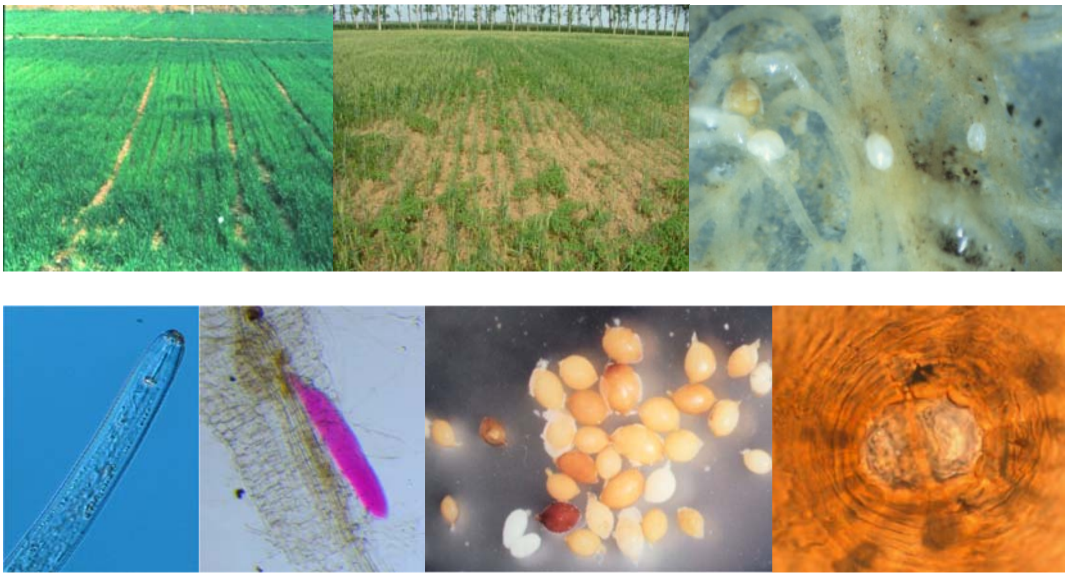
# The 4th International Cereal Nematodes Initiative Workshop

## 第四届国际禾谷类线虫病害学术研讨会

**August 22-24, 2013**

**Beijing·China**



**Sponsored by**

Institute of Plant Protection, Chinese Academy of Agricultural Sciences

International Maize and Wheat Improvement Center (CIMMYT)

China Agricultural University

State Key Laboratory of Biology of Plant Diseases and Insect Pests

Chinese Society for Plant Pathology

Chinese Society of Plant Nematologists

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# General Information

## Letter of Invitation

**Dear Friends ＆ Colleagues,**

It is our pleasure to invite you and your collaborators to attend the 4$^{th}$ International Cereal Nematodes Initiative Workshop (ICNI) in Beijing, China. This event, which will take place on 22$^{nd}$-24$^{th}$ August 2013, and provide a stimulating platform for exchanging research developments and tracking technical progress on cereal nematodes research.

The 4$^{th}$ ICNI is a Pre-workshop included in the program of the 10$^{th}$ International Congress of Plant Pathology (ICPP), to be held in Beijing from 25$^{th}$ to 31$^{st}$ August 2013, thus allowing participants to attend both 4th ICNI and ICPP minimizing time and costs.

The 4$^{th}$ ICNI has been jointly sponsored by the Institute of Plant Protection, Chinese Academy of Agricultural Sciences (IPP-CAAS), CIMMYT International Maize and Wheat Improvement Center, China Agricultural University and is supported by the State Key Laboratory of Biology of Plant Diseases and Insect Pests (SKLBPI), and the Chinese Society for Plant Pathology (CSPP).

We sincerely invite you to come together with us, make this workshop enjoyable and profitable.

We wish you have a good stay in Beijing.

On behalf of the 4th ICNI organizing committee
Prof. Deliang Peng and Dr. Francesco Di Serio

## Organizers

**Dr. Deliang Peng,** Institute of Plant Protection, Chinese Academy of Science, China, Email: dlpeng@caas.net.cn
**Dr. Amer A. Dababat,** CIMMYT, Ankara, Turkey, Email: a.dababat@cgiar.org

## Scientific Committee

**Dr. Deliang Peng** – IPPCAAS, China, Email: dlpeng@caas.net.cn
**Dr. Amer A Dababat,** CIMMYT, Ankara, TURKEY, Email: a.dababat@cgiar.org
**Dr. Julie Nicol** – CIMMYT International, Email: j.nicol@cgiar.org (Chair ICCNI)
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**Dr Zahra Tahna Maafi** – Iran, Email: tanhamaafi@yahoo.com
**Prof Halil Elekciouglu** – Cukurova University, Turkey, Email: halile@cu.edu.tr

## Local Organizing Committee

Dr. Wenkun Huang (China)
Dr. Lingan Kong (China)
Prof. Heng Jian (China)
Prof. Jinling Liao (China)
Prof. Yuxi Duan (China)
Prof. Julian Chen (China)
Ms. Wenting He (China)
Prof. Shulong Chen (China)
Associate Prof. Yanhua Wen (China)
Prof. Jingwu Zheng (China)
Prof. Hongmei Li (China)
Prof. Xianqi Hu (China)

## Workshop Topics

1. Global status of the distribution of cereals nematode (CN)
2. The economic importance and population dynamics of CN on wheat
3. Control strategies of CN in wheat using host resistance
4. Control strategies of CN other than host resistance
5. Use of molecular tools for research with CN (such as pathogen diagnostics, phylogeny studies and host resistance).
6. CN genome and parasitism genes
7. Biological control and IPM

## Registration and Abstract Submission

Registration and abstract submission of this workshop are bundled with the 10th ICPP registration system.

**Early bird registration before Jan. 15, 2013: RMB 1,600; Onsite registration: RMB 2,100**

Official published proceedings will be made from this workshop. If you would like to contribute to the workshop please kindly submit your abstract of no more than 250 words via 10th ICPP web and also email to Dr. Deliang Peng (dlpeng@ippcaas.cn) and Dr. Amer Dababat (a.dababat@cgiar.org) and the scientific committee for the workshop will review applications and inform contributors of acceptance as poster/paper by 1 June 2013. Contributors with accepted papers will be asked to prepare a 2-4 page paper by June 16, 2013. Please follow the format provided below.

## Accommodation

Hotel costs are RMB 490/single room at Friendship Hotel Grand. The hotel is located 40 minutes away from airport by car and can be reached with Shuttle Bus for about 16 RMB. Please contact workshop organizer Deliang Peng at dlpeng@caas.net.cn; phone +86-10-62815611; fax +86-10-62896114 for hotel reservations.

## Weather

August in Beijing is sunny, warm and humid. The average daily temperature is 25-33°C（77-95F）.

## Insurance

The registration fees do not include insurance for the participants regarding accidents, sickness or loss of personal property. You are advised to make necessary arrangements for a short-term health and accident insurance before leaving your home country.

## Currency Exchange

In China, only RMB is used. Exchange centers can be found at airports, hotels. The exchange rate is set by the Bank of China, which is now about US $1.00=RMB 6.15. When exchanging money, you should keep your receipt in case you want to change any RMB back to foreign currency when leaving China.

Credit cards (Visa, Master, American Express, Diners Club and JCB) are accepted in many department stores and hotels. It is possible to draw cash from ATM machine by above credit cards.

The Bank of China and most of the hotels can cash traveler cheques issued by most foreign banks or financial institutions.

## Arrival in Beijing

From Beijing Capital International Airport to Friendship Hotel Grand. The hotel is located 40 minutes away from airport by car and can be reached with Shuttle Bus for about 16 RMB. It takes about 30 mins by taxi, and the charge is around RMB 100 for one way.

**Most important: Please print the following picture and show it to TAXI driver, who may deliver you to the Friendship Hotel Grand.**

请送我去友谊宾馆 3 号楼迎宾楼

地址：北京海淀区中关村南大街 1 号

电话::010-68498888

Get the Taxi note: please send me to Friendship Hotel Grand, Building 3, Yingbin Lou

Address: zhongguancun Nan Da Jie，No.1

Tel. :010-68498888

## Check-in

There will be reception staff to help you check-in at 8:00 am-22:00 pm on August 22, at the Building 2，Jingbing Lou，Friendship Hotel Grand.

