.1-60%), VS = very susceptible (>60%).

Table 6. Distribution of *Heterodera* species in fields within a 20 km^2 area of Washington[1]

***Heterodera* spp.**		**Identified[3] by:**	
Density[2]	**Species**	**PCR**	**RFLP**
40	*avenae*	x	x
4,080	*avenae*	x	x
8,712	*avenae*	x	x
760	*avenae + filipjevi*	x	x
20,724	*avenae + filipjevi*	x	x
240	*filipjevi*	x	x
4,320	*filipjevi*	x	x
6,864	*filipjevi*	x	x

[1] Selected data from Smiley and Yan (2015).
[2] *Heterodera* spp./kg of soil) includes juveniles extracted from soil plus eggs + juveniles from cysts.
[3] Molecular identifications of species using species-specific PCR assays and PCR-RFLP procedures.

DISCUSSION

Wheat and barley cultivars differed in resistance and tolerance to invasion by four species of *Pratylenchus* and *Heterodera* that reduce small grain yields in the PNW. Similar observations have been made for non-cereal crops invaded by *P. neglectus* and *P. thornei* (Smiley *et al.* 2014). Crop management guidelines in the PNW are now specified according to the species of these genera present in individual fields. However, commercial diagnostic laboratories generally identified these nematodes only to the genus level, and our findings demonstrated important discrepancies when several labs were asked to specify the distribution of individual species of *Pratylenchus* that they were reporting. It became clear that more precise and more rapid methods must be developed and incorporated into these laboratory protocols before growers could be-

gin to utilize results of the management-related research we were conducting. Multiple DNA-based methods were therefore developed and optimized to identify and, in some cases, to enumerate the four most important species affecting crops in our region. Several of those new diagnostic methods are now provided as a readily accessible and affordable commercial service to agriculturalists in our region.

ACKNOWLEDGMENTS

Funding was from the Idaho Wheat Commission, Oregon Wheat Commission, Washington Wheat Commission, Idaho Agricultural Experiment Station, Oregon Agricultural Experiment Station, and USDA-Agricultural Research Service's Root Disease and Biological Control Unit at Pullman, WA. Technical assistance was provided primarily by J Gourlie, P Okubara, and A Skantar. We thank J Gourlie for reviewing this paper prior to submission.

REFERENCES

- Smiley RW (2009a) Root-lesion nematodes reduce yield of intolerant wheat and barley. *Agronomy Journal*, 101, 1322-1335.
- Smiley RW (2009b) Occurrence, distribution and control of *Heterodera avenae* and *H. filipjevi* in the western USA. In: 'Cereal Cyst Nematodes: Status, Research and Outlook.' (Eds. IT Riley, JM Nicol, AA Dababat) pp. 35-40. (CIMMYT: Ankara, Turkey).
- Smiley RW, Yan GP (2015) Discovery of *Heterodera filipjevi* in Washington and comparative virulence with *H. avenae* on wheat. *Plant Disease*, 99, 376-386.
- Smiley RW, Yan GP, Gourlie JA (2014) Selected Pacific Northwest crops as hosts of *Pratylenchus neglectus* and *P. thornei*. *Plant Disease*, 98, 1341-1348.
- Yan GP, Smiley RW (2010) Distinguishing *Heterodera filipjevi* and *H. avenae* using polymerase chain reaction-restriction fragment length polymorphism and cyst morphology. *Phytopathology*, 100, 216-224.
- Yan GP, Smiley RW, Okubara PA (2012) Detection and quantification of *Pratylenchus thornei* in DNA extracted from soil using real-time PCR. *Phytopathology*, 102, 14-22.
- Yan GP, Smiley RW, Okubara PA, Skantar A (2013a) Species-specific PCR assays for differentiating *Heterodera filipjevi* and *H. avenae*. *Plant Disease*, 97, 1611-1619.

- Yan GP, Smiley RW, Okubara PA, Skantar A, Easley SA, Sheedy JG, Thompson AL (2008) Detection and discrimination of *Pratylenchus neglectus* and *P. thornei* in DNA extracts from soil. *Plant Disease*, 93, 1480-1487.
- Yan GP, Smiley RW, Okubara PA, Skantar AM, Reardon CL (2013b) Developing a real-time PCR assay for detection and quantification of *Pratylenchus neglectus* in soil. *Plant Disease*, 97, 757-764.

AUTHOR INDEX

F

G

H

I

J

K

L

M

N

NEMATODES OF SMALL GRAIN CEREALS
CURRENT STATUS AND RESEARCH

Fifth International
Cereal Nematode Initiative Workshop

12-15 September 2015,
Ankara, Turkey

ABDELFATTAH A. DABABAT
HAFIZ MUMINJANOV
RICHARD W. SMILEY
Editors

Layout and design:
Madibaev Timour
(it@log.tj)

ISBN 978-92-5-108878-4

9 789251 088784

I4934E/1/09.15

CIMMYT MR
International Maize and Wheat Improvement Center

ICARDA
Science for Better Livelihoods in Dry Areas

GRDC
Grains Research & Development Corporation
Your GRDC working with you

syngenta

REPUBLIC OF TURKEY MINISTRY OF FOOD AGRICULTURE AND LIVESTOCK

DUPONT

serene

bisab
Plant Breeders Sub-Union of Turkey