





This proceeding is dedicated to



for the continuous support to the  
Soil Borne Diseases Program at CIMMYT, Turkey  
and, in particular, the  
International Cereal Nematodes Symposium series

The International Maize and Wheat Improvement Center, known by its Spanish acronym, CIMMYT, is a non-profit research and training organization with more than 400 partners in over 100 countries. The Center works to sustainably increase the productivity of maize and wheat systems and thus ensure global food security and reduce poverty. For this it applies the best of biotechnology, traditional agronomy and breeding, socio-economics, agricultural extension, and capacity building to create sustainable solutions with lasting impact and a strong focus on climate change, hunger, rural community development, and the environment.

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**Abstract:** The Sixth International Cereal Nematodes Symposium, held in Agadir, Morocco during 11-15 September 2017, is an update to the 1<sup>st</sup> International Cereal Cyst Nematodes Initiative Workshop held in 2009 in Antalya, Turkey. The 6<sup>th</sup> International Cereal Nematodes Symposium involved more than 80 scientists from wheat and barley producing regions in 16 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 five abstracts in this proceeding 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.

**Image on Cover:** Newly produced cysts of *Heterodera filipjevi*  
Courtesy of Drs. Shree Pariyar and Abdelfattah Dababat

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



**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**



**Fifth International Cereal Nematodes Initiative Workshop  
12-16 September 2015, Ankara, Turkey (UTEM)**

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## Foreword

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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<sup>1</sup>. 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 to 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 parasites. Three decades ago, it was estimated<sup>2</sup> 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<sup>3</sup> indicates yield losses equivalent to about 10% of global production.

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<sup>1</sup>Alexandratos, N., and 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*.

<sup>2</sup>Sasser, J.N., and Freckman, D.W. (1987) A world perspective of nematology: The role of the Society. In. 'Vistas on Nematology' (Eds. J.A. Veech and D.W. Dickson). pp. 7-14. Society of Nematology. Hyattsville, Maryland.

<sup>3</sup>Dixon J., Braun, H.J., Kosina, P., and Crouch, J. (Eds.). 'Wheat Facts and Future 2009.' (CIMMYT, Mexico)

<sup>4</sup>Smiley, R.W., and Nicol, J.N. (2009) Nematodes which challenge global wheat production. In. 'Wheat: Science and Trade.' (Ed. B.F. Carver). pp. 171-187. (Wiley-Blackwell: Ames, Iowa).

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).<sup>4</sup> 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. 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 symposium 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, included 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. Richard W. Smiley**

Professor Emeritus, Oregon State University, Pendleton, Oregon

**Dr. Abdelfattah A. Dababat**

CIMMYT Wheat Improvement Program, Ankara, Turkey

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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), The Food and Agriculture Organization (FAO) of the United Nations, DuPont, The Plant Breeders Sub-Union of Turkey (BISAB), INRA Morocco, Labomine, and Agrifutur. All are gratefully acknowledged for their support.

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### **Dr. Abdelfattah A. Dababat**

CIMMYT Wheat Improvement Program, Ankara, Turkey



**CIMMYT** (International Maize and Wheat Improvement Centre) and **ICARDA** (International Centre for Agricultural Research in Dryland Agriculture) are non-profit International Research Centres with the mandate to improve agriculture in developing countries as part of the Consultative Group of International Agriculture Research (CGIAR). CIMMYT's mandate is to improve the productivity of wheat and maize systems through sustainable management and improvement. The Turkey office works in West Asia, Central Asia, and North Africa. ICARDA programs are currently coordinated from Lebanon, with technical facilities being coordinated from Jordan. ICARDA works regionally in dryland areas on cereal, legume and animal production systems, once again improving productivity of these through sound management practices. ICWIP (ICARDA-CIMMYT Wheat Improvement Program) is the collective effort of both centres to address food security for cereals in West Asia, Central Asia, and North Africa. CIMMYT and ICARDA are gratefully acknowledged for scientific leadership in research on cereal nematodes, technical input, international capacity building and financial support towards this workshop. For further information see [www.cimmyt.org](http://www.cimmyt.org) and [www.icarda.org](http://www.icarda.org).



**INRA**, Morocco is a century-old, public organization mandated to undertake research for Agricultural development in Morocco. INRA has greatly contributed to the modernization of the agricultural sector and agro-systems, and to the improvement of the competitiveness of the country's agriculture. Moreover, INRA is a member of several regional networks and maintains bilateral cooperation with several countries. For more information see <http://www.inra.org.ma>.



**LABOMINE** (Laboratory of Mining and Agricultural Analyzes), is a leading player in this field. This laboratory is an independant, privately owned company specialized in agronomy analysis including soil, water, plant and nematode analyzes and pesticide residues in order to optimize crop profitability while respecting the environment. The lab contains modern certified technical equipments guaranteeing the quality and reliability of the results and a multidisciplinary, qualified and well trained staff. The main objectives of Labomine are to a) help farmers make effective use of fertilizer to increase their financial returns, b) benefit society with improved environment and water quality through efficient use of fertilizer on farms, lawns and gardens, c) maintain a soil analysis data base for research that will improve soil testing and fertilizer application, and d) characterization and quantification of different plant parasitic nematodes associated with different crops in all Moroccan regions in order to develop good strategies for controlling the main plant parasitic nematodes.



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The Australian **Grains Research and Development Corporation** (GRDC) is one of the world's leading grains research organisations. GRDC's primary objective is to drive the discovery, development and delivery of world-class innovation to enhance the productivity, profitability and sustainability of Australian grain growers and benefit the industry and the wider community. The GRDC is funded primarily by grower levies and Australian Government contributions. Levies are collected for production of 25 crops, including small grain cereals. GRDC is thanked for their financial support to the Soil Borne Pathogens Program at CIMMYT-Turkey. For further information see [www.grdc.com.au](http://www.grdc.com.au).

**Syngenta** is one of the world's leading companies with the goal of improving crop productivity in nearly 100 countries. Through world-class science, global reach and commitment to our customers we help to increase crop productivity, protect the environment and improve health and quality of life. Thanks are given for their support towards capacity building and being a key sponsor to this workshop. For further information see <http://www.syngenta.com>

The Republic of Turkey's **Ministry of Food, Agriculture and Livestock** (MFAL) is responsible for food safety, veterinary and phytosanitary policy development, and enforcement activities for food and feed safety, animal health, animal welfare and plant health in Turkey. MFAL is responsible coordinating agricultural research and development projects in cooperation with the General Directorate of Agricultural Research and Policy (GDAR). The GDAR is the center of the national agricultural research system in Turkey, with 49 research stations located to best serve the needs of citizens throughout the Republic. GDAR coordinates research on plant breeding and production, plant protection, animal breeding and husbandry, animal health, fishery and aquaculture, food and feed, postharvest technologies, biodiversity/genetic resources, organic agriculture, bio-safety, and soil and water resource management. Thanks is given to MFAL for hosting and financially supporting this workshop. For further information see [www.tagem.gov.tr](http://www.tagem.gov.tr).



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The **Plant Breeders Sub-Union of Turkey (BISAB)** is active in Turkey at the national level. It represents common interests of its 165 members, which includes individuals or private sector companies that are involved in crop breeding and agricultural research. BISAB has been the voice of the professional interests of Turkish plant breeders for nearly six years since being founded in 2008. BISAB provides valuable services to Turkey's agricultural sector with successful ventures and services. BISAB meets the needs of the internal market with the work in the seed sector that has experienced intense competition. Likewise, the plant breeders have opened foreign markets by developing high-quality products and other important contributions to the seed sector, thereby reducing dependence on foreign companies. They are thanked for being a sponsor of this workshop and for enriching the capacity building in the region. For more information see <http://en.bisab.org.tr>.



The **Food and Agriculture Organization (FAO)** of the United Nations is an intergovernmental organization of 194 member nations plus two associate members and the European Union. FAO has headquarters in Rome, Italy and has staff in over 130 countries. The main goals of FAO are to eradicate hunger, food insecurity and malnutrition, to eliminate poverty and improve economic and social progress for all, and to foster sustainable management and utilization of natural resources (land, water, air, climate and genetic) for the benefit of present and future generations. FAO has endorsed the Rome Declaration on Nutrition and the Framework of Action, which enshrines the right of everyone to have access to safe, sufficient and nutritious food, and commits governments to prevent malnutrition in all its forms. FAO is support in capacity building throughout the world and particularly for printing this book. For further information see [www.fao.org](http://www.fao.org).

### **Disclaimer**

This volume contains abstracts voluntarily contributed by participants of the Sixth International Cereal Nematodes Symposium, 11-15 September 2017, Agadir, Morocco.

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## Program: Sixth International Cereal Nematodes Symposium

11-15 September 2017

Agadir, Morocco

### 11 September 2017 - Monday

16:00 – 18:00 Registration of participants

### 12 September 2017 - Tuesday

Opening by:

09:00 – 09:30 CIMMYT  
Turkish Ministry of Food, Agriculture and Livestock  
INRA - Morocco  
ICARDA

### Session 1: Distribution of cereal nematodes: Global status

Chairs: Richard Smiley and Abdelfattah Dababat

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09:30 – 10:00	Wheat Production in a Changing Environment: The Impact of Nematode Parasitism on Root Architecture and Implications for Root Health Management	Richard SIKORA	GERMANY	2
10:00 – 10:30	Economic Importance, Population Dynamics and Control of <i>Pratylenchus thornei</i> in Wheat Crops in Southern Australia	Grant HOLLAWAY	AUSTRALIA	3
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10:50 – 11:05	Occurrence and Distribution of Wheat Seed Gall Nematode, [ <i>Anguina tritici</i> (Steinbuch) Filipjev] in Wheat ( <i>Triticum aestivum</i> L.) Growing Areas in Thrace Region of Turkey.	Adnan TULEK	TURKEY	4
11:05 – 11:20	Long Term Research on the Root Lesion Nematodes ( <i>Pratylenchus neglectus</i> , <i>P. thornei</i> ) and Cereal Cyst Nematodes ( <i>Heterodera avenae</i> , <i>H. filipjevi</i> , <i>H. latipons</i> ) in Turkey	Halil ELEKCIOĞLU	TURKEY	5
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11:35 – 11:50	Current Status of the Cereal Cyst Nematodes in Morocco	Fouad MOKRINI	MOROCCO	7
11:50 – 12:05	Surveying Wheat Growing Area in Kazakhstan for Plant Parasitic Nematodes with a Main Focus on the Cereal Cyst and Root Lesion Nematodes	Nurgul AMANGELDI	KAZAKHSTAN	8
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12:30 – 13:30	<b>Lunch-break</b>			
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Chair: Fouad Mokrini				
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Chairs: Deliang Peng and Halil Elekcioglu

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### Session 3: Management strategies using host resistance (*continued*)

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Chairs: Tesfamariam Mengistu and Henricka Fouri

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Chairs: Richard Sikora and Grant Hollaway

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Chairs: Timothy Paulitz and Luma Al Banna

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<b>16:45 – 17:00</b>	Prospects of Transgenic Research for Cereal Nematode Resistance	Muhammad Amjad ALI	PAKISTAN	55
<b>17:00 – 17:15</b>	RNAi Silencing of Different Functional Classes of Genes in <i>Heterodera avenae</i> Singly and in Combination	UMARAO	INDIA	56
<b>17:15-17:30</b>	From Genes to Biological Control: A High-Throughput Sequencing Approach to Identifying Potential Nematode Suppressive-Soil Microbial Communities	Tesfa Mengistu	USA	57
<b>17:30 – 17:50</b>	<b>Discussion and symposium closing</b>			
<b>19:30 – 22:00</b>	<b>Gala dinner: "A typically Moroccan evening in Chems Ayour"</b>			

## 14 September 2017 - Thursday

<b>08:00 – 9:30</b>	<b>Visit to the nematology laboratory (Labomine)</b>
<b>09:30 – 17:00</b>	<b>A sightseeing tour to the historical wonders around Taroudant city (visit Tiout)</b>



# **Session 1**

## **Distribution of Cereal Nematodes: Global Status**

**Wheat Production in a Changing Environment: The Impact of Nematode Parasitism on Root Architecture and Implications for Root Health Management**

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Sustainable production of rainfed crops exposed to moisture stress, especially those growing under arid and semi-arid conditions, are being impacted by climate change induced hotter and drier soils. In some regions soil temperatures will rise to levels that prevent production of important crops such as maize. The cereal nematodes - root rotting complex is underestimated as a serious yield limiting factor. Breeders of course need to stress yield and resistance to the more obvious pests and diseases of the crop. Currently, climate change has shifted stress toward research on water use-efficiency. It is imperative to recognize that a plant's ability to secure adequate amounts of water is severely impacted by the destabilizing effects of nematodes and root rotting fungi on root architecture. Integrated crop health management approaches, using both modern cultivars with resistance/tolerance to these organisms, as well as, technologies that simulate root health and growth coupled with modern nematode management strategies are needed for sustainable production in the ever drier environments that are now a reality in many areas of the world.