For those who have not paid registration fee online, you will need to pay it on-site.

After checking-in, you will get an information pack, meal vouchers, and workshop documents.

On-site registration cash (RMB) only, 2100 RMB for each delegate and 600 RMB for each family member.

Please you in advance change to RMB at the airport or Friendship Hotel Grand.

## Preliminary Schedule

8:00 - 20:00, August 22, 2013   Yinbinlou, Friendship Hotel Grand, Registration;
8:30 - 19:00, August 23, 2013   Jiabinlou, Friendship Hotel Grand，Scientific program;
19:30 - 22:00, August 23 2013   Welcome reception and banquet ,Yuegongfu, Xueyuan South Road;
8:30 - 12:30, August 24, 2013  Jiabinlou, Friendship Hotel Grand，Scientific program;
14:00- August 24, 2013  Check out，Workshop end

## Scientific Program

The conference will be held in August 23rd-24th, 2013, in Beijing. The conference covers a wide range of topics on cereal nematodes.

All meetings will take place in the first Meeting Room at Friendship Hotel Grand, Yibing Lou, on August 23-24, 2013.

For the speakers, please copy your PPT files to the computer between 14:00 pm and 22:00 pm on August 22 on Registration Site at Friendship Hotel Grand, Jingbin Lou or 7:30 am-8:20 am and 13:00-13:30 pm on August 23-24 at meeting room.

**Agenda for the 4th International Cereal Nematodes Initiative Workshop**

**August 22-25，2013，Beijing，China**

| **Organizers: Prof. Deliang PENG & Dr. Amer DABABAT** | |
|---|---|
| **August 22, 2013** | **Registration: Lobby of Building No 2(Jingbinlou)in Friendship Hotel, Beijing(北京友谊宾馆 3 号楼，敬宾楼大厅)**<br>**Drs. Wenkun HUANG & Lingan KONG** |
| **August 23, 2013** | **Building 8, Jiabinlou，First Meeting Room(8 号楼，嘉宾楼第一会议室)** |
| **8:30- 9:10** | **Opening Ceremony, Photos** |
| **SESSION I :**<br>**9:10-10:30** | **Global status of the distribution of cereal nematodes:**<br>**Chairman: Prof. Halil ELEKCIOĞLU Prof. Heng JIAN** |
| **9:10-9:30** | 1. Amer DABABAT: Cereal nematodes management strategies in wheat |
| **9:30-9:50** | 2. Richard W. SMILEY: Resistance and tolerance to *Heterodera avenae* in North American spring wheat |
| **9:50-10:10** | 3. Deliang PENG: Current research status and perspective of cereal cyst nematode, (*Heterodera aveane*，*H. filipjevi*) on wheat in China |
| **10:10-10:30** | 4. Zahra Tanha MAAFI: Cereal cyst nematodes and its research perspectives in Iran |
| **10:30-10:45** | **Tea Break** |
| **SESSION II**<br>**10:45-12:00** | **The economic importance and biology of cereal nematodes**<br>**Chairman: Dr. Amer DABABAT, Prof. Jinling LIAO** |
| **10:45-11:00** | 5. Shulong CHEN: The biology of cereal cyst nematode in Hebei Province, China: implications on its control |
| **11:00-11:15** | 6. Alireza AHMADI: Crop loss assessment of *Heterodera filipjevi* on some cultivars of wheat, barley and triticale under field condition of Southwest of Iran |
| **11:15-11:30** | 7. Ricardo HOLGADO: Importance of identification cereal cyst nematodes species and patothypes in breeding for resistance and for management strategies |
| **11:30-11:45** | 8. Jie ZHANG: The effect of the cereal cyst nematode, *Heterodera avenae*, on wheat yield in Shangdong province, China |
| **11:45-12:00** | 9. Mustafa İMREN: Studies on cereal cyst nematodes, *Heterodera avenae* Wollenweber, in South East Anatolia and Eastern Mediterranean Regions in Turkey |
| **12:00-13:30** | **Lunch** |
| **SESSION II**<br>**13:30 -14:30** | **The economic importance and biology of cereal nematodes**<br>**Chairman: Dr. Zahra Tanha MAAFI, Dr. Shulong CHEN** |
| **13:30-13:45** | 10. M.M.M. ABD-ELGAWAD: Spatial distribution of the cereal cyst nematodes in Egyptian bread wheat (*Triticum aestivum* cv. Giza 68) field |

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| **13:45-14:00** | 11. Hongxia YUAN: Field dynamics and hatching characteristics of two species of wheat cereal cyst nematode in Henan province, China |
| **14:00-14:15** | 12. Honghai ZHAO: The effect of the rotation with peanut on CCN population and wheat yield |
| **14:15-14:30** | 13. Fouad. MOKRINI: Morphometrical and molecular characterization of root lesion nematodes (*Pratylenchus* spp.) on wheat in Morocco |
| **Session III**<br>**14:30-15:45** | **Control strategies of CCN in wheat using host resistance**<br>**Chairmen: Prof. Richard W. SMILEY, Prof. Hongmei LI** |
| **14:30-14:45** | 14. Halil ELEKCIOĞLU: Screening of international spring wheat germplasms against the cereal cyst nematode (*Heterodera avenae*) and the root lesion nematodes *(Pratylenchus thornei* and *P. neglectus*) in-vitro conditions in 2012, Turkey |
| **14:45-15:00** | 15. Hongjie LI: Toward management of cereal cyst nematode with host resistance: identification of effective resistant sources in China |
| **15:00-15:15** | 16. Halil TOKTAY: New sources of resistance against the root lesion nematodes, *Pratylenchus thornei* Sher et Allen in some national and international wheat germplasm |
| **15:15-15:30** | 17. Andreas WESTPHAL: Identifying a novel source of resistance against *Heterodera filipjevi* in spring barley 'Steptoe' by analysis of a double haploid population Steptoe x Morex |
| **15:30-15:45** | 18. Kh.A MOUSTAFA: Selection of certain wheat genotypes for resistance to cereal cyst nematode (*Heterodera avenae*) based on growth parameters and molecular markers |
| **15:45-16:00** | **Tea Break** |
| **Session IV**<br>**16:00-17:00** | **Control strategies of CCN other than host resistance**<br>**Chairmen: Dr. Ricardo HOLGADO, Dr. Yuxi DUAN** |
| **16:00-16:15** | 19. Richard W. SMILEY: Influence of abamectin and *Bacillus firmus* on *Heterodera avenae* and on spring wheat yields in the USA |
| **16:15-16:30** | 20. M.M.M. Abd-Elgawad: Effect of essential oils of some medicinal plants on phytonematodes in Egypt |
| **16:30-16:45** | 21. Al-Hazmi: *Verticillium chlamydosporium*, a fungal parasite of the cereal cyst nematode (*Heterodera avenae*) in the Saudi fields |
| **16:45-17:00** | 22. Hongmei LI: Toward integrated management of cereal cyst nematodes in China: an example for research work in Jiangsu Province |
| **Session V**<br>**17:00-17:45** | **Molecular diagnosis of cereal cyst nematodes**<br>**Chairmen: Prof. Andreas WESTPHAL, Prof. Yanhua Wen** |
| **17:00-17:15** | 23. Gaofeng WANG: Sensitive and direct detection of *Heterodera filipjevi* in soil and infected wheat by species-specific SCAR-PCR assays |
| **17:15-17:30** | 24. Fateh TOUMI: Quantitative detection of *H. avenae* and *H. filipjevi* using qPCR |
| **17:30-17:45** | 25. Guiping YAN: Developing species-specific PCR assays for identification of *Heterodera filipjevi* and *H. avenae* |