Tap root deformation (root-knot and ectoparasites), induction of shallow root architecture due to abnormal stunting and branching (cereal cyst nematodes), and overall root biomass degradation (root lesion nematodes and fungal pathogens) reduces translocation of moisture and nutrients to the root and stem and influences grain formation and yield. Bio-assays that incorporate root growth factors such as root architecture, biomass, and degree and speed of root depth penetration need to be part of nematology research programs designed to screen for resistance to pests and for moisture use-efficiency. Testing without exposure to the natural limiting factors that influence root architecture will prove inadequate.

Root architecture scanning equipment and software as well as appropriate bio-assays are in some cases available or need to be refined for greenhouse and field investigations. Agronomic approaches for improved root growth exist and need to be tested on nematode infested soils under moisture stress conditions with improved cultivars for root health impact. These tools include seed treatments that enhance emergence and vigor and improve root biomass and depth penetration through plant growth and health promoting microorganisms and agrochemicals. Data will be presented showing climatic trends and soil temperature shifts, effects of seed care technologies on root architecture and plant health, and technologies for their study.

Increased research funding is required for a more holistic approach to plant health management. Nematologists, breeders and agronomists need to work together to find a solution to the complex issues facing agricultural production and to use multidisciplinary approaches to move forward in insuring food security for all.

**Economic Importance, Population Dynamics and Control of *Pratylenchus thornei* in Wheat Crops in Southern Australia**

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In south eastern Auspariyartralia, the root lesion nematode (RLN), *Pratylenchus thornei* is one of several important soil borne pathogens of wheat. These nematodes are widespread, and the losses they cause are related to their density in the soil, the tolerance of the wheat cultivar grown and seasonal conditions. Although losses in this region are generally less than 15%, and often go unrecognised, their widespread distribution means that across the region their economic loss to Victorian wheat production can be up to \$6.3AUD million per annum. Australian farmers use a pre-sowing DNA based diagnostic test (PreDicta B) to detect fields with yield limiting densities of *P. thornei*. Field screening of both cereal and pulse crops for resistance/susceptibility has identified rotational options for growers to minimise *P. thornei* density in their fields, and therefore yield loss to subsequent crops. A META analysis of field resistance screening data demonstrated that varietal resistance/susceptibility rankings were highly correlated across sites and seasons. Nematode multiplication was influenced by initial nematode density, with multiplication reducing as initial nematode densities increased.

**Occurrence and Distribution of Wheat Seed Gall Nematode, [*Anguina tritici* (Steinbuch) Filipjev] in Wheat (*Triticum aestivum* L.) Growing Areas in Thrace Region of Turkey.**

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Wheat is an important crop for human nutrition in the world, and is grown on 218.4 million hectares. Wheat is also one of the major and strategic crops in Turkey in terms of economy, nutrition and employment. Wheat in Turkey is grown on 7.8 million hectares, with a total production of 22 million metric tons. Wheat seed gall nematode, *Anguina tritici*, is considered an economically important pest of wheat in substandard agricultural conditions in some parts of the Thrace region of Turkey, where it is found in limited fields. In this study, seed samples were collected from farmers' storages in 2015 for determining the wheat gall nematode occurrence and distribution across wheat fields in Thrace Region provinces, which are on the European side of Turkey. Evaluation was done with 685 wheat seed samples from Edirne, Kırklareli and Tekirdağ provinces, which have together a share of 5.6% in wheat production areas and 7.2% in total production amount. A total of 507 seed samples were from Edirne province, 156 samples were from Kırklareli province, and 21 samples were from Tekirdağ province. Seed gall nematode was found to contaminate 13 samples; with 10, 3, and 0 samples being found to be contaminated with wheat gall nematode from Edirne, Kırklareli and Tekirdağ provinces, respectively. Wheat gall nematodes were present in fields of the farmers who do not utilize certified seed and modern seed cleaning techniques.

**Long Term Research on the Root Lesion Nematodes (*Pratylenchus neglectus*, *P. thornei*) and Cereal Cyst Nematodes (*Heterodera avenae*, *H. filipjevi*, *H. latipons*) in Turkey**

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The cereal cyst nematodes (CCN) (*Heterodera* spp.) and root lesion nematodes (RLN) (*Pratylenchus* spp.) are widely distributed parasites to cause serious economic damage in cereal crops worldwide. The first comprehensive research on cereal nematodes in Turkey was started in 1993, establishing the distribution of nematodes in cereal growing areas in both East Mediterranean region and Central Anatolia. Until now, almost all cereal growing areas were surveyed to identify the occurrence and distribution of CCN and RLN. Three important species of CCN (*Heterodera avenae*, *H. latipons* and *H. filipjevi*) and two important RLN species (*Pratylenchus neglectus* and *P. thornei*) have been found. *Heterodera filipjevi* is widespread and more suited to continental environments, such as in the Central Anatolia, Northwest Anatolia and East Anatolia regions, whereas *H. avenae* and *H. latipons* are found in the East Mediterranean, Southeast Anatolia, and West Anatolia regions. Some biological parameters such as hatching, pathotype of CCN, optimizing of methods for both CCN and RLN studies, screening of wheat varieties and international germplasm to find resistance and to identify new sources of resistance. Yield loss studies were done in Central Anatolia and Southeast Anatolia conditions. Under in-vitro conditions, highest hatching percentages of *H. filipjevi* (94%, 92% and 75%) were obtained at temperatures of 15°C, 10°C and 5°C, respectively. The highest cumulative hatching of *H. avenae* (82%) was obtained at a constant temperature of 10°C for 252 days, and the lowest cumulative hatching (19%) was obtained at 25°C for 252 days. The average yield loss was 20% - 47% in Central Anatolia where *H. filipjevi*, *Pratylenchus neglectus* and *P. thornei* were found. Yield losses caused by *H. avenae* varied between 4% and 26% depending on wheat varieties in the South East Anatolia conditions. More than 2,000 national and international winter and spring wheat lines and cultivars from Turkish National and International (CIMMYT and TCI (TURKEY/ CIMMYT/ICARDA) and other International) were screened in order to determine their resistance against *H. filipjevi*, *H. avenae*, *H. latipons*, *P. neglectus* and *P. thornei*.

**Global Importance of the Root-Knot Nematodes (*Meloidogyne* Spp.) Associated with Cereals**

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Root-knot nematodes (*Meloidogyne* spp.) are obligate plant parasites that are distributed throughout the world and are considered as the most important genus of plant-parasitic nematodes. Although 98 *Meloidogyne* species are identified to date, *M. incognita*, *M. javanica*, *M. arenaria*, *M. chitwoodi*, *M. fallax* and *M. hapla* are the most widely distributed species and account for more than 95% of the occurrences of this genus. *Meloidogyne incognita*, *M. javanica* and *M. arenaria* are the most prevalent species in warmer climates, while *M. hapla*, *M. naasi*, *M. chitwoodi* and *M. fallax* exist in cooler conditions. Up to now, studies on root-knot nematodes have remained behind that of cyst nematodes and root lesion nematodes in cereals. Cereals are the main plants in rotation systems used for root-knot nematode management in the vegetable and potato-producing areas in many countries. *Meloidogyne artiellia*, *M. chitwoodi* and *M. naasi* are the most devastating root-knot species in cereals in cool climates and *M. microtyla* and *M. ottersoni* are the other root-knot nematode species attacking cereals in cool climates. *Meloidogyne graminis*, *M. kikuyensis* and *M. spartinae* are important pests in warm climates for Poaceae. *Meloidogyne incognita*, *M. javanica* and *M. arenaria*, the most damaging and widespread nematode in the whole world, also attack cereal crops. Resistance has been determined in some cereals for some of these tropical root-knot species and races. Temperature is the most important limiting factor damaging the tropical species in cereals. In addition, these species have more potential to incur damage on cereals as a result of global warming. Root-knot nematodes are parasites of almost every species of vascular plant and this situation makes them difficult to be controlled by crop rotation. Nematicides and soil fumigants are effective control methods for root-knot nematodes, but their use in cereals is extremely limited due to high cost. Therefore, the most effective control measure for *Meloidogyne* spp. in cereals is the use of resistant cultivars. We recommend screening of wheat genotypes in terms of root-knot nematodes at the Soil Borne Diseases Program at CIMMYT-Turkey.

**Current Status of the Cereal Cyst Nematodes in Morocco**

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The occurrence and distribution of cereal cyst nematodes (CCN) have been recently surveyed in Morocco by a research project supported by the Islamic Development Bank in collaboration with Ghent University and the Institute for Agricultural and Fisheries Research (ILVO). All CCN species were identified on the basis of their morphology and morphometrics, complemented by ITS-rDNA sequences. The CCN represent two species, viz. *Heterodera avenae* and *H. latipons*. *Heterodera avenae* was the most prevalent species, occurring in 13% of the fields, and was associated with wheat in the three regions where this nematode was found. *Heterodera latipons* was detected in one sample originating from Ain Jmaa (Saiss) and is herewith reported for the first time in Morocco. Although, *H. avenae* is widely distributed in the three regions studied, there is important variation in incidence between different regions. The lowest (15%) incidences were found in Saiss and Zaer, whereas the highest (20%) frequencies were detected in Chaouia. The population densities of CCN in soil samples ranged from 6 to 155 cysts/100 g soil. The highest levels of infestation were found in Marchouch; 155 cysts/100 g soil. In view of the estimates of nematode densities obtained in this survey, one can assume that these nematode probably damage cereals in many cases. Field studies on the population dynamics and the damage function are necessary to estimate the economic impact of these nematodes on cereals. Further investigations are necessary to identify the pathotype of Moroccan CCN populations. International collaboration, mainly with CIMMYT, is needed to reinforce the research of resistant genotypes against CCN and RLN.

**Surveying Wheat Growing Area in Kazakhstan for Plant Parasitic Nematodes with a Main Focus on the Cereal Cyst and Root Lesion Nematodes**

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Kazakhstan is an important producer and exporter of high-quality wheat. The average annual production is about 13 million tons, but output is highly dependent on weather and in recent years has fluctuated between 10 and 17 million tons. Between 2 and 8 million tons is exported annually, mainly to destinations in Europe (including Russia and Ukraine), northern Africa, and Central Asia. Accordingly, the greatest attention in Kazakhstan (as well as throughout the country) was paid to the study of nematode fauna of the most important crops (cereals, vegetables, fruits, etc.) (Z.A. Balbaeva, 1967, 1972, 1981; E.N. Kuanshalieva, 1963, 1972; A.A. Razzhivin, 1969; M.T. Sofrygin, 1973; L.A. Vladimirova, 1975; R.I. Isatulyeva, 1967, 1981, 1987; D.S. Chinasilov et al., 1987). The main purpose of such studies was to maximize the knowledge of species diversity of nematodes associated with specific agricultural crops, mainly on a qualitative basis. The detection of the presence of harmful phytoparasitic species in this case was secondary and disordered. For example, as a result of long-term studies of the nematode fauna of cereal crops, 62 species belonging to 14 families and 4 orders were identified. Phytoparasitic species among them were only 7 species from the family of hopllyaimids, and cystogenic and root nematodes were not detected (Z.A. Balbaeva 1967, 1979). In the Kustanai region, 116 species of nematodes related to 49 genera, 25 families, and 5 orders were detected on wheat, but only 24 phytohelminths without information on their harmfulness and prevalence (A.Z. Balbaeva, D.S. Chinasilov, 1981). Therefore, at the time, the most harmful pathogen is cereal cyst and root lesion nematode, which have been documented to cause economic yield loss on rainfed wheat production systems in several part of the world. In 2017 a survey was carried out in the main wheat growing areas (South Kazakhstan – Almaty region, East Kazakhstan – Ural, North Kazakhstan – province Shortandy) and a total of 230 soil samples were collected. Preliminary results showed that cyst nematodes were extracted from 24 of the 42 samples collected from province of Shortandy. Performing microscopic identification of intercepted nematodes, the following species of plant parasitic nematodes were identified – *Aphelenchus spp.*, *Aphelenchoides spp.*, *Tylenchus spp.*, *Filenchus spp.*, *Pratylenchus spp.*, *Parapratylenchus spp.*, *Ditylenchus spp.* from Ural province; as well as *Aphelenchus spp.*, *Aphelenchoides spp.*, *Tylenchus spp.*, *Filenchus spp.*, *Pratylenchus spp.*, *Parapratylenchus spp.*, *Ditylenchus spp.*, *Merlinius spp.* from Almaty province. The results of this study of 230 samples will be presented more thoroughly at the 6th International Cereal Nematodes Symposium.

**Infestation of Cereal Plots by the Cyst Nematode (*Heterodera* spp.) in Western Algeria**

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The current state of infestations and the distribution of species of *Heterodera* in western Algeria has been the subject of this study. The nematode analysis of 1787 samples representing 156 communities and 11 willayas showed that this nematode genus was present in 53% of the samples. The frequency of infestation varied between 48 and 51%, depending on the region. The infestations were caused by five species: *Heterodera avenae*, *H. latipons*, *H. hordecalis*, *H. mani*, and *H. filipjevi*. These species were found either in pure or mixed populations. *Heterodera avenae* was the dominant species in only 50% of surveys, and was supplemented by *H. latipons* in 34% of the localities and 34% of the samples. *Heterodera mani*, sometimes declassified by *H. filipjevi* or *H. hordecalis* in certain localities, is often the third most common species; 7% of localities and in 34 (4%) of samples. *Heterodera hordecalis*, a species especially on barley, was fourth most common; reported in 6% of localities and in 35 (4%) of samples. *Heterodera filipjevi*, a species very close to *H. avenae*, appears to be important in some localities; 2% of localities, and in 19 samples (3%) of samples.



## **Session 2**

### **Economic Importance and Population Dynamics**

**Distribution of cereal cyst nematodes (*Heterodera* spp.) and root lesion nematodes (*Pratylenchus* spp.) in wheat fields in Tadla region of Morocco**

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The distribution of cereal cyst nematodes (CCN) and root lesion nematodes (RLN) in the Tadla region of Morocco was investigated by systematic surveys of cereal fields conducted in March 2017. Cysts were extracted from soil using flotation and decanting through 200µm sieves. Vermiform stages were extracted from soil using Baermann funnel method. Nematodes were identified up to species level using the morphological method. Cereal cyst nematodes were found only in 10% of soil samples and root lesion nematodes in 80% of soil samples. Cereal cyst nematodes were identified as *Heterodera avenae*. Population densities of CCN in soil samples were 2 cysts/200 g soil, possibly less than the critical threshold for damage. The root lesion nematodes, *Pratylenchus thornei* and *P. penetrans* were the most widely distributed species of root lesion nematode in the surveyed area. Densities of mobile stages of RLN in wheat fields ranged from 15 to 35/100 g of soil.

**Occurrence and Distribution of Root Lesion Nematodes (*Pratylenchus* Spp.) on Wheat in Jordan**

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A survey was conducted in spring 2016 to determine the distribution of root lesion nematodes (RLN) in major wheat growing areas in Jordan. A total of 25, 16, 5, and 4 fields were sampled from Northern Mediterranean, Southern Mediterranean, Jordan Valley and Eastern Desert, respectively. RLN were recovered from roots of wheat plants in 38 locations comprising 76% of the surveyed fields. Of these 38 fields, RLN were recovered from 18, 15 and 5 locations in Northern Mediterranean, Southern Mediterranean and Jordan Valley phytogeographical regions, respectively. No RLN were recovered from Eastern Desert wheat fields. Morphological and molecular characterization revealed that populations of RLN *P. thornei* (Sher & Allen, 1953) were found in most surveyed locations. RLN belonging to *P. neglectus* (Rensch, 1924) were recovered from one location and this is the first report of the occurrence of this species on wheat in Jordan. Such findings of the occurrence and distribution of RLN on wheat in Jordan is considered the first step for further epidemiological studies to understand the impact of such a nematode on wheat in Jordan. Once the epidemiology of this pest is fully studied, management strategies will be set and control methods can be employed.



## **Session 3**

### **Management Strategies Using Host Resistance**

**International Winter Wheat Improvement Program: Diversifying Genetic Basis for Resistance to Abiotic and Biotic Stresses**

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The International Winter Wheat Improvement Program ([www.iwwip.org](http://www.iwwip.org)) is a partnership between the 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 high priority. Annually, 800-1000 crosses are made which are subjected to a 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, more than 70 varieties were released in the region occupying more than two million hectares. Diversification of the genetic basis of resistance to abiotic (drought and heat) and biotic stresses represents an important objective. Three groups of germplasm have been utilized in the crossing program: modern germplasm; landraces from Turkey, and primary hexaploidy synthetics. All of them contribute to enhancement of resistance though their utilization methodology is slightly different. Cereal cyst nematodes (CCN) represent high priority for IWWIP breeding, especially for rainfed conditions. Several thousands of genotypes have been screened and more than 200 CCN resistant entries have been identified and evaluated for a number of traits. Most of them are higher yielding an exhibit 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 F2 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.

**Phenotypic Analysis of F<sub>4</sub> Wheat Families for Resistance to Cereal Cyst Nematode**

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Wheat cultivars grown in Saudi Arabia today are susceptible to *Heterodera avenae* (CCN). Therefore, a breeding program has been initiated in the College of Food and Agricultural Sciences to determine the genetic nature of wheat resistance to CCN, as well as to characterize and map the quantitative trait loci (QTLs) linked to CCN resistance genes using SSR markers. The mapping population was developed from the cross between two local bread wheat genotypes, *Triticum aestivum* L., namely; KSU 118 (the susceptible parent) and LNM-136 (the resistant parent). Phenotyping was conducted on naturally-infested soil collected from wheat fields at Hail region. The parents and 160 F<sub>4</sub> families were evaluated phenotypically for CCN resistance in an outdoor pot experiment during the wheat growing season 2013/2014. The results of CCN score revealed that the frequency distribution of parental parents and F<sub>4</sub> families was found to be continuous and slightly skewed towards resistance. The number of F<sub>4</sub> families with resistance and susceptible CCN score were 122 and 38, respectively. This observed ratio was tested using chi square test and was found to fit the theoretical expected ratio, 3:1 ( $\chi^2 = 1.63$ ). This result suggested that a single locus was controlling this trait, and a single dominant gene for resistance to CCN was present in the LNM-136 × KSU 118 population.

**Influence of Infestation with the Cereal Cyst Nematode *Heterodera avenae* on the Resistance of Wheat Lines to Root Lesion Nematodes (*Pratylenchus penetrans* and *P. thornei*)**

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The root lesion nematodes *Pratylenchus penetrans* and *P. thornei* cause high yield losses in rainfed wheat fields in Morocco, as well as worldwide. Growing resistant varieties is one of the most effective methods for controlling nematodes. Therefore, a collection of 14 lines of spring wheat and 11 lines of winter wheat (*Triticum aestivum* and *T. durum*), developed at CIMMYT, were screened for resistance to *P. penetrans* and *P. thornei* in tubes (15 x 20 x 120 mm<sup>3</sup>) under greenhouse conditions. The resistance level was evaluated based on the numbers of nematodes extracted from roots and soil nine weeks after infestation. The number of *P. penetrans* or *P. thornei* were determined visually using a microscope and with a qPCR assay. Three lines (L9, L12 and L13) were found moderately resistant to *P. thornei* and one of these (L9) was also moderately 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*) on the three moderately resistant lines. Our results showed that the lines L9 and L9, L12, L13 remained moderately resistant to *P. penetrans* and *P. thornei*, respectively, in the presence of *H. avenae*. These findings are promising; however, the field performance of these lines against root lesion nematode attacks should be evaluated.

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**NEMARES: Importance of Root Lesion Nematodes in German Crop Production and Strategies to Breed Resistant Varieties**

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Severe damage in German cereal production has been reported as being caused by root lesion nematodes (RLN) and it is regarded by farming experts as a major threat for cereal production. Narrow crop rotation, early-sowing dates, and mild winters which are expected due to climate change increase the damage by this pest. Since the assessment of RLN in the field as well as in the greenhouse is cumbersome and time consuming, this pest has been largely disregarded by European cereal breeders so far. Aiming at the identification of wheat (*Triticum aestivum*) lines with low RLN infection rates and unravelling the genetic mechanisms for RLN resistance in barley (*Hordeum vulgare*) and wheat, we are continuing a previous project in which resistant barley lines have been identified and major QTLs for RLN resistance have been mapped. To identify RLN QTL(s) present in European wheat material, a panel of 193 lines is being characterized for their resistance level against *Pratylenchus neglectus* and *P. penetrans*. To overcome the bottlenecks of the phenotyping tests, we are developing a qPCR based quantification method. For association analysis, genotypic data from 15k, 20k, or 90k Chip-arrays is available. We will estimate the effect of RLN resistance on yield and other agronomical important parameters by performing field trials in highly infested soils using 20 selected wheat lines varying according to their resistance level to RLN. In a final step, we will compare resistance bearing regions from barley and wheat exploiting the high conservation of gene order and sequences between both species.

**Assessment of Crop Loss Caused by *Heterodera filipjevi* and the Relationship of Initial Population Density and Grain Yield in Wheat Cultivars Under Field Conditions in Iran**

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The cereal cyst nematode, *Heterodera filipjevi*, is the dominant species of CCN in most cereal growing areas of Iran and is distributed in different regions of the country. The experiments were conducted in two fields infested with *H. filipjevi* in Isfahan province, Iran during the wheat growing season in 2014-15. The trials were conducted in a factorial experiment in a complete randomized block design with six treatments including three winter wheat cultivars (viz, Back-cross Rowshan, Pishtaz and Parsi, the most commonly grown cultivars in Isfahan province) planted with and without the nematicide Aldicarb 10G (Temik 10G). Each treatment consisted of 6 m<sup>2</sup> (2×3 m) plots which were replicated five times. Seven irrigations were done for all plots using the sprinkler irrigation method. The initial and final nematode population of *H. filipjevi* in each plot was determined before sowing the seeds and after harvesting, respectively. The nematode reproduction factor was calculated. The grain yields were recorded and the variables of two years experiment evaluated by linear regression analysis. Comparison the mean of cultivars × nematicide treatment combinations in the first and second years showed that *H. filipjevi* significantly affected grain yield in all three studied cultivars. The results showed significant reduction of grain yield by 25%, 25% and 20% in Back-cross Rowshan, Pishtaz, and Parsi cultivars, respectively. The nematode reproduction factor ranged from 0.32 to 1.76 in plots with or without nematicide application, respectively. The analysis showed a linear inverse relationship between the initial population ( $P_i$ ) and the yield of three wheat cultivars in check plots without nematicide application; the grain yield was decreased by increasing the  $P_i$ .