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| **18:00-21:00** | **Banquet Yuegongfu (粤公府), Xueyuan South Road No.59, 学院南路 59 号 (农科院南门向东 100 米,魏公村路口东 500 米)**<br>**Contact Dr. Wenkun HUANG** |
| **August 24, 2013** | **Buliding 8, Jiabinlou，First Meeting Room（8 号楼，嘉宾楼第一会议室）** |
| **Session VI**<br>**8:30-9:30** | **Genome and parasitism genes**<br>**Chairmen: Prof. Mike JONES, Dr. Deliang PENG** |
| **8:30-8:45** | 26. Mike JONES: Gene silencing in root lesion nematodes significantly reduces reproduction in host plants |
| **8:45-9:00** | 27. Joong-Ki PARK: Comparative analysis of complete mitochondrial genome sequences confirms independent origins of plant-parasitic nematodes |
| **9:00-9:15** | 28. Dan YANG: Comparison of transcriptome pre- and post-parasitic stages of the nematode *Heterodera avenae* |
| **9:15-9:30** | 29. Changlong CHEN: Identification of an annexin-like parasitism gene from cereal cyst nematode, *Heterodera avenae* |
| **9:30-9:45** | **Tea Break** |
| **Session VII**<br>**9:45-11:45** | **Biological control and IPM**<br>**Chairmen: Prof. Joong-Ki PARK，Prof. Jingwu ZHENG** |
| **9:45-10:00** | 30. Amer DABABAT: Control options against the cereal cyst nematodes *Heterodera filipjevi* in Turkey |
| **10:00-10:15** | 31. Haiyan WU: Occurrence and Development of the Cereal Cyst Nematode (*Heterodera avenae*) in Shandong, China |
| **10:15-10:30** | 32. Yuxi DUAN: The researches of *Heterodera glycines* and resistant soybean germplasm in China |
| **10:30-10:45** | 33. Jie ZHANG: Biocontrol of cereal cyst nematode by two bacteria agents |
| **10:45-11:00** | 34. Kan ZHUO: First report of cystoid nematode in China with notes on *Cryphodera sinensis* n. sp.(Nematoda: Heteroderidae) |
| **11:00-11:15** | 35. Hai LONG: Diagnosis of *Cryphodera brinkmani* intercepted on *Juniperus chinensis* imported into China from Thailand |
| **11:15-11:30** | 36. Xiangxia BU: Temperature-manipulated development of novel cellular and mycelium stages of *Pasteuria penetrans* |
| **11:30-11:45** | 37. Congli WANG: QTL analysis of gene RKN2 in *Gossypium barbadense* which clusters with gene rkn1 in *G. hirsutumfor* transgressive nematode resistance |
| **11:45-12:00** | **Closing remarks** |
| **12:00-14:00** | **Lunch** |
| **14:00** | **Workshop end** |

# Abstracts of Scientific Program

## SESSION I : Global status of the distribution of cereal nematodes

## August 23, 9:10-10:30

## Chairman: Dr. Prof. Halil ELEKCIOĞLU

### 1 Cereal nematodes management strategies in wheat

*A.A. Dababat, G. Erginbas-Orakci, H.J. Braun, and A. Morgounov*

*Amer Dababat: CIMMYT Global Wheat Program, Ankara-Turkey,*

*Corresponding author:a.dababat@cgiar.org*

Soil borne pathogens (SBPs) including the Cereal Cyst Nematode (CCN) caused by *Heterodera* species and the Root Lesion Nematodes caused by *Pratylenchus* species are attack roots of cereal crops and resulting in a high yield loss and reduce grain quality. The damage caused by these nematodes is accelerated in areas where drought exists. A few control options are being used to reduce CCN damage through keeping the population level below damage threshold such as; chemical, biological, cultural, and genetic (resistance/tolerance) practices. Resistance is environmentally friendly and biologically effective once identified. However, up to now, resistance has only been identified against one of the CCN nematodes, *Heterodera filipjevi* in Turkey and foreign wheat germplasm though this resistance is not yet present in high yielding cultivars. Resistance to the other nematodes in the CCNs complex is still being sought. Therefore, alternative approaches limiting the damage caused by CN to wheat are needed. As a result of screening wheat germplasm against the CN hundreds of moderately resistant germplasm to *H. filipjevi* in winter wheat and to both *Pratylenchus* species in spring wheat germplasm are available. In 2012, germplasm with multi disease resistance including *H. avenae*, *Pratylenchus thornei* and *P. neglectus*, and *H. filipjevi* were distributed to international collaborators. The preliminary results of using seed treatments showed that seed treatment of wheat susceptible germplasm gave up to 47% reduction in number of *Heterodera filipjevi* cyst per plant but did not reduce the number of cyst in the resistant germplasm since the cyst number was low and no room to decrease it further.

**2 Resistance and tolerance to *Heterodera avenae* in North American spring wheat**

*R.W. Smiley, J.M. Marshall and G.P. Yan*

*Oregon State University, Pendleton, Oregon, 97801, U.S.A.*

*Corresponding author:richard.smiley@oregonstate.edu*

The cereal cyst nematode, *Heterodera avenae*, reduces wheat production efficiency by >US$3.4 million annually in the Pacific Northwest states of Idaho, Oregon and Washington. Spring wheat trials were conducted in naturally-infested fields in Idaho and Washington during 2012.Twenty cultivars were planted as a split-plot design with each cultivar planted into six replicates of 1.8 × 9-m plots that were either treated with nematicides or were untreated. All plants exhibited the typical root knotting symptom in untreated soil. Mean number of white females was greater ($P < 0.01$) in untreated (9/plant) than in treated soil (<1/plant). In untreated soil, fewer white females were produced on Ouyen and WB-Rockland (1/plant) than on other cultivars(9-29/plant). Post-harvest density of *H. avenae* eggs (from cysts)was higher (>12,000/kg of soil) following growth of susceptible cultivars than following Ouyen and WB Rockland (<5,000/kg of soil). The latter density was similar to that in plots of all cultivars produced in treated soil, indicating a natural background density of *H. avenae* remaining from cysts that had been produced on cereal crops one or two years earlier. Ouyencontains*Cre1* resistance but was intolerant, with grain yield being lower in untreated than in treated plots. WB-Rockland was both highly resistant and tolerant. Two cultivars (Buck Pronto and UI Stone) werehighly susceptible but very tolerant. These were the first field trials in North America that demonstrated the benefits that can be expected fromdeveloping cultivars with resistance plus tolerance to *H. avenae*.

**3 Current Research status and perspective of cereal cyst nematode, (*Heterodera aveane,H. filipjevi*) on wheat in China**

*Deliang PENG, Wenkun Huang, Huan Peng*

*State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, China.*

*Corresponding author::pengdeliang@caas.cn*

The cereal cyst nematode (CCN), *Heterodera avenae* is one of the most important plant parasitic nematode and distributes throughout nearly all cereal growing areas in China. Since 2009, The occurrence and distribution of the cereal cyst nematodes (*H. avenae*) has been confirmed to occurrence in four new provinces including Ningxia, Tianjing, Xizang (Tibet) and Xijiang based on the morphological identification and molecular characterisation, so CCN has been confirmed to distribute 16 provinces in China. Yield losses were tested with inoculation and field, in Henan, Hebei, Beijing suburb,Qinghai, yield losses reach up to18%-35% in Henan,15-20% in Hebei, 11-18% in Beijing Suburb and 10%-28.24% in Qinghai, The population dynamics and life cycle of H.avenae were investigated in Beijing, Hebei, Jiangsu and Shandong from 2010 December to 2012 December. The results showed that there is one generation of *H. avenae* per year in those area above mentioned. There are two cyst nematodes *(H.avenae and H. filipjevi )* occurrence in the wheat production area of China, the *H.avenae* is dominate species.The molecular diagnosis methods based on SCAR-PCR were developed to identify and early detect *H. avenae* and *H. filipjevi* from infested field. The pathotypes of twenties population of *H. avenae* from Beijing, Hebei, Jiangsu and Shandong were tested and identified by using the International Test Assortment (CIMMYT provide). The results showed that Qingyundian population (*H.avenae*) of Beijing was different from the 13 pathotypes which have been described and nominated. It`s slightly similar with pathotype Ha23. More than two huandrands cultivars were tested and evaluated resistance to *H. avenae* and *H. filipjevi* in greenhouse and the field respectively. The results showed that no immune materials were found. Five cultivars including VP1620, BATAVIA, SUNR23, AUS4930 6.5/GS50a and Taikong 6 were high resistance to CCN Beijing population.