**Phenotype and Cellular Response of Wheat Lines Carrying *Cre* Genes to Pathotype Ha91 of *Heterodera avenae***

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Cereal cyst nematode (CCN, *Heterodera avenae*), a major limitation of wheat production worldwide, is widespread in most wheat-growing regions in China. Therefore, screening and characterization of resistant (R) wheat sources against *H. avenae* are very important. In this study, we screened 51 wheat lines collected from the International Maize and Wheat Improvement Center (CIMMYT). The 51 lines carried different *Cre* genes (*Cre1*, *Cre2*, *Cre3*, *Cre5*, *Cre7*, *Cre8*, *CreR* and *Pt*). For *H. avenae* pathotype Ha91, we identified one immune (M) cultivar, with no females produced, and five resistant cultivars, with the number of females under 5. The *Cre3* gene conferred the most effective resistance against *H. avenae* pathotype Ha91 in both field and greenhouse assays. Conversely, the *Cre1* and *CreR* genes conferred the lowest effective resistance. Using Pluronic F-127 gel and a staining assay, juvenile nematodes invading wheat roots were observed, and nematode development was analyzed. Compared to R and M roots, those of the susceptible (S) wheat cultivar Wenmai19 were more attractive to *H. avenae* second-stage juveniles (J2s). We observed the retardation of nematode development in R cultivars and tiny white female cysts protruding from the R cultivar VP1620. Nematodes in M roots either disintegrated or remained J2s or third-stage juveniles (J3s) and failed to complete their life cycle. Molting was also suppressed or delayed in R and M genotypes. For both S and R cultivars, syncytia were characterized by cell wall perforations and dense cytoplasm in hypertrophied syncytium component cells. Syncytial size increased gradually with nematode development in S cultivars. Moreover, an incompatibility reaction occurred in M wheat roots: the syncytium was disorganized, exhibiting disintegration and condensed nuclei. These sources of genetic resistance against CCN can potentially be planted in severely infested fields to reduce economic loss or can be used for introgression in breeding.

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**Sources of Resistance to *Heterodera filipjevi* in Irrigated and Rainfed Iranian Wheat Cultivars**

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Resistance in 30 irrigated and 13 rainfed wheat cultivars (*Triticum aestivum* L.) recommended for cultivation in Iran was screened against the cereal cyst nematode *Heterodera filipjevi* in greenhouse and field conditions. The number of cysts, second stage juveniles, and eggs were analyzed. Also, reproductive factors and percentage reductions and/or increases in nematode populations were taken into consideration. Statistical and clustered analysis data were carried out by Duncan's multiple range test (DMRT), SAS, and SPSS statistical softwares, respectively. The genetic variability by SSR marker, genomic sequencing and gene expression were also run on the related cultivars. The results showed that, in the greenhouse, the lowest milky cyst production was in Heydari cultivar, with 4 cysts per plant. The lowest numbers of CCN eggs, second stage juveniles, and reproductive factors were in the Pyshtaz cultivar, with the average of 0.49 per g of soil. The cultivars were classified into three ranges; resistant, tolerant and susceptible. In the field, the lowest number of brown cysts were for the Bam cultivar, with 113 per 200 cc of soil. Whereas, the lowest numbers of CCN eggs and second stage juveniles and reproductive factors were in the Parsi cultivar, with 0.69 per g of soil, followed by the Aflak cultivar, with 1.69 in two different statistically groups. Also, cluster analysis classified the cultivars into three different categories, which also showed remarkable similarities to Duncan's test results. The results on genetic variability by SSR marker, classified the genotypes into resistant ranges accordingly. Also, genomic sequencing and gene expression indicated significant results on the related genotypes in terms of resistant and or susceptible genotypes.

**Evaluating Spring Wheat Cultivars' Resistance to *Heterodera avenae* in Southeastern Idaho, USA**

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Over 140 years have passed since Kühn made the first record of *Heterodera avenae*. Hundreds of studies have been published so far, but nematologists are still struggling with identification, systematics, biology and control of cereal cyst nematode. The widespread occurrence and high populations throughout the PNW are responsible for consistent losses of a minimum \$US3.4 million in Idaho, Washington and Oregon annually. Host diversity to most Poaceae, variable resistance response, and lack of effective nematicides make the management strategy limited, especially in wheat. To evaluate the resistance response of seven spring wheat cultivars of major interest in the region, we set up a randomized complete block design experiment with six replicates in a heavily infested field in St. Anthony, Idaho. Roots from plants of each plot were collected at Feekes growth stage 10.5.1, and cysts per root system were counted after processing and staining. Soil was sampled before planting and after harvest, and changes in abundance of eggs and juveniles in cysts and in soil were quantified for assessment of multiplication rate. The cultivars WB-Rockland and Espresso were moderately resistant (3-6 cyst per plant) and moderately susceptible (6 to 12), respectively. WB9668 and Patwin515 were both susceptible (12 to 25), whereas Snowcrest, WB9411 and WB936 ranked as very susceptible (>25). The co-occurrence of stripe rust and CCN in Southeastern Idaho and the regulation of both pathogens with closely located resistance genes provides an efficient means of testing for both in the field in the development of new commercial cultivars.

**Wheat Breeding for Multiple Stresses Tolerance at ICARDA: Achievements and Prospects**

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Wheat is the principal food crop in Central and West Asia and North Africa (CWANA) region, where average consumption is 200 kg/capita/year. The productivity of wheat in the region is very low (2.5 t/ha) principally due to drought, heat and stripe rust, among others. In the year 2050, the CWANA population is expected to increase from the current 0.9 billion to 1.4 billion, and the demand for wheat will rise from the present 164 million tons to 268 million tons. Fulfilling this demand is challenging especially in the face of climate change where its effects are predicted to be more pronounced in the CWANA and Sub Saharan Africa (SSA) regions. The wheat breeding program at ICARDA has developed high yielding wheat genotypes with increased water-use efficiency, heat tolerance and resistance to major diseases and pests following inter-countries shuttle breeding and key location testing approaches. In the last five years, more than 2000 bread wheat genotypes have been distributed through international nurseries and more than 30 varieties of ICARDA origin have been released by the national programs in the CWANA and SSA regions. Some of the current elite genotypes produced up to 2.5 and 11 t/ha under drought (250-300 mm) and irrigated/optimum (550 mm) conditions, respectively. Major genes and minor genes for resistance to stem rust and yellow rust have been deployed. Most of the elite genotypes have protein levels of 12 to 16% with the 5+10 (Glu-D1), 7+8 (Glu-B1) and 2\* (Glu-A1) alleles. Molecular markers linked to heat tolerance and yellow rust resistances have been identified. Pedigree analysis showed that resistance sources for heat and drought in such elite germplasm were introgressed from synthetic wheats and wild relatives, mainly *T. dicoccoides*. These genotypes have been distributed to the NARS for potential direct release and/or parentage purposes. Rapid deployment of such wheat varieties with improved crop management technologies will help to increase and enhance sustainable wheat production across the CWANA and SSA regions. In 2017, a set of 161 wheat lines developed at ICARDA breeding program and resistant to the above mentioned traits was sent to the Soil Borne Pathogens Program at CIMMYT, Turkey to be screened for nematodes and *Fusarium*. Then the most resistant germplasm will be pyramided to develop a new germplasm resistant to those soil borne diseases in the CWANA region.

**Distribution of *Heterodera filipjevi*, in Wheat Fields of Chaharmahal va Bakhtiari Province, Iran Based on Interpolation by Geographic Information System**

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This study was conducted to identify the cereal cyst nematodes (CCN), distribution and population density in wheat fields in Chaharmahal va Bakhtiari province in Iran based on interpolation using geographic information system (GIS). Totally, 212 composite soil and root samples were randomly collected from different wheat fields of nine districts of the province during 2013-2014. The soil samples were processed for cyst extraction. Species were identified based on morphological and morphometric features and molecular characters. Analysis for GIS was performed by Arc GIS software using interpolation technique for determination of raster maps of population density. *Heterodera filipjevi* was the only species of cereal cyst nematode identified in the province. Based on the obtained results, *H. filipjevi* was found in 42% of soil samples containing an average of 994 eggs and J2 (200 g soil)<sup>-1</sup>. The maximum infestation occurred in the Dehsahra region in Lordegan district with an average of 4720 eggs and J2 (200 g soil)<sup>-1</sup>. Wheat fields of Kohrang, Kiyar and Farsan districts showed low population density while moderate infestation was observed in the Ardal, Ben, Saman and Borujen districts. High population density of *H. filipjevi* was found in Shahrekord and Lordegan districts. The eastern parts of Shahrekord and western parts of Lordegan districts were predicted as regions with high infestation to *H. filipjevi* based on interpolation of population density and interpolation maps, and were considered as hotspots for this disease. The results of prediction of population density through interpolation were validated by the presence of *H. filipjevi* with high population density in the predicted regions of the province after soil sampling for verification.

**Association Analysis for Resistance to Cereal Cyst Nematodes and Root Lesion Nematodes in a Panel of Durum and Spring Bread Wheat Lines**

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Wheat is one of the most important food crops in the world. It is grown on 20% of the global cultivated land area and is the main food resource for 40% of the world's population. Cereal production is limited by several biotic factors, one of which is plant parasitic nematodes. The cereal cyst nematode (CCN) and root lesion nematodes (RLN) are considered to cause the most damage to temperate cereals and are the most economically important nematode species worldwide. Both nematode groups can be managed by crop rotation and different tillage techniques. However, the use of resistant and tolerant wheat varieties offers the most effective, economic, and environmentally friendly option of controlling nematodes. Sources of resistance to CCN and RLN include cultivated durum and bread wheat varieties, synthetic hexaploid wheat and wheat wild relatives, but usually they are not well studied. So far, 11 CCN resistance genes conferring predominately dominant or partial resistance have been reported. In addition, a limited number of QTL have been found by traditional bi-parental mapping. Genome wide association studies (GWAS) are an alternative approach to bi-parental mapping that do not require the development of parental crosses and show a higher mapping resolution investigating many more meiotic recombination events. In view of these advantages, GWAS should be applied to develop molecular markers for nematode resistance in wheat. In this study, a panel of durum and spring bread wheat lines was genotyped with SNP markers. The panels were screened for the CCN *Heterodera filipjevi* and the RLNs *Pratylenchus neglectus* and *P. thornei* in order to investigate their resistant reaction against those nematodes. The results will be shown.

**Screening Resistant Genes (*Cre*) in Wheat Germplasms Against Cyst Nematode (*Heterodera latipons*) with Molecular Markers**

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The cereal cyst nematode (CCN) (*Heterodera latipons*, Franklin) is an important key pest of wheat (*Triticum aestivum* L.) that significantly reduces grain yields in temperate countries. The use of resistant varieties is one of the most effective options in management of CCN. Recently, resistant genes (*Cre*), obtained from wild wheat genotypes, have been transferred into wheat and barley varieties to control the CCN species. As such, the development of CCN-resistant cultivars may be accelerated by the use of molecular markers. This study aimed to screen wheat lines for resistant genes by using specific primers for known resistant genes. Thirty genetically diverse wheat and barley genotypes with and without *Cre* genes were used. The reactions of wheat and barley germplasms against *H. latipons* populations were investigated under *in-vitro* conditions. Additionally, microsatellite markers linked to *Cre1*, *Cre3*, *Cre5*, *Cre8*, *CreX*, and *CreY* genes were screened in the wheat and barley germplasms. The results revealed that *Cre1*, *Cre3*, *Cre5* and *CreX* were found in the screened genotypes whereas *Cre8* and *CreY* were not detected. *Cre1* gene was located on chromosome 2B and 2BL by using G035/Crecon, *Cre3* gene was located on chromosome 2DL by using Xgwm301- 2D marker; the two genes provided high levels of resistance to CCN in wheat genotypes. However, *Cre5* and *CreX* gene located on chromosome 2AS, provided an insufficient resistance to CCN in wheat genotypes using Xgwm140 and Xgwm636- 2A marker. In conclusion, *Cre1* and *Cre3* had high potentials to aid in the development of resistant cultivars against CCN in wheat breeding programs.

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**Current Status of Cereal Cyst Nematodes (*Heterodera avenae* and *H. filipjevi*) in China**

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Cereal cyst nematodes (CCN) *Heterodera avenae* and *H. filipjevi* have been confirmed to occur and are distributed in 16 provinces in China. *H. filipjevi* was first reported from Henan in 2010. Now it has been reported from Anhui, Ningxia and Qinghai, in densities exceeding those so far reported for CCN in wheat. Yield losses reach up to 18-35% in Henan, 15-20% in Hebei, 11-18% in Beijing Suburb, and 28-24% in Qinghai. *H. avenae* and *H. filipjevi* have one generation per year in Beijing, Hebei, Jiangsu and Shandong. Four diagnostic methods based on SCAR-PCR and LAMP were developed to detect *H. avenae* and *H. filipjevi* from infested fields. A new pathotype of *H. avenae* named Ha91 was found in Beijing and Qinghai. Using *H. avenae* second stage juvenile a cDNA library was constructed to explore parasitism genes; 5800 ESTs were generated and 2568 unigenes were obtained. A predicted effector gene (HaEXPB2) from *H. avenae* was cloned and transiently expressed in *Nicotiana benthamiana*. Infiltration assays showed that HaEXPB2, a predicted expansin-like protein, caused cell death in *N. benthamiana*. *In situ* hybridization showed that *HaEXPB2* transcripts were localised within the subventral gland cells of the pre-parasitic second-stage nematode. It had the highest expression levels in parasitic second-stage juveniles. Subcellular localization assays revealed that HaEXPB2 could be localized in the plant cell wall after *H. avenae* infection. We found that HaEXPB2 bound to cellulose and its carbohydrate-binding domain was required for this binding. The infectivity of *H. avenae* was significantly reduced when *HaEXPB2* was knocked down by RNA interference *in vitro*. Integrated management strategies for CCN control were developed in China, including crop rotations, tolerant and resistance varieties, decrease soil surface for sowing, seed-coating techniques and teasaponin granules. Effects of six different seed-coatings, Gannong I, Gannong II, Gannong III, Abamectin AV1, AV2 and emamectin benzoate were evaluated for control of CCN on wheat. The taxonomic status of CCN in China has been the subject of discussion. Recently, on purely molecular data, the species “*Heterodera sturhani*” was proposed (Subbotin, 2015) and suggested to be endemic to China. We reject the new designation because of the lack of morphological, biological and ecological differences from *H. avenae*, and insufficient evidence that the molecular differences justify species-level distinction. Therefore, “*Heterodera sturhani*” is considered an invalid species; “*Heterodera sturhani*” is a junior synonym of *Heterodera avenae*. This research was supported by Chinese Special R & D Fund for Public Benefit Agriculture (201503114, 200903040) and Nature Science Foundation of China (31171827).

## **Session 4**

### **Management Strategies Other Than Host Resistance**

## **Cereal Nematodes in the Pacific Northwest USA: Current Knowledge and Future Needs**

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The Inland Pacific Northwest (PNW) has a semi-arid continental climate. Summers are dry and warm, and most precipitation occurs during winter. Rainfed winter wheat is the primary crop in vast areas where annual precipitation averages 250 to 400 mm. The wheat is planted during alternate years, with a 14-month fallow between crops. Winter wheat is rotated with barley, mustard, potato, or a pulse crop in wetter areas or where crops can be irrigated. Root diseases caused by soilborne plant-pathogenic fungi and plant-parasitic nematodes are favored by the dry summers and either the winter wheat monoculture or a short rotation. Comprehensive studies of soilborne fungal pathogens, cereal cyst nematodes (CCN) and root-lesion nematodes (RLN) have occurred in the PNW for 80, 40 and 20 years, respectively. The CCN *Heterodera avenae* is much more prevalent than *H. filipjevi*. The RLN *Pratylenchus neglectus* is more prevalent than *P. thornei*. Mixtures of CCN and/or RLN are found in some fields. The greatest economic damage is caused by *Pratylenchus neglectus*, *P. thornei* and *H. avenae*. These species reduce productivity of wheat by an estimated US\$55 million annually in the states of Idaho, Oregon and Washington. Since 1975, management studies for *H. avenae* have included crop rotations, tillage intensity, screening of nematicides, screening of cultivars for tolerance and resistance traits, and management of weed grasses and volunteer cereals. Since 1999, these same tests plus host preference studies were conducted for each of the RLN species. Workers at our small research facility could not reliably distinguish individual species of CCN and RLN based on morphological traits. We therefore developed PCR tests that could detect, identify and quantify these species quickly and accurately. Our PCR tests revealed that a change from winter wheat to spring wheat or spring barley caused a change in dominance from *P. neglectus* to *P. thornei*. PCR-RFLP tests also revealed that our collection of '*H. avenae*' samples from four states included several from Oregon and Washington that were actually *H. filipjevi*, or were mixtures of *H. avenae* and *H. filipjevi*. Those tests therefore led to the first discoveries of *H. filipjevi* in North America, and they showed that development of new RLN and CCN management strategies would be more complex than we had realized. Our PCR tests therefore proved to be very valuable. They are now being used routinely in several commercial nematode diagnostic laboratories and in many research laboratories internationally. We also identified wheat and barley cultivars that express resistance to *H. avenae*, *H. filipjevi*, *P. neglectus* or *P. thornei*, or combinations of resistance plus tolerance to *H. avenae*, *P. neglectus* or *P. thornei*. Before this knowledge can become truly useful for commercial agriculture, it will be necessary to develop cultivars with pyramided resistances plus tolerances to both species of *Pratylenchus* and to both species of *Heterodera*. Cultivars that express resistance but are not tolerant are unlikely to become widely accepted by farmers. Other future needs for nematode management will also be discussed.

**Research on Cereal Cyst Nematode in Eastern Washington**

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Cereal cyst nematode (*Heterodera avenae*) was first detected in North America in western Oregon in the 1970s, but by the 1980s had moved to the dryland wheat production area of northeastern Oregon. Cereal cyst nematode was not considered a problem in eastern Washington until 2010. From 2010 to 2016, surveys showed that 25% of fields were infested in the high precipitation annual cropping area (the Palouse) in the far eastern part of Washington. This region typically has a 3-year winter wheat/spring wheat/legume rotation. There were few detections in the drier area, wheat summer fallow, where winter wheat is grown every other year. In 2014, *H. filipjevi* was detected in eastern Washington. To facilitate species identification, we developed a direct sequencing method with DNA extracted from single cyst using ITS-rDNA primers. To date, the infestation of *H. filipjevi* is confined to a small geographical area in southern Whitman County. Since 2011, we have focused on screening germplasm and varieties of spring wheat, using a heavily infested field plot that could be planted in the spring after nematodes had hatched. Both species require a cold vernalization before hatching is induced. We then expanded resistance screening to the greenhouse, by collecting infested field soil in April as the nematodes were hatching. We developed a method of collecting soil in the fall, keeping it at 4°C for 2 months, and then conducting greenhouse trials. This soil could be stored at 4°C for 6-9 months and remain infective. Finally, we have developed pot cultures for both species. Last year we were able to screen over 500 lines in the greenhouse. Resistance to *H. filipjevi* does not appear to be effective against *H. avenae*. For example, the cultivar ‘WB Rockland’ is resistant to *H. avenae* and the cultivar ‘SY Steelhead’ is resistant to *H. filipjevi*, but not vice-versa. Surprisingly, we have found a number of resistant winter soft white and club wheat lines already adapted and developed by ARS, including ARS Crescent, ARS Selbu, Cara, Prichett and a number of soft durum lines. Current work is being done with *Cre* markers and pathotyping of *H. filipjevi* using differentials. We have been interested in fungal parasites and the microbiome of *H. filipjevi*. Using next-generation sequencing (Illumina MiSeq), we sequenced and identified 800 fungal OTUs from 10 individual cysts. These included species of *Ophiosphaerella*, *Exophiala*, *Pseudogymnoascus*, *Oidiodendron*, *Penicillium*, *Cistella*, *Chalara*, *Podospora*, and *Fusarium*. Only one parasite, *Metacordyceps chlyamdosporia* was identified from DNA sequences, and this was also readily isolated from cysts. The most dominant fungi in the cysts were also detected in the soil, but at much lower abundance. Many of the most abundant fungi could only be identified to the level of Ascomycetes, suggesting there are a number of fungi that have not been identified and deposited in the sequence database. Finally, using species-specific primers, we identified the parasite *Dactylella* in soil and cysts, but it was not detected with next-generation sequencing methods, and we were unsuccessful in isolating it.

**Influence of Berseem *Trifolium alexandrinum* on the Development of *Heterodera avenae* Woll., 1924 Population in Algeria**

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The objective of this project was to study the influence of berseem clover in a rotational program on population dynamics of *Heterodera avenae*. The study was conducted over three years, from September 2012 to August 2015, on the rotation (bread wheat (variety Anza)/berseem (variety Miscawi)/durum wheat (variety Vitron) in the region of Oued Smar (03°09E / 36°42'N). The results showed an average annual reduction of 26% of second stage juveniles (J2) of *H. avenae*. The estimated population densities in terms of number of cysts/100g of soil do not reflect this decline, because many cysts were obtained before and after crop. The results in J2/g of soil show a reduction in nematodes J2 of up to 26.1% just after the berseem crop. Analysis of variance reveals a very highly significant difference in the degree of infestation (J2 / g of soil) with respect to the period of sampling of soil samples for the three crops: (df = 5, F = 86.922, P <0.0001). The use of berseem will support the management strategy of the nematode to reduce and/or maintain the population under the economic threshold level. Cultural practices to control *H. avenae* are important tools to reduce the damage of nematodes especially because the high cost of nematicides causes chemical treatments to be used only rarely in Algeria.