A cDNA libarary from the second stage juvniles of *H. avenae* was constructed for exploring more candidate parasitism genes. 5800 ESTs were generated and 2568 unigenes were obtained. Three β-1,4-endoglucanase genes (*Ha-eng-1a,Ha-eng-2* and *Ha-eng-3*) expressed in the pharyngeal glands of the sedentary cyst nematode(*H. avenae*,)were cloned. The cDNA of *Ha-eng-1a* encoded a deduced 463-amino acid sequence containing a catalytic domain and a cellulose binding module separated by a linker. The genomic DNA of Ha-eng-1a is 2,129-bp long, containing eight introns ranging from 56 bp to157 bp and nine exons ranging from 70 bp to 299 bp. Southern blot analysis revealed that two copies of the *Ha-eng-1a* gene are present in *H. avenae*. *In situ* hybridization showed that the *Ha-eng-1a* transcripts specifically accumulated in the two subventral gland cells of the second-stage juveniles.

**Acknowledgement:** This Research was supported by Chinese Special R & D Fund for Public Benefit Agriculture（200903040） and Ministry of Science and Technology (2009DFB30230).

## 4 Cereal cyst nematodes and its research perspectives in Iran

*Tanha Maafi, Z[1], Ahmadi, A.[2] and Dababat, A.[3]*

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[2]*Agricultural Research and Natural Resources Centre of Khuzestan, Ahvaz, Iran. alirahmadi2000@ gmail.com;*

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Wheat is considered one of the most important strategic crops in Iran. Reducing the biotic and abiotic factors affecting the wheat yield production is one of the main priorities for the plant protection research sector in the country. Cereal nematodes are plant pathogenic agents combined with other fungal, viral and bacterial pathogens could cause severe damage on wheat production in Iran. Cereal cyst nematodes (CCN) have been identified in the main wheat producing regions, comprising of three species, in order of their abundances are *Heterodera filipjevi*, *H. latipons* and *H. avenae* type B. Progress has been made in the morphological and molecular identification, due to high overlapping in morphometric characters of *H. avenae* type A, B and *H. filipjevi* confirmation of morphological identification by molecular marker such as rRNA-ITS-RFLP has become an essential component of the recognition. Several research studies on the distribution and population density indicated that cereal cyst nematodes are widely distributed in most wheat producing fields with various population densities. The economic importance assessments demonstrate that these three species possess the potential to damage the grain wheat particularly under the rain-fed and drought conditions, the situations which were observed in most of the sampled regions. Although the damage threshold should be studied for each region and determined under different environmental conditions such as: availability of water and nutrition, genotypical factors *i.e* tolerance and resistance of cultivars. The other main priority in CCNs researches should be concentrated on the sources of genetic resistance to cereal cyst nematodes in Iranian cultivars and landraces of wheat that are unknown. Meanwhile, pathotype characterization of the Iranian populations of cereal cyst nematodes that could assist to some extent to determinant their genetic diversity is the other principal subject for achieving the resistance resources.

## SESSION II The economic importance and biology of cereal nematodes
## Chairman: Dr. Amer DABABAT, Prof. Jinling LIAO

### 5 The biology of Cereal Cyst Nematode in Hebei: implications on its control

*Li Xiuhua Ma Juan Gao Bo Wang Rongyan Chen Shulong*

*Institute of Plant Protection, Hebei Academy of Agricultural and Forestry Sciences/ IPM Center of Hebei Province/ Key Laboratory of Integrated Pest Management on Crops in Northern Region ofNorth China, Ministry of Agriculture, P. R. China, Baoding 071000, Hebei Province, China*

Cereal cyst nematode is one of most important diseases in wheat production in Hebei, China. The biology of the nematodes was studied systematically for optimizing the control strategy. The nematode species were identified by morphological and molecular characteristics. The natural weed hosts in wheat field were investigated. The resistance of 41 local cultivars to the nematodes was evaluated in the pot and the field by counting the number of cysts produced. The hatch characteristics were tested with the cysts sampled from the natural fields in different periods. Effects of different temperatures on the nematode penetration and development were tested in mini pots. Life cycle was investigated in three fields for three years. The toxicity of different nematicides to eggs and second stage juveniles were tested in vitro. Effect of rotation, fallow and continues cropping on population dynamics of the nematodes were evaluated in natural fields. The results showed that only Heterodera avenae were detected in Hebei. Avena fatua and Aegilops squarrosa were the most important wild hosts for H. avenae in wheat fields. Three cultivars were resistant among the 41 cultivars tested, and four cultivars were highly tolerance to the nematode infection. The nematodes hatched at 5～10℃ but not for over 15℃ in 19 weeks for the newly formed cysts (June), while the hatching peak of H.avenae collected in wheat sowing period (October) was in 10～12 weeks and in 6～8 weeks at 5℃ and 15℃ respectively, but the optimum temperature for hatching of the nematode collect from natural field in December was 20℃.The optimum temperature for penetration is 16℃ and the optimum temperature for nematode development and cyst forming is 18～22℃. Second stage juveniles could be extracted from soil all year round except the early and mid ofJune. There was a small peak of hatch before soil freezing, but most of nematodes hatched in the early and mid of April. The peaks of third stage juveniles, fourth stage juvenile and female occurred in the early, mid to late and late of May respectively. Second stage juveniles were more sensitive to the nematicides tested than the eggs or cysts. Population of H. avenae reduced by 89.8% after one year's fallow. The reducing rate was 93.8% after rotation with eggplant for one year, while it was 90.7% and 98.8% after rotation with the muskmelon or melon for one and two years respectively. In the natural disease fields, the population of the nematodes was increased by 36.8% and by 49.2% after wheat continuous cropping for one and two years respectively. The control of the H. avenae should integrate the resistance cultivars, weed eradication, fallow or rotation and chemical control.

**Acknowledgments**: We appreciate the financial support provided by the Ministry of Agriculture of China (200903040) and Ministry of Science and Technology of China (2009DFB30230).

### 6 Crop loss assessment of *Heterodera filipjevi* on some cultivars of wheat, barley and triticale under field condition of Southwest of Iran

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Khuzestan province is ranked as the first cereal producing province in Iran during 2011-2012. The cereal cyst nematode, *Heterodera filipjevi* is widespread in the main producing cereal fields in the region. It was found in 38% of the surveyed wheat fields of the province according to the recent studies. The damage assessment of *H. filipjevi* was performed on different yield components of four spring wheat cvs. Chamran, Virinak (bread wheat), Yavarus and Behrang (durum wheat), barley line wb/19/10 and Triticale cv. Juvanilo in an experiment under *Heterodera filipjevi* infested field in Ramshir region during 2008-2009. The trial was arranged in a randomized complete block design. eEach treatment replicated seven times, consisting of plots with or without nematicide application (aldicarb 10G) at sowing time. The population density was determined before sowing to evaluate the initial population (Pi) and after harvesting for the final population (Pf). The obtained results indicated significant reduction of grain yield, biomass, shoot weight, shoot height and tillering by 52 (40-73), 40 (14-53), 38 (6-67), 15 (8-21) and 24 (10-39) percent in the different cultivars, respectively. The initial population was 10 eggs and juveniles/ g of soil. The nematode reproduction factor in plots plus and minus nematicide were ranged between 0.7-2.14 and 0.9- 4.52 respectively. This is the first evidence of the cereal yield losses caused by *H. filipjevi* in natural infested field conditions in Iran.

## 7 Importance of identification cereal cyst nematodes species and patothypes in breeding for resistance and for management strategies

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Cyst nematodes are usually recognised by the morphology of the adult female (cyst) and by host plant associations. But, there are risks of misidentification if identification only relay on host plant. For more than 90 year the potato cyst nematode, the beet cyst nematode and cereal cyst nematode were considered as one species. In 1923 Wollenweber classified *Heterodera rostochiensis* as separate species. The cereal cyst nematode and the beet cyst nematode were considered as two races until 1940 when Franklin demonstrated a difference in length of second stage juveniles (J2).Later it was agreed to designate cereal cyst nematode as *Heterodera avenae* Wollenweber.It is known that cereals crops can be parasitised by a group of cereal cyst nematode involving a number of species, which differ morphologically and biologically. The most common and important species is *H. avenae*, but the "*Heterodera avenae*-complex" (Stone and Hill, 1982) includes also *H. arenaria*, *H. avenae*, *H. aucklandica*, *H. australis*, *H. bifenestra*, *H. filipjevi, H. hordecalis, H. latipons, H. mani, H. pratensis, H. spinicauda and H. ustinovi* (*synonym H. iri*). The virulence on cereal cultivars differs between and within species and several pathotypes occur among them. Borderlines between species and pathotypes often appear unclear. Identification usually requires morphometric studies including cyst size, colour, cuticular patterning, and several featuresof the cyst cone. The latter include the length of the vulval opening, the structure of the cyst wall around the vulva and in the perineal area, as well as internal structuresof the vulval cone (bullae, underbridge, and vaginal sheath). Specific diagnostics also rely upon features of the J2, notably body length, number of lateral lines on the cuticle, and length and shape of the stylet and tail. Identification could be assisted by molecular approaches that can distinguish some species. Recognition and definition of pathotypes as a sub-specific grouping depend upon tests with plants with genetically distinct resistance named host differentials. True pathotypes are not yet distinguishable by molecular tools. Species of cereal cyst nematodes have had a long co-evolution with the host and progenitors. Consequently, there are many host resistance genes matching the virulence genes in the nematode populations. As breeding new cereals varieties may reduce the numbers of resistance genes; a decisive consideration for resistance breeding is to be aware of the degree of heterogeneity in cereal cyst nematode species and populations. Furthermore it is essential to pay attention to the fact that field population may contain several species and patothypes.

Nematode management practices must be based on the knowledge of the population dynamics, the population density required to cause economic damage and the measures capable of reducing or keeping the population density below the threshold for economic damage.

In Norway management systems based on precise identification of nematode species and pathotypes and good knowledge on appropriate resistant cultivars are in operation. Resistant barley is generally recommended when nematode populations are high due to its high tolerance compared to resistant oats. Farmers implementing this program have reported increased cereal yields on the average of 1000 kg /ha. It has been calculated that by implementing this program in full, the county of Vestfold could make an economic gain of 800 000 € annually.

## 8 The effect of the cereal cyst nematode,*Heterodera avenae*, on wheat yield in Shangdong province

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Forevaluating theimpact of*H. avenae* on yield of wheat in Shandong province of China,pot and field trials were conducted with different initial population densities (*Pi*) inoculated in soils. In pot trials, as the increasing of eggs per gram soil from 5-30, the chlorophyll contents of flag leaf decreased from 10.89% to 34.46%, dry weight of roots decreased from 30.65% to 73.95%, dry weight of shoots decreased from 13.93% to 58.68%, number of spikes decreased from 30.48% to 53.33%, grain numbers decreased from 3.97% to 36.93%, weight of spikes from 30.14% to 68.84%. The reproduction factors significantly decreased from 3.40 to 1.52 with the increase of initial egg density of cereal cyst nematode from 5 to 30 per gram soils, which negatively correlated with the production indexes. While under the field conditions in Feicheng city, there had no significant effect of different initial egg densities of cereal cyst nematode on the above production indexes of wheat.

**Key words:** Cereal cyst nematode;initial densities; yield, Fitting model

**Acknowledgement:** This Research was supported by Chinese Special R & D Fund for Public Benefit Agriculture（200903040).

## 9 Studies on cereal cyst nematodes, *Heterodera avenae* Wollenweber, in South East Anatolia and Eastern Mediterranean Regions in Turkey

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Cereal cyst nematodes (*Heterodera* spp.) are a major constraint to cereal production in many parts of the world and contain at least 12 species that invade roots of cereals and grasses. *Heterodera avenae* (Wollenweber, 1924), *H. filipjevi* (Madzhidov, 1981), *H. latipons* (Franklin, 1969) are recognized as the most economically important species in this genera. *H. avenae* has wide distribution in temperate wheat-producing regions and it was first detected in Turkey in 1974 and is now known to occur in many cereal growing parts in Turkey. The objectives of this study were to focus on identification of cereal cyst nematodes (CCN) in cereal fields of South East Anatolian and Eastern Mediterranean region and finding out inter and intra species genetic variations of *Heterodera* species; to determine the biological features (hatching) of *Heterodera avenae* under in vitro; to investigate the pathotypes of *Heterodera avenae* using international host differential test materials in Eastern Mediterranean region; and to screen some wheat varieties (international, national wheat and wild wheat relatives) against *Heterodera avenae* and estimating yield loss caused by *H. avenae* in field condition. The result, based on phylogenetic analysis using ITS-rDNA sequences and morphological analysis; *H. avenae*, *H. filipjevi*, *H. latipons* species were identified. The highest cumulative hatching of 85.6% was obtained at a constant temperature of 15 ºC for 283 days, the lowest cumulative hatching of 16.6% was obtained at 25 ºC for 283 days. Nematode populations were found completely belong to Ha1 group Ha21 pathotype and this study is the first report to determine *H. avenae* pathotype in Turkey. Four national wheat varieties, seventeen wheat wild genotypes and twenty three international wheat genotypes were found to be moderately resistant against Ha1 group, Ha21 population of *H. avenae*. The average yield loss varied from 1,19% to 21,8% in Eastern Mediterranean region.

**Key words:** *Heterodera* spp., hatching, pathotype, screening, yield loss

## 10 Spatial distribution of the cereal cyst nematodes in Egyptian bread wheat (*Triticum aestivum* cv. Giza 68) field

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The spatial distribution of the cereal cyst nematodes, *Heterodera* spp., in 12 different 350-$m^2$-plots of bread wheat (*Triticum aestivum* cv. Giza 68) field was studied. The dispersion parameter, viz. mean–variance test, of the nematode larvae in soil indicated agreement with a Poisson model at the 95% probability level and the hypothesis of randomness was not disproved in any of the sampled plots. Chi squared ($x^2$) test for goodness-of-fit across all the plots proved that the same model was a good fit to the original nematode counts too. Yet, Taylor's Power Law was not fit ($P \leq 0.05$) to *Heterodera* spp. population data obtained from the plots. Sample size optimization needed to achieve a predetermined level of sampling error for the nematodes was calculated. For example, to sample cereal cyst nematode larvae with a 0.20 or 0.1 standard error to mean ratio and 11 nematodes/100 gm soil, one could collect 2 or 7 samples of 5 cores each, respectively. Integrated nematode management practices in the light of their random distribution were discussed.

## 11 Field dynamics and hatching characteristics of two speices of wheat cereal cyst nematode in Henan province

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The distribution and infection dynamics of *Heteroderaavenae* and *H. filipjevi* were studied in the fields in Henan province.The hatching characteristics of *H. avenae* and *H. filipjevi* at different temperatures were studied. The preliminary results were as follows.

(1) The test field in Xuchang(*H. filipjevi*) had 60 plots, the field in Xingyang(*H. avenae*) had 48 plots, and each plot was 49m$^2$. The horizontal samples analysis result indicated that every plot had cereal cyst nematodes, but the densities of cyst were different in different plot. The highest cyst density was 36 per 100g soil, the lowest was 4.3 per 100g soil in Xuchang field. The highest cyst density of plot was 38.7 per 100g soil, and the lowest density of plot was 4.3 per 100g soil in Xingyang field. The vertital samples analysis result indicated the number of cysts was the highest in 5-15cm soil layer. The number of cysts was tapering along with depth increasing of soil layer. The cysts of field in Xingyang were mainly distributed in 10-15 cm soil layer, the average number was 98.3 per 100g soil. The cysts of field in Xuchang were mainly distributed in 5-10 cm soil layer, the average number was 66.7 per 100g soil.