**Effect of Conservative Tillage and Cultivation Pattern on Population Density of Cereal Cyst Nematode, *Heterodera avenae* in Southwestern of Iran**

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Khuzestan province has first place for grain production in Iran. The cereal cyst nematodes (CCNs) are a major pest of wheat and barley throughout the world. Recently the CCNs have been detected in 38% of the wheat and barley fields in Khuzestan province with a population average of 280 eggs and second stage juveniles (J2) /100 g of soil. The impact of conservation tillage and cultivation pattern on the population density of CCN, *H. avenae*, and wheat yield, was evaluated under field conditions. The trial was conducted as strip plot in a randomized complete block design with 5 treatments and 3 replicates during 2014-16 in Behbahan district. Treatments were conventional tillage method (control), reduced tillage #1 (two passes of disc harrow), reduced tillage #2 (chisel plowing), reduced tillage #3 (rotavator), and direct drill (no tillage) method. Cultivation patterns were wheat-fallow and wheat-corn in the first and second year, respectively. The initial nematode population, final population, and reproduction rate index were calculated before planting and at harvesting time. The results of wheat-fallow showed that in the first year low tillage methods, including two passes of disc harrow, chisel plowing, rotavator and no tillage could reduce nematode population by 99, 96, 92 and 99%, and increase grain yields by 13, 12 and 3%, respectively, as compared with the control. Grain yield in no-tillage dropped 3% when compared with the control. In the wheat-corn pattern two passes of the disc harrow treatment reduced nematode population by 86% while chisel plowing and rotavator increased the nematode population by 96% and 6%, respectively, as compared with the control. The multiplication rate of nematodes in the no-tillage treatment was 60 times of that in the control. These methods increased grain yield by 17, 14, 10 and 33% when compared to the control.

**Investigation of Cellular Variation in Some Wheat Varieties Caused by the Cereal Nematodes (*Pratylenchus thornei*, *P. neglectus*, *Heterodera avenae*, and *H. latipons*)**

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Root lesion nematodes (*Pratylenchus thornei* and *P. neglectus*) (RLN) and cereal cyst nematodes (*Heterodera avenae* and *H. latipons*) (CCN) are economically important nematodes that feed entoparasitically on root hairs. Until now, most studies were published on *P. thornei* and *P. neglectus* and CCN to study their identification, biology and control methods, such as resistant varieties in Turkey. In this study, wheat varieties infected by *P. thornei* and *P. neglectus* were investigated in order to determine cellular variation due to the nematode damages. Wheat lines were infected by second stage juveniles of RLN and CCN. After 12 weeks, wheat lines were washed, fixed in wax, cut by microtome, fixed as slides, and examined under a light microscope. Throughout the examination, it was observed that RLN always migrated from epidermal to cortical cells by breaking down cell walls along the nematode pathway. Especially, most of the wheat lines showed destruction in cortical cells below the epidermis. Moreover, it was observed that many RLN nematodes were fed collectively in the same region.

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**Nematode Biodiversity as Soil Quality Indicator in Wheat Growing Areas in the South of Morocco: Relationship between Nematodes Community Structure with Soil Factors and Land Uses**

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Nematodes are major organisms that reduce wheat production in many regions of the world. But they are key components of soil biodiversity and represent valuable bio-indicators of soil food webs and their assemblage can indicate the potential of soil for biological regulation of pest species. Numerous community indices have been developed in order to track variations in nematode-mediated soil ecosystem processes mainly under anthropogenic stresses. To fill the gap in relation to the poor availability of data about the biodiversity of nematodes associated to wheat crops in Morocco, in this study, we propose to identify associated nematode communities to wheat crops and to determine how soil factors and land uses can affect the community structure and the trophic diversity using nematodes derived ecological indices. Nematode communities were sampled in different regions that reflect edaphic factor variation in their texture under different land uses. For every site, the Shannon-wiener Index (SWI), Maturity Index (MI), Plant Parasitic Index (PPI), Channel Index (CI), Enrichment Index (EI), Structure Index (SI) and nematode metabolic footprints were calculated. The indices were calculated using the nematode families identified and nematode genus when possible. More than 20 nematode taxa were enumerated representing different trophic groups. These taxa are affected qualitatively and quantitatively by soil texture and some agricultural practices. This work revealed that the nematodes associated with wheat crops are diversified and their soil community study can serve as an important tool for the biomonitoring of soil conditions in wheat production.

**Impact of Some Non-host Crop Rotations on Population Densities of Cereal Cyst Nematodes in Algeria**

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Repeated cereal cultivation causes important yield losses. Crop rotations including non-host crops are an effective method of controlling cereal cyst nematodes (CCNs) since these rotations can greatly reduce the densities of CCNs. The aim of this work was to follow the evolution of population levels of these parasites in cereal fields where rotations were practiced. Monitoring of the effect of certain non-host crops on the evolution of CCN populations levels was studied for two consecutive years (September 2015 to September 2016) in four cereal plots naturally infested by CCNs. The assessment was made by collecting a constant number of soil samples from plots after each harvest (before and after the establishment of non-host crops) and counting the cyst numbers. The results showed that rapeseed, berseem (bersim), chickpea, and potato contributed to significant decreases of the number of cysts, with reductions of 12%, 22%, 33% and 10%, respectively. Although these data are preliminary from the perspective of the short time of the follow-up, such effects encourage an expansion of the planting of these non-host crops in both intensive and traditional rotation systems.

**Phytonematodes Parasitising Grain Crops in South Africa and Problems Experienced to Control Such Pests**

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*Meloidogyne* and *Pratylenchus* are the most abundant nematode pests present in the South African grain crop production areas. *Meloidogyne* in particular have been shown to cause severe yield losses of up to 60% to local maize crops. Other phytonematodes such as Criconematidae, Hoplolaimidae, *Paratrichodorus/Nanidorus*, *Tylenchorhynchus* and others also infect the crop. *Meloidogyne incognita*, followed by *Meloidogyne javanica* are predominant in maize fields in South Africa. Due to the presence of extremely high population densities of this genus in diagnostic and research studies since the middle 1990s and the reality of climate change occurring, a need existed to update the identity of *Meloidogyne* spp. attacking local maize crops. Identification of *Meloidogyne* spp. from 69 populations obtained during two surveys was pursued using morphological (for 9 populations) and molecular approaches (for 60 populations). Three (*M. arenaria*, *M. incognita* and *M. javanica*) of the four economically most important *Meloidogyne* spp. were identified, either as single- or mixed species populations. *Meloidogyne incognita* dominated, followed by *M. javanica* and *M. arenaria*. The use of synthetically-derived nematicides, which represented the main traditional and also the current management strategy are discussed as well as other strategies (host plant resistance used in crop rotation, use of alternative products) investigated and envisaged to reduce population densities of such pests to allow sustainable crop production.

**Sulfur and Metal Sulfide Nanoparticles to Suppress Cereal Nematodes**

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The objective of this research work was to investigate the effect of sulfur nanoparticles (SN), iron sulfide (FeS) nanoparticles, and zinc sulfide (ZnS) nanoparticle synthesized by green route on cereal nematodes. The SN, FeS, and ZnS nanoparticles were synthesized using aqueous extract of different plants at room temperature. The tested nanoparticles were characterized using scanning electron microscopy and X-ray diffraction. Several bioassays were conducted to study the effect of different concentrations of SN, FeS, and ZnS nanoparticles on hatching and survival of the second stage juveniles (J2) of root knot nematode, *Meloidogyne javanica*, and cereal cyst nematode, *Heterodera latipons*. Results showed that the studied nanoparticles varied in their effects and SN nanoparticles at 100 and 200 ppm was the most effective treatment in suppressing both hatching and survival of J2 of the two nematodes. Further, the beneficial effects of these sulfur nanoparticles on enhancing root and shoot growth of wheat were investigated. Field work should be continued to optimize the application method and dose to achieve the suppression of cereal nematodes as well as the enhancement of wheat growth and thus the possibility of the use of such nanoparticles to act as a potential component in integrated wheat management.

**Interactions Among Two Important Soil Borne Diseases and Drought on Morpho-physiological Traits in Bread Wheat**

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The cereal cyst nematode *Heterodera filipjevi* and the crown rot caused by *Fusarium* species are significantly important diseases facing cereal production around the world. They attack cereal crops and cause significant yield loss. The losses accelerate when those diseases coexist with other abiotic factors such as drought. Therefore, the main objective of this study was to investigate the interaction among those two diseases and drought on different wheat germplasm having different levels of resistance to both diseases and drought. The results showed that germination of wheat germplasm was negatively affected when exposed to drought, *Fusarium culmorum*, and *Heterodera filipjevi*. The damage worsened when the plants were exposed to dual or triple stresses compared to a single stress. Crown rot assessment showed that damage of *F. culmorum* under water stress condition was more severe (average score in all cultivars = 3) when compared with *F. culmorum* under normal irrigation (average of crown rot value in all cultivars = 2.45). The results indicated that the cyst number significantly reduced when *H. filipjevi* was co-inoculated with *F. culmorum*, revealing antagonistic interaction between the two pathogens. The minimum cyst number was observed under the combination treatment of normal irrigation plus *F. culmorum* inoculation, while the maximum cyst number was obtained from the susceptible cultivar (Seri) to *H. filipjevi* under water stress condition. Single water stress caused significant reduction in plant height. Single *F. culmorum* stress did not significantly affect plant height but when combined with water stress it was significantly reduced. Grain yield and thousand kernel weight were significantly reduced by any of the single stresses, and the losses were greater when the plants were exposed to double or triple stresses. Water stress caused significant reduction in spike weight and seed per spike, and this was accentuated when seedlings were co-inoculated with *F. culmorum*. Chlorophyll content and relative water content of wheat leaves were negatively affected by water stress, and *Fusarium* inoculation significantly increased this effect.

**Effect of Direct and Conventional Seeding with Crop Rotations on *Heterodera avenae* Populations**

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The study of plots in the region of Sétif and Bouira, in Algeria, revealed different levels of infestation depending on the type of soil, climate and the farming technics. The density of full cysts is high in the semi-arid region of Sétif, with 13 cysts /100 g of soil. In Bouira, the density is about 1 cyst/100 g of soil. The analysis of the record plot of Sétif highlights the proportion of 91% of full cysts compared to 9% of empty cysts. The crop rotation practiced in this plot is (cereal/cereal – cereal/fallow-potato/cereal) using the conventional seeding in 30 cm of depth. Concerning the region of Bouira, the percentage of full cysts observed compared to empty ones is 70% and 30%, respectively. The seeding of wheat was practiced during three consecutive years with the same variety (Chen'S). In order to know about the effect of direct and the conventional seeding on the evolution of *Heterodera* populations we have done a nematologic analysis of four plots of each region, Bouira and Sétif. Each plot was specified by its seeding system. Concerning Sétif, the first plot was grown in wheat during three years but using the conventional seeding. On the other hand, the first plot of Bouira was grown in wheat during two years of direct seeding and one of conventional seeding. The second one was grown in wheat followed by a worked mallow and no worked one. The results of this analysis revealed a remarkable number of cysts in Sétif when grown using the direct seeding compared to conventional seeding associated to other technics. In fact, the number of cysts per 100 g of soil by direct and conventional seeding reached up to 18 and 8 cysts, respectively. In Bouira, the direct seeding repeated seeding during two seasons with one conventional seeding revealed 1 full cyst per 100 g of soil. The technique of practicing a conventional seeding, a mallow with a rest of the ground for three years, we recorded 0.64 with the same weight of soil analyzed.

**State of Infestation of Some Cereal Plots by the Cyst Nematode *Heterodera* sp. and Means of Lute Biological**

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The study of the state of infection cereal crops in some wilayas in Algeria by the cyst nematode *Heterodera* sp. shows that all the surveyed cities are infected by this parasite. Furthermore, the values of the densities (number of cysts/100 g soil) do not reflect the actual state of a plot infection. We found that the densities recorded Djendel (Ain Defla) (2,3 cysts/100 g soil) and Oued Smar (Alger) (2,35 cysts/100 g soil) are very close to each other. But, comparing their respective degrees of infection (7,8 and 4,6 J2/g soil) shows a clear difference regarding the state of infection of their plot. The isolation of the microflora from *Heterodera* sp. cysts allowed us to identify six fungi: *Fusarium* sp., *Ulocladium* sp., *Penicillium* sp., *Aspergillus* sp., *Rhizopus* sp. And *Rhizomucor* sp., and a bacteria, namely *Erwinia* sp. The use of two fungi, *Fusarium* sp. and *Ulocladium* sp., in direct confrontation with the eggs of two populations of *Heterodera* sp. (Mouzaïa and Dahmouni) revealed an antagonistic effect against the nematode.