(2) The wheat roots were infected by the second-stage juveniles two weeks (early November) after planting in the two fields. A few third-stage juveniles were found four weeks after planting. The first infection peak of the second-stage juveniles comes in the 6$^{th}$ week after the planting.With the temperature got lower 60 days after planting in the winter, the number of the larva of different stages remained stable.120 days after planting, with the temperature rising in the spring, the number of the second stage juveniles started to increase and reached to the second peak in the late March and early April,but the number of juveniles which invaded the wheat roots in the second peak were apparently less than the first one.The larva gradually developed into white females and cysts.The infection dynamics of two species was approximately alike,but the third stage,fourth stage of the larva and the white females of the *H.filipjevi* appeared one week earlier than that of *H.avenae*.

(3) The cysts were collected from the fields of Xuchang and Xingyang in the middle of August.The influence of the temperature on the hatching of the two species was studied indoors. The results showed that *H. avenae* could hatch at 5°C,10°C,15°C,20°C and 25°C,*H.filipjevi* could hatch at 5°C,10°C and 15°C,but it could hardly hatch at 20°C and 25°C.The most suitable temperature of hatching was at 10°C or 15°C. The second-stage juveniles started to emerge on the 20$^{th}$ day. The hatching peak appeared on the 50$^{th}$to the 60$^{th}$ day. There were more second-stage juveniles of *H.filipjevi* than that of *H.avenae.*The hatching continued until the 75$^{th}$ day. Changing the temperature could increase the number of the second-stage juveniles dramatically, and the hatching rate reached 83%.

**Acknowledgement:** This Research was supported by Chinese Special R & D Fund for Public Benefit Agriculture（200903040).

12 **The effect of the rotation with peanut on CCN population and wheat yield**

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*Heterodera avenae*（CCN）is the most important nematode damaging wheat in China. It was numerously reported that juvenile emergence from eggs stopped in newly formed brown cysts and the break of dormancy required low temperature treatment for a relative long time. Free juveniles were also found out of the egg shell in newly formed brown cysts with different juvenile-egg ratio during our CCN investigations. The test was implemented to determine the activity of free juveniles and eggs in newly formed brown cysts. Free juveniles and eggs were isolated under room temperature (22~30 ℃), afterwards some of them were inoculated onto wheat seedlings immediately and some were treated at low temperature (5℃) for different length of time before the inoculation. Wheat seedlings were earlier planted in paper cups, and incubated under 15~16℃ in light incubator after each inoculation. Wheat roots were taken out and stained to detect infection situations after 3~4 weeks of incubation. It was found that no infection occurred for both juveniles and eggs without low temperature treatment; infection occurred only for the juveniles which had been treated with low temperature for 20, 30 and 45 d, and for the eggs treated with low temperature for 10, 20, 30 and 45 d. The results indicated that dormancy happened on the juveniles as well as eggs in newly formed brown cysts and the encysted juveniles required low temperature treatment to activate the ability of infection or even emergence from cysts.

**Key words:** *Heterodera avenae*; newly formed brown cysts; encysted juvenile; dormancy

**Acknowledgement:** The research was supported by Special Fund for Agro-scientific Research in the Public Interest in China (No.200903040)

## 13 Morphometrical and molecular characterization of root lesion nematodes (*Pratylenchus* spp.) on wheat in Morocco

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Root-lesion nematodes of the genus *Pratylenchus* have a worldwide distribution and are regarded as severe production constraints for numerous important crops. A rapid and accurate method to identify *Pratylenchus* to the species level is necessary to develop appropriate management strategies. During a survey of wheat-growing areas of Morocco from May to June 2011, 18 populations of the root-lesion nematode were collected. The nematodes were extracted from root and soil using an automated apparatus based on centrifugal floatation. They were identified on the basis of their morphological and morphometrical characters, complemented by molecular methods. The morphometrical observations of the collected females and males demonstrated the occurrence of *Pratylenchus penetrans* in most of the samples; *Pratylenchus thornei and P. pseudo coffeae* were only detected in samples from Zaere and Settat, respectively. After morphometrical identification, DNA was extracted from a single individual that was hand-picked. The duplex PCR primers described by Waeyenberge *et al*. (2009) were used to identify *P. penetrans*; the species-specific forward primer PTHO and the common reverse primer D3B (Al-Banna *et al*., 2004) were used to identify *P. thornei.* For the remaining populations that were not identified by species-specific primers, the D2D3 expansion segments of the 28SrRNA gene were amplified with the forward D2A and reverse D3B primers (Joyce *et al*., 1994). The purified PCR products were sequenced using the same primers. The obtained sequences were compared with those of *Pratylenchus* species available in the GenBank database (www.ncbi.nlm.nih.gov). This comparison confirmed the morphological identifications. The study of the phylogenetic relationship of the Moroccan *P. penetrans* populations together with other populations of *P. penetrans* and some species of which the sequence was available in GenBank (NCBI) showed a high similarity (98,5%) between all *P. Penetrans* populations from Morocco. This is the first report on molecular characterization of *Pratylenchus* populations from Morocco.

## Session III：Control strategies of CCN in wheat using host resistance
## Chairmen: Prof. Richard.W. SMILEY, Prof. Hongmei LI

### 14 Screening of International spring Wheat Germplasms against the Cereal Cyst Nematodes (*Heterodera avenae*) and the Root Lesion Nematodes (*Pratylenchus thornei* and *P. neglectus*) *in-vitro* Conditions in 2012, Turkey

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The occurrences and distribution of Cereal cyst nematodes (*H. avenae*, *H. filipjevi* and *H. latipons*) and Root lesion nematodes (*P. thornei* and *P. neglectus*) have been surveyed in research projects in different part of Turkey. The most environmentally sound methods to control these nematodes is the use of resistant germplasm. Therefore, international, varieties have been screened for their resistance reactions to those nematodes at the Biological Control Research Station in Adana-Turkey. The International Maize and Wheat Improvement Centre (CIMMYT), Turkish Food, Agriculture and Livestock Ministry and in collaboration with Cukurova University have been conducted screening project to understand the resistant reactions of International spring wheat varieties and lines against to *H. avenae*, *P. thornei* and *P. neglectus* in nematology part of the Soil Borne Pathogen Project since 2001. In this aspect 29 SAWSN was investigated resistance against to these nematodes in 2012. The experiments were established with 7 replicates and two biological repetitions. Each line were germinated and planted individually in small tubes filled with 80 g soil mixture. 400 nematodes for *P. thornei*, *P. neglectus* were inoculated to each tubes on seventh planting day and 200 $J_2$ for *H. avenae* onplanting day. Plants harvested after 9-12 weeks and the number of *P. thornei, P. neglectus* and *H. avenae* cysts and juveniles ($J_2$) per plant were counted. Totally 5292 seeds were tested against to *P. thornei, P. neglectus* and *H. avenae* during the 2012 screening program. The results indicated that several wheat lines with multiple resistances to *H. avenae*, *P. thornei* and *P. neglectus* have been identified. This result gave significant contribution to genetic resistance against *H. avenae*, *P. thornei* and *P. neglectus* in international wheat breeding programs.

**Key words:** Screening, Resistance, *H. avenae*, *P. thornei, P. neglectus*

**Acknowledgment:** CIMMYT and the Turkish Ministry of Agriculture would like to thank the Grain Research and Development Corporative GRDC for funding this project and Syngenta for partial money support to present this work at this meeting.

## 15 **Toward management of cereal cyst nematode with host resistance: identification of effective resistant sources in China**

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Recent prevalence of cereal cyst nematode (CCN, *Heterodera avenae* and *H. filipjevi*) throughout the important wheat growing regions of China has attracted serious attentions. Unfortunately, the shortage of resistant resources has limited the use of host resistance to control CCN. Since 2008, a project has been initiated to identify effective sources of resistance to the prevalent pathotypes of *Heterodera* spp. indigenous to China. Based on a three-year field test, resistance of the wheat cultivar Madsen from Washington State University, USA was effective against both species of *Heterodera*. Results of histological analysis indicated that the number of juveniles invaded the roots of Madsen was fewer than the susceptible control Wenmai 19. Inoculation tests with 11 pathotypes of *H. filipjevi* and *H. avenae* from Henan, Anhui, and Shandong provinces demonstrated that Madsen was effective against all the populations of both species of *Heterodera*. Genetic analysis indicated that a single dominant gene was associated with the resistance of Madsen to *H. filipjevi*, which permits the transfer of its resistance to CCN in to more adapted local cultivars. Some advanced lines have been produced, which have shown to be resistant to *H. filipjevi* and *H. avenae* as effective as the donor parent Madsen. In addition to resistance to *H. filipjevi* in Madsen, a number of *Triticum durum* and wheat-*Thinopyrum* derivatives, *Triticale*, *T. dicoccum*, and *T. dicoccoides* accessions exhibited excellent resistance against CCN in the field tests. Since most commercial wheat cultivars currently grown were susceptible, these resistant germplasms provide valuable sources for improvement of CCN resistance in China.