## **Session 5**

**Use of Molecular Tools for Research  
(such as pathogen diagnostics, phylogeny studies,  
and host resistance)**

**First Report of Cereal Cyst Nematode (*Heterodera filipjevi*) on Winter Wheat in Xinjiang Uygur Autonomous Region, China**

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The cereal cyst nematode, *Heterodera filipjevi*, is considered to be one of the most important plant parasitic nematodes attacking most cereals crops and causing significant crop losses (Sikora 1988). In China, this nematode was first recorded in Henan province in 2009 and the distribution area is continuously expanding to Anhui province (Peng et al., 2016). During 2015-2016, a survey for important nematodes was undertaken in Xinjiang Uygur Autonomous Region of China. Fifty soil samples were collected and the cysts and second stage juveniles (J2) were extracted from soil using the sieving-decanting method. Morphological and molecular studies of cysts and J2 confirmed the identification of *H. filipjevi* in two samples from Xinyuan county of Yining city (Subbotin et al. 2010). The cysts were characterized by a lemon shaped with light to dark brown cyst wall. Subcrystalline layer visible, ambifenestrate, bullae large and numerous, with underbridge. The key morphometrics of cysts (n=10) were length excluding neck (range = 705 - 887  $\mu\text{m}$ , mean =  $775 \pm 30.5 \mu\text{m}$ ); width (478 - 694 $\mu\text{m}$ ,  $571 \pm 15 \mu\text{m}$ ); length of fenestra (45.2 - 63.9  $\mu\text{m}$ ,  $54.7 \pm 2.1\mu\text{m}$ ); width of fenestra (26.9 - 34.8  $\mu\text{m}$ ,  $28.4 \pm 1.2 \mu\text{m}$ ); length of vulval slit (7.5 - 12.5  $\mu\text{m}$ ,  $9.4 \pm 0.5 \mu\text{m}$ ); length of underbridge (71.4 - 84.5 $\mu\text{m}$ ,  $76.4 \pm 4.8 \mu\text{m}$ ). Measurements of second-stage juveniles (n = 20) included length of body (range = 481.1 - 581.5  $\mu\text{m}$ , mean =  $540 \pm 7.1 \mu\text{m}$ ), stylet (24.5 - 27  $\mu\text{m}$ ,  $24.7 \pm 0.2 \mu\text{m}$ ), tail (53.4 - 65.3  $\mu\text{m}$ ,  $61.2 \pm 1.4 \mu\text{m}$ ), and hyaline tail terminus (30.4 - 42.2  $\mu\text{m}$ ,  $36.5 \pm 0.7\mu\text{m}$ ). Genomic DNA was isolated from single cyst (n=5), the internal transcribed spacer (ITS) regions were amplified with primers TW81 (5'-GTTTCCGTAGGTGAACCTGC-3') and AB28 (5'-ATATGCTTAAGTTCAGCGGGT-3') (de Ley et al. 1999). The obtained sequences (GenBank accession KY448472 and KY448473, 1054bp) were found to be 99-100% identical to those of *H. filipjevi* from the United States (KP878490.1 and GU079654.1), Turkey (KR704304.1 and KR704308.1) and China (KT314234.1 and GU083595.1). The specific primers were used and produced single bands specific for *H. filipjevi* (Peng et al., 2013). Pathogenicity of the cyst nematode was confirmed by infection and reproduction on wheat. Wheat (*Triticum aestivum* cv. Wenmai 19) was cultured in a glasshouse in 300 cm<sup>3</sup> volume pots containing autoclaved soil, and 800 eggs were inoculated into the soil near the wheat roots of 4-day-old seedlings. The parasitic J2s, third-stage juveniles, fourth-stage juveniles, and adult females were observed in roots stained with acid fuchsin at 7, 14, 28, and 35 days after inoculation (DAI), and an average of 51 cysts per pot were extracted at 50 DAI. The new cysts' morphological and molecular characteristics were identical to the cysts from the original soil samples. Those results supported its identity as *H. filipjevi*. To our knowledge, this is the first report of *H. filipjevi* in Xinjiang Uygur Autonomous Region, China.

This research were supported by grants from the Special Fund for Agro-scientific Research in the Public Interest (201503114 and 200903040) and the National Key Basic Research Program of China (973 Program, 2013CB127502).

**Morphological and Molecular Variations within *Heterodera filipjevi* Populations from Iran Compared to Other Populations**

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Cereal cyst nematodes (CCN), *Heterodera avenae*, *H. latipons*, and *H. filipjevi*, are considered major nematode species that cause significant yield losses of cereals around the world. Of these, *H. filipjevi* is widely spread in wheat fields in Iran, and causes yield losses. An *in-silico* study indicated that the sequences of the ITS regions of ribosomal DNA of *H. filipjevi* populations from Iran showed low similarity (97%) with sequences of the populations of the same species available in GenBank. Hence, further studies were conducted to clarify this phenomenon. DNA was extracted from 16 populations of *H. filipjevi* originating from six countries and included eight populations collected from different provinces in Iran. The ITS-rDNA region and mitochondrial cytochrome oxidase subunit 1 (COI) region were amplified and sequenced, and the obtained sequences were aligned. The alignment of the ITS region showed distinct differences in three positions between the Iranian and the other *H. filipjevi* populations (insertion of one nucleotide, 1 SNP, and a deletion of two nucleotides in another position). Also, the alignment of the COI sequences indicated clear differences between the Iranian populations and the other populations. Furthermore, deviating morphological characteristics for some of the Iranian *H. filipjevi* were noticeable, including variability in the underbridge (from “hardly observed” over “very weak” to “strongly developed”), and the bullae (from “very conspicuous and numerous” over “very weak and few” to “hardly found”). Further investigation is needed of the Iranian *H. filipjevi* populations to see to what extent these molecular and morphological variations reflect differences in pathogenicity and impact yield, and for discriminating the Iranian populations of *H. filipjevi* in different localities.

**Diversity of Root-lesion Nematodes (*Pratylenchus* spp.) Associated with Wheat (*Triticum aestivum* and *T. durum*) in Morocco**

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Root-lesion nematodes (*Pratylenchus*) have a worldwide distribution and cause severe production constraints on numerous important crops. During a survey of the wheat-growing area of Morocco, 17 populations of root-lesion nematode were collected. They were identified on the basis of their morphological and morphometric characters, and by molecular methods. Microscopic observations of females and males demonstrated the occurrence of *P. penetrans* in 13 of the 17 samples; *P. thornei* and *P. pseudocoffeae* were detected in four samples from Zaers and a single sample from Settat, respectively. A duplex PCR primer set was used to confirm the presence of *P. penetrans* while the species-specific forward primer PTHO and the common reverse primer D3B were used for *P. thornei*. For the remaining populations, the D2-D3 expansion segments of the 28S rRNA gene were amplified and the obtained sequences were compared with those of *Pratylenchus* species in the GenBank database. This comparison confirmed the morphological identifications and revealed a population of *P. pinguicaudatus*. The study of the phylogenetic relationship of the Moroccan *Pratylenchus* populations showed a high similarity (99-100%) between all *P. penetrans* populations. The population dynamics of six *Pratylenchus* populations from Morocco were evaluated on carrot-disk cultures at 4, 8 and 12 weeks after inoculation, and at 10, 15, 20 and 25°C. The optimum temperature for reproduction of all populations was 20°C. After eight weeks at this temperature, nematode numbers increased up to 458-fold, 310-fold and 252-fold for the four populations of *P. penetrans*, the *P. thornei* and the *P. pseudocoffeae* population, respectively.

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**Identification of Cereal Cyst Nematode, *Heterodera filipjevi***  
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Plant parasitic nematodes are known one of the utmost threats causing economical yield losses in wheat growing areas in the world. Many nematode species attack almost every part of the wheat plant including roots, stems, leaves and seeds. The most economically important and globally recognized nematode on wheat is the cereal cyst nematode (CCN). The CCN complex is represented by a group of closely related species: *H. avenae*, *H. filipjevi*, *H. latipons*, and others, collectively known as the “*Heterodera avenae* group”. *Heterodera filipjevi* within the CCN is distinguished from other species by small differences of cysts and second stage juveniles (J2). In this study, cysts were collected at the harvest in 2014 from wheat fields in the Eastern Anatolian region of Turkey for morphological and molecular identification. The cysts were characterized based on: body length without neck (490-852 µm), body width (309-620 µm), l/w ratio (1.18-1.68), fenestra length (40-61 µm) and width (19-40 µm), heavy underbridge (71.1-94.8 µm), vulval slit (7.5-13 µm), and many bullae. The cysts were identified using morphological characters and morphometric measurements. Three different real-time PCR primer sets were designed from the 28S ribosomal region of the cysts for molecular identification. The species-specific PCR assays were successfully performed to separate *H. filipjevi* populations collected from different countries all over the world, as compared to the East Anatolian region. Future works are still undergoing on screening some varieties from this region under the East Anatolian wheat nematode project (Tubitak, 112O565).

**Implementation of Nematode Community DNA-Metabarcoding in Assessing the Health Status of Agricultural Soils**

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Although the health status of soils usually is described based on bacteria, fungi and earthworm counts, soil health can also be measured by the community of nematodes present in that soil. Soil-borne nematodes are often dominant in terms of numbers compared with other members of the soil fauna. But more important, they are extremely diverse and are present in different trophic groups taking care of a variety of tasks within different levels of the food chain. Some nematodes feed on algae or plants. Others use bacteria or fungi as a food source influencing both directly and indirectly the mineralization of nutrients. Finally, a group of nematodes are predators and omnivores, feeding mainly on insects, protozoa and also nematodes. If the latter are plant-parasitic, the predators and omnivores can play a role in biological control of these nematode species. Unfortunately, the main method for determining the nematode community is still based on morphology. This is time-consuming, requires great expertise and thus impedes research on assessment of soil health based on nematodes. As a consequence, little is known about nematode communities in agricultural fields in general. Particularly, the influence of mineral fertilizers, soil amendments like compost and green manures, chemical biocides, and soil-disturbing activities such as tillage, on the biodiversity and abundance of nematodes are rarely studied. Therefore, we are developing a DNA metabarcoding protocol to characterise nematode communities in a faster way, independent of expertise based on morphological identification. Once the technique is sufficiently tested and validated, it will become an important instrument to assess soil health, to study nematode soil communities and their functions, including soil suppressiveness and biocontrol, with a final goal to give valuable advice to farmers.

**Two New Records of *Pratylenchoides ritteri* and *Geocenamus* Species Associated with Wheat in Jordan**

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During a survey to investigate the occurrence of plant parasitic nematodes on wheat grown in major areas of Jordan, root lesion nematodes (RLN) and stunt nematodes were recovered from roots and rhizosoil of wheat from different locations in Northern Mediterranean phytogeographical regions in Jordan. Nematodes were identified based on morphological and morphometric characteristics, using identification keys. Molecular characterization was performed by the analysis of sequence data obtained from the D2/D3 expansion segments of 28S rDNA. Both morphological and molecular analyses confirmed that *Pratylenchoides ritteri* Sher, 1970 was one of the recovered RLN. While the morphological data revealed that the recovered stunt nematodes belonged to undetermined species of *Geocenamus*. This is the first report of the occurrence of those two species in Jordan. It was recorded that species of *Pratylenchoides* and *Geocenamus* occurred in varying frequencies on cereal fields and caused considerable damage to cereals. Not only that but also species of stunt nematodes are associated with soil-borne zoosporic fungi that cause root rot of wheat. Thus, it is of great importance to address these two nematodes and more intensive surveys should be conducted to determine their distributions and their frequencies in Jordan and followed by further epidemiological studies to assess the impact of such species on cereal production.



## **Session 6**

### **Cereal Nematodes Genomes and Parasitism Genes**

**Genetic Dissection of Cyst Nematode Resistance in Wheat**

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Cereal cyst nematodes are obligate biotrophic parasites posing a serious threat to global wheat production. Damage accelerates further in the regions where drought is prevalent. Resistant cultivars are the most preferred method of controlling cyst nematodes. In past, conventional approaches have been applied to identify the resistance sources, though they are labor intensive and time consuming. Association mapping is a powerful tool to detect associations between phenotypic variation and genetic polymorphisms; in this way favorable traits such as resistance to nematode can be located. Here, we genotyped 161 diverse wheat accessions by 90K iSelect SNP Chip and identified 11 novel quantitative trait loci (QTLs) on chromosome 1AL, 2AS, 2BL, 3AL, 3BL, 4AS, 4AL, 5BL, and 7BL. Eight of these QTLs were linked to putative genes known to be involved in plant-pathogen interactions whereas, two other QTLs on 3BL and one QTL on 7BL were linked to putative genes known to be involved in abiotic stress. To get insight into the details of parasitism, we compared nematode invasion, development, and reproduction between resistant and susceptible accessions, and found all these parameters were significantly reduced in resistant accessions. By comparative genomic approaches, we further identified an amino acid permease 6 (TaAAP6) linked to the QTL on chromosome 2B. High expression of TaAAP6 in infected roots of two susceptible wheat accessions were recorded, whereas the expression remained unchanged in infected roots of two resistant accessions. Further, we also identified an orthologue in *Arabidopsis* and found reduced nematode development in a knock-out mutant. We confirmed the high abundance of AtAAP6 in syncytia at 1, 10 and 15 days post nematode infestation. Thus, we conclude AtAAP6 and TaAAP6 are important factors for nematode parasitism.

**Molecular Characterization and Function Analysis of a putative effector Ha16674 from the Cereal Cyst Nematode *Heterodera avenae***

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Cereal cyst nematodes are sedentary biotrophic endoparasites that maintain a complex interaction with their host plants. Nematode effector proteins are synthesized in the oesophageal glands and are secreted into plant tissue through the stylet. One predicted effector named Ha16674 from *Heterodera avenae* was cloned and identified. It encoded a calreticulin protein, which could suppress the cell death induced by Bax when expressed in *Nicotiana benthamiana*. The result showed that HaCRT1 was localized in the endoplasmic reticulum in plant cells. Ha16674 gene expression in *Arabidopsis thaliana* increases susceptibility to *Pseudomonas syringae*. We tried to identify which signaling Ha16674 inhibited Bax induced cell death depend on yeast, and study the function of Ha16674 in host innate immunity. The results will supply evidence to understand the interaction mechanism between cereal cyst nematode and their hosts, and the plant nematode pathogenic molecular mechanism. These findings will be very important for making control strategies and ultimately increasing food security.

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**Identification and Expression Analysis of a New Pectate Lyase Gene *Ha-pel-1* from *Heterodera avenae***

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The cereal cyst nematode (*Heterodera avenae*) is one of the important plant parasitic nematodes which seriously threatened cereal crops and cause huge economic losses to agricultural production in China. However, its pathogenic mechanism and effective prevention and control methods still need further research. The objective of this study is to provide a theoretical basis for further study on the gene function of Ha-pel-1 and its interaction with host plants, and to give new ideas for the control strategies of cereal cyst nematode based on the cloning and expression analysis of a new pectate lyase gene Ha-pel-1 from *H. avenae*. A novel pectate lyase gene Ha-pel-1 was cloned from *H. avenae* using homology cloning combined with RACE technology, and its nucleotide sequence and amino acid sequence were analyzed by related bioinformatics softwares and online tools, such as DNAMAN, Clustal, SignalP 4.0 Server, and GSDS. A phylogenetic tree was also constructed using MEGA 5.0. The tissue localization and developmental expression characteristics of Ha-pel-1 were analyzed by *in situ* hybridization and a semi quantitative PCR method. A novel pectate lyase gene (Ha-pel-1, GenBank accession number GQ998895) was cloned successfully from *H. avenae*. Ha-pel-1 was 1717bp in length which contained a 1563-nucleotides-long open reading frame (ORF) encoding a protein of 521 amino acid residues. The molecular weights of Ha-pel-1 encoding protein were 57.5kD and isoelectric point was 8.52. The full length of genomic sequence of Ha-pel-1 was amplified from the nematode genome DNA which contains 7199 nucleotides. Gene structure analysis showed that the Ha-pel-1 genome contains 14 exons and 13 introns, except for the third intron splice sites are GC-AG, the other 12 introns are in line with the rules of the eukaryotic gene splicing site GT-AG. The results of homologous comparison showed that the C-terminal sequence of the putative Ha-PEL-1 had a 67% identity and a similarity of 83% with that of soybean cyst nematode HG-PEL-1 and beet cyst nematode HS-PEL-1. In addition, after the N-terminal signal peptide, the putative Ha-PEL-1 had a sequence of 254 amino acid residues more than other reported plant parasitic nematodes pectate lyases. In this sequence, 184 amino acid residues closing to the N-terminal had no similarity with protein database, while 70 amino acid residues (Lys205-Glu274) close to the C-terminal had an identity of 32% and a similarity of 47% with the methyltransferase domain of Wesselsbron virus NS5 (Registration No. 3ELD). The amino acid sequence analysis revealed that the predicted protein contained a signal peptide of 20 amino acid residues, as well as four highly conserved regions and several conserved cysteine residues characteristic of class III pectate lyases (PL3). A phylogenetic analysis revealed that Ha-pel-1 and other nematodes pectate lyase genes are gathered in a large branch with bacterial and fungal sources PEL. *In situ* hybridization analyses showed that the transcripts of Ha-pel-1 was mainly expressed in the two subventral gland cells of *H. avenae*. A semi-quantitative RT-PCR analysis confirmed that its transcriptions were highly expressed in the pre-parasitic and parasitic second-stage juveniles. A new pectate lyase gene Ha-pel-1 from *H. avenae*, closely related to the infection and parasitic process of cereal cyst nematode, was found and analyzed.

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**Prospects of Transgenic Research for Cereal Nematode Resistance**

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Since domestication, improvements in crop plants regarding pest resistance and higher yields have been preferential research areas in plant sciences, and have usually involved targeted genetic exploitations through natural means. These exploitations were based on available genetic diversity among the crop plants. With continuous improvement, there is a change in basic assumptions in genetic plant resources with reduced genetic diversity. To overcome such genetic erosion, transformation of genes from other sources has been a subject of modern plant molecular biology. The application of transgenic strategies for enhancement of resistance against biotic stresses is not a new idea. Transgenic crops with engineered resistance against insects, microbial pathogens and viruses are under cultivation worldwide. In the last two decades, genetically modified crops (GMCs) have brought a revolution in agriculture sector for enhanced crop productivity and sustainability. Thanks to this revolution, during 2016 around 18 million farmers in 26 countries planted 185.1 million hectares of GMCs with an increase of 3% over the area of last year. Cotton, maize and soybean were the main GMCs grown in these countries. The other crops are potato, eggplant, pineapple, canola, sugar beet, alfalfa, papaya and squash. In these engineered crops, major objectives are higher yields by controlling insect pests, microbial pathogens and establishing herbicide tolerance. However, generation, adoption and commercialization of GMCs with an objective to overcome assault of nematodes is not so common. Nematodes attack cereals like maize, rice and wheat and they are a serious global concern. Most of these nematode species belong to family Heteroderidae and cause enormous losses in cereal crops annually. There is a great potential to use genetically engineered crop plants for the said purpose. Plenty of basic research, with wide acceptance and validation in the scientific community is available in the field of transgenic resistance against plant parasitic nematodes. This could be utilized to engineer cereal crop plants with transgenic resistance against cereal nematodes in future. This is the subject matter of this update. Furthermore, the perspectives of latest technologies including CRISPR-Cas9 and TALENs have been also discussed along with established transgenes. This information will be valuable in the paradigm shift of cereals resistance against nematode and will attract the attention of the scientific community towards this neglected area.

**RNAi Silencing of Different Functional Classes of Genes in *Heterodera avenae* Singly and in Combination**

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Cereal cyst nematode (CCN), *Heterodera avenae* is an important biotic stress in wheat and barley cultivation causing considerable yield losses worldwide. The availability of transcriptome of *H. avenae* is an advantage as it provides a repertoire of genome wide gene expression that can be used for the selection of genes involved in various biological activities for any kind of downstream analysis. In the present study, we choose 40 genes of CCN for functional validation that could be categorised into four major group's viz., neuropeptides, proteases, cell wall degrading enzymes and secretory proteins. All these target genes were cloned from cDNA of second stage juveniles (J2) of CCN and used for *in vitro* RNAi studies. Initially, the genes were silenced singly to determine the effect on nematode infection, development and reproduction. Further, the promising genes were selected for combinatorial RNAi. The roots of freshly germinated wheat seedlings were infected with CCN juveniles soaked in dsRNA of selected target genes singly to establish the short and long term effects of gene silencing. Effect of RNAi silencing was evaluated on nematode penetration at seven days and reproduction after about 75 days after inoculation respectively to assess the short term and long term effects of gene silencing. The results revealed that silencing of individual genes led to about 25-83% reduction in penetration compared to control. Similarly, we have also observed 10 to 93% reduction in number of cysts produced and 13 to 93% reduction in number of eggs per cyst in single gene silenced nematodes. Likewise, silencing of some of the selected genes in combination was also found to be effective in reducing the number of cysts by 5 to 93% and eggs per cyst by 9 to 83%. Multiplication factor (MF), which is important for determining the population pressure on the subsequent crop, was reduced by 10 to 30% in single gene knock-down studies, and was reduced 9 to 40% by combinatorial silencing. The results were supported by perturbation in the expression of target genes as indicated by RT-qPCR. However, the genes belonging to CAZymes and secretory proteins showed significant effect (singly or in combination) in reducing both the initial host invasion and reproduction. Some of the promising genes are being tested using host delivered RNAi in wheat.

**From Genes to Biological Control: A High-Throughput Sequencing Approach to Identifying Potential Nematode Suppressive-Soil Microbial Communities**

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Plant-parasitic nematodes are of great concern in global agriculture due to the significance damage they cause to crops and the increasing management costs. Soil is a reservoir of microbial organisms with potentials for application as biological control agents and new DNA sequencing technologies have made it progressively attractive to screen the microbiological genomic resource in soil. Metagenomic analyses of the soil microbial communities in peanut-producing counties in Central and Northern Florida was conducted by sequencing for the bacterial 16S ribosomal RNA and fungal internal transcribed spacer (ITS2) marker genes. The metagenomic DNA was extracted from soil samples and amplified with universal primers targeting the hypervariable regions 4 and 5 (V4-V5) of the 16S rRNA and the fungal ITS2. Here we report genomic information we garnered from analyses of intra- and inter-field soil microbial community diversities, their relative abundances as well as their functional significance. We demonstrate that a high-throughput DNA sequencing approach coupled with appropriate bioinformatic tools can generate relevant synthesis of genomic data into a biologically meaningful form. This work will help development of a genome-centric predictive framework for understanding and harnessing of potential beneficial soil biota for management of plant-parasitic nematodes.

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