**Acknowledgement:** The research was supported by Special Fund for Agro-scientific Research in the Public Interest in China (No.200903040)

## 16 New sources of Resistance against the Root Lesion Nematodes, *Pratylenchus thornei*Sher et Allen in some National and International Wheat Germplasm

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Root Lesion Nematodes (RLNs) are widespread throughout the grain growing regions of Turkey. *Pratylenchus thornei* Sher et Allen is the predominant species of RLN in the and causes a yield losses of up to 19% in the Eastern Mediterranean Region. Due to the huge range of hosts to the RLN makes crop rotation is a weak option to control its damage. Therefore, breeding wheat germplasms for resistance to this destructive nematode will be an effective and economical method of minimizing crop losses by preventing nematode reproduction and reducing the overall disease problem by leaving fewer nematodes in soils to attack subsequent crops. Globally there are several known sources of genetic resistance in wheat against root lesion nematodes, However, these sources are still not well documented whether they are effective in Turkey or not. In this study, 90 international and 30 national wheat varieties have been screened under the *in vitro* conditions against a common root lesion nematode (*P. thornei*) at the Biological Control Research Station in Adana-Turkey. The results of this screening indicated that eight national wheat varieties and fifty-nine international wheat genotypes were found to be moderately resistant against Adana populationof *P. thornei.* Among these genotypes, the national bread wheat variety, Adana 99 (PFAU/SERI82//BOG"S") approved gave resistant reaction to *P. thornei*. In conclusion, those national and international genotypes are of high value to the Turkish and regional breeding programs. However, testing more varieties against more population of *P. thornei* is still needed.

**Key words:** Root Lesion Nematodes, *Pratylenchus thornei,* Screening.

## 17 Identifying a novel source of resistance against *Heterodera filipjevi* in spring barley 'Steptoe' by analysis of a double haploid population Steptoe × Morex


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Cereal cyst nematodes (CCN) damage small grain crops in many regions of the world. In Europe, these pests have become known for their infectivity on emerging spring cereals. A newly recognized species of the CNN complex is *Heterodera filipjevi*, which is similarly spread as *H. avenae* and causes damage in arid areas among others in the Pacific North West of the US, the Mediterranean and China. Resistance to *H. avenae* may not be effective against *H. filipjevi*. A resistant response to *H. filipjevi* was found in *Hordeum vulgare* 'Steptoe' while this cultivar was susceptible to *H. avenae*. The objective of the current study was to determine the chromosomal location of the resistance to *H. filipjevi* in Steptoe by use of a Steptoe × Morex double haploid population. In the greenhouse in small growth containers, a total of 93 double haploid lines, along with the resistant and susceptible parents, were tested if they permitted *H. filipjevi* to reproduce. Genetic mapping using a dense framework of single nucleotide polymorphism (SNP) markers allowed for precise positioning of the resistance locus on chromosome 3H. The development of markers appropriate for marker assisted selection will help breeders to effectively incorporate resistance into elite germplasm.

## 18 Selection of Certain Wheat Genotypes for Resistance to Cereal Cyst Nematode (*Heterodera avenae*) Based on growth parameters and Molecular Markers

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The cereal cyst nematode (CCN), *Heterodera avenae* Woll. causes severe damage to wheat (*Triticum aestivum* L.) production in the Kingdom of Saudi Arabia. This study aimed to screen certain wheat genotypes for resistance to CCN to aid in the development of new resistant cultivars of bread wheat adapted to Saudi Arabian conditions. The field performance of 17 genetically diverse wheat genotypes (local and international materials) were evaluated for two successive years (2009 and 2010) in a *H. avenae*-naturally-infested field. Results showed that the tested wheat genotypes were significantly different in their field performance, and in their resistance to CCN. Ten local wheat genotypes were designated as resistant. The local cv. KSU 119 was the most resistant one (no. cysts/plant= 0.7) among all the tested genotypes. However, the Australian cv. AUS-30851 and the SIMMYT cvs. 15 SAWYT-30, 15 SAWYT-31, 15 SAWYT-38, and 15 SAWYT-42 plus the susceptible standard Yecora Rojo were found to be the most susceptible (no. cysts/plant= 18-28) genotypes in this study.

Microsatellite markers linked to *Cre1* and *Cre3* genes were used in this study. It was found that ten out of 17 wheat genotypes (LNM-72, LNM-99, LNM-126, LNM-136, KSU118, L11-8, L11-17, L11-21, KSU 119, and AUS-30851) had both *Cre* genes. The dendogram generated using SSR data divided wheat genotypes into two main clusters. Genotypes LNM-72, LNM-99, LNM-126, LNM-136, KSU118, L11-8, L11-17, L11-21, and KSU 119 were found in the same sub-cluster. These genotypes were found to be the most resistant to CCN. Therefore, amplification conditions for *Cre3* and *Cre1* loci were optimized and are now used in our marker-assisted selection (MAS) programs to identify CCN-resistant wheat genotypes.

**Key words:** Wheat, *Heterodera avenae*, Pathotypes, PCR, Marker Assisted Selection (MAS).

## Session IV: Control strategies of CCN other than host resistance
## Chairmen: Dr. Ricardo HOLGADO, Dr. Yuxi DUAN

### 19 Influence of abamectin and *Bacillus firmus* on *Heterodera avenae*and on spring wheat yields in the USA

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The cereal cyst nematode, *Heterodera avenae*, reduces wheat yields in the Pacific Northwest (PNW) states of Idaho, Oregon and Washington. Documentable annual economic losses have been estimated at US$3.4 million but actual losses are thought to be much greater. Seed treatments that are effective against plant-parasitic nematodes on other crops were evaluated on spring wheat. Abamectin (Avicta) and *Bacillus firmus* (Votivo) were examined in field trials in each of the PNW states. Aldicarb (Temik), banded with the seed, was included as a treatment for comparative purposes. The initial density of *H. avenae* eggs plus juveniles, from cysts, in the three fields averaged 2332, 2860, and 6666/kg of soil. The first two locations were also infested by high, economically important densities of *Pratylenchus neglectus*; 2602 and 13596/kg of soil, respectively. Abamectin and *B. firmus* had no effect on grain yield or on the incidence or severity of the root knotting symptom caused by *H. avenae*. These products also failed to reducethe post-harvest density of *H. avenae* or *P. neglectus*. Aldicarb increased the mean grain yield by 949 kg/ha, valued at $312/ha. Aldicarb also reduced the severity of root symptoms and reduced the post-harvest density of *H. avenae* by 38% to 93%. However, aldicarb is not, and cannot, be registered for commercial use on wheat in the USA. We concluded that management of cereal cyst nematode can be achieved most effectively through further emphasis on crop rotations and on development of wheat cultivars with both resistance and tolerance to *H. avenae*.

## 20 Effect of essential oils of some medicinal plants on phytonematodes in Egypt

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The phyto-extracts have advantages over synthetic nematicides because of their new compounds – with usually less concentrate than synthetic chemicals - that nematodes are not able to inactivate, multiple action modes, and formation of renewable sources. Therefore, ethanol extract of *Tagetes lucida*, a medicinal plant recently introduced into Egypt, was tested and compared with other plant species *Achillea millefolium*, *Cymbopogon citratus*, *Artemisia annua* and *Calendula officinalis* for their nematicidal activity against plant-parasitic nematodes of common occurrence. All extracts inhibited ($P \leq 0.05$) motility of *Meloidogyne incognita*, *Criconemella* spp., *Helicotylenchus* spp., and *Pratylenchus* spp. *A. annua* was generally more effective in reducing the numbers of active nematodes tested than others except *T. lucida*-root extract which was superior to herbal extract. When transferred to water, the total nematodes that regained their activeness ranged from 15% for *T. lucida* to 38% for *A. millefolium* after 24 hours and from 4% for *C. officinalis* to 39% for *A. millefolium* after 72 hours.The extracts inhibited *M. incognita*-juvenile hatching in the range 46.8% for *A. millefolium* to 88.8% for *C. citratus* compared to 5.1% at the controls. The nematicidal activity of the isolated materials from the plants was concentration dependent. The main compound(s) of each plant extract determined by GLC analysis was presented and their mechanisms of action were discussed. Further studies are warranted to obtain insights on rates and timing of their possible application as bio-nematicides, as well as growth parameters of treated plants under greenhouse then field conditions in Egypt.

## 21 ***Verticillium chlamydosporium*, a fungal parasite of the Cereal Cyst Nematode (*Heterodera avenae*) in the Saudi Fields**

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Cereal cyst nematode (*Heterodera avenea*) is a devastating root parasite of wheat and barley in Saudi Arabia. During a survey of *H. avenae* in the infested wheat and barley fields in Hail and Tabuk regions, north Saudi Arabia, we noticed a heavy colonization of some extracted cysts with a fungal mycelium. The fungus colonized the encysted eggs as well, and was morphologically identified as *Verticillium chlamydosporium*. The fungus had optimum growth on yeast extract peptone-glucose liquid medium at 25 ºC. Pathogenicity of the fungus on the eggs and newly formed cysts of *H. avenae* was evaluated under laboratory conditions. Further studies are being carried-out to identify the strain of the fungus on molecular basis, and also to determine the role of the fungus as a biocontrol agent for *H. avenae* on wheat plants

**Key words:** barley, egg parasites, infected cysts, nematophagous fungi, wheat

## 22 Toward Integrate management of cereal cyst nematode (CCN) in China: an example for research work in Jiangsu Province

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The cereal cyst nematode on wheat was first discovered in Jiangsu Province during May of 2009. The wheat growing area of Jiangsu province is about 2.3 Mha and listed as $5^{th}$in China. The well understanding of disease caused by CCN is urgently needed for making effective control strategies.

A systematic investigation of CCN distribution was carried out during 2009-2011. CCN was detected from 301 out of 580 investigated fields which from 152 towns of 47 counties in Jiangsu province. The widely distribution has caused serious damage to wheat production and the population densities of CCN in some areas were much higher than the economic threshold of losses. All Jiangsu populations of CCN were identified as *Heterodera avenae* by morphological and molecular characters and the status of *H. filipjevi* is unknown.

The life cycle of *H. avenae* on winter wheat in Peixian, Jiangsu Province was investigated systematically during two wheat growing season in 2010-2012. Peixian population finished only one life cycle during the whole growth season. Only few hatched second stage juveniles (J2) infected roots before wheat over-wintering and none of them developed in normal. The hatch peak appearing in soil and massive infection to roots was happened at the end of February in each year. Attassel period, the white females appeared on roots which can be seen by naked eyes and some males can be observed from roots and soil. At maturation period of wheat, the white females changed into brown cysts and fell into soil for over-summering.

Planting resistant cultivars is the most economical and practical measure for controlling the soil-borne disease caused by CCN. Optimizing the conditions of bioassay is important for the accuracy of resistance/susceptible evaluation for wheat cultivars. The cysts pre-treated at 4°C for 8 weeks and further incubated at 15°C obtained a large amount hatching J2 in short period, which can be used as inoculum for bioassay. The wheat seedlings planted in different size of containers with inoculum density of 4 juveniles per $cm^3$ soil produced the largest number of white females. The screening bioassay using the optimized conditions revealed only Huamai No.1, Wenliang 58 and Yumai 66-18 out of 40 wheat cultivars was evaluated as highly resistant to Peixian population of *H. avenae.* At the meantime, the field trial evaluated only Huamai No.1as resistance, which can be used as the prospective resistant cultivar planting in CCN heavily infested area of Jiangsu province.

The control effect of five granular nematicides (GR) with different usages was evaluated on CCN in wheat field during regreening stage. Although the numbers of cysts in soil collected from different treatments were clearly dropped after nematicide application, there is no significant difference in corrected cyst reduced rates between different nematicide treatments. Due to the effect of 0.5% Avermectin GR on inhibiting the reproduction of nematodes and improving the growth of wheat, the usage of 30 kg/ $hm^2$ was suggested to be applied in regreening field heavily infested with CCN, which might decrease the yield loss to some extent. Furthermore, economical seeds-coating chemicals were screened and tested for CCN control. The result revealed the self-patented Gannong seed coating Ⅲ not only has the better control efficacy for CCN, but also has characteristics of environmental safety, lower toxicity, labor and cost saving, which is suitable for widely application in China.

**Key words**: *Heterodera avenae*; distribution; species identification; life cycle; bioassay optimization; resistance evaluation; integrated control

**Acknowledgement:** The research was supported by Special Fund for Agro-scientific Research in the Public Interest in China (No.200903040)

## SessionV：Molecular diagnosis of cereal cyst nematodes
## Chairmen: Prof. Andreas WESTPHAL, Prof. Heng JIAN

### 23 Sensitive and direct detection of *Heterodera filipjevi* in soil and infected wheat by species-specific SCAR-PCR Assays

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Cereal cyst nematodes (CCN), especially *Heterodera avenae* and *H. filipjevi*, are the most economically important plant-parasitic nematode on cereal crops in wheat production area of the world. Morphological identification these species is time-consuming and laborious because there are only slight differences. In this study species-specific SCAR-PCR assay for detection and identification of *H. filipjevi* from infected wheat roots and soil were developed. The species-specific primers were designed according to the randomly amplified polymorphic DNA (RAPD) markers amplified with random primer OPK16. A 646bp specific fragment of sequence was generated, which characterized amplified regions (SCAR) in *H. filipjevi*. The detection limitation of PCR assay was as low as 0.125 µl second-stage juvenile lysate, 3.9×10-3µl adult female lysate and 10-3 µl cyst lysate. The method was able to detect the various developmental stages of *H. filipjevi*, and a single of nematode in 0.5g soil. Two of six field samples (TYHN and XCHN) were detected as *H. filipjevi* by the method. In addition, we determined that the TYHN sample contained a mixed population of *H. filipjevi* and *H. avenae*. This present study is the first to provide a definitive diagnostic assay for *H. filipjevi* in wheat roots and soil using SCAR primer. The discovery of *H. filipjevi* in the Tangyin, Anyang city, Henan province represents a new record for the occurrence of this species in China.

**Acknowledgement:** The research was supported by Special Fund for Agro-scientific Research in the Public Interest in China (No.200903040)

## 24 Quantitative detection of *Heterodera avenae* and *H. filipjevi* using qPCR

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Twelve *Heterodera* species are considered of major economic significance in wheat and barley. *Heterodera avenae*, *H. filipjevi* and *H. latipons* are the most important ones. Precise identification and quantification of these nematodes are necessary to develop effective integrated pest control. We report the results of a qPCR assay that we developed for the quick detection and quantification of *H. avenae* and *H. filipjevi*. Two qPCR primer sets comprising two primers and a probe, were designed and optimized. Both developed qPCR were able to detect a single second stage juvenile (J2). Their specificity was confirmed by the lack of amplification of DNA extracted from 13 other *Heterodera* species. A qPCR using DNA extracted from 106 and 114 J2 + mature eggs of *H. avenae* and *H. filipjevi*, respectively, resulted in steady Ct-values (Ct = 22.33 ± 0.1 and Ct = 21.83 ± 0.05, respectively). Dilution series of DNA extracted from known numbers of J2 of *H. avenae* and *H. filipjevi* were made. The qPCR resulted in a standard curve showing a highly significant linearity between the Ct-values and the dilution rates ($R^2$ = 0.99; slope = -3.05 and $R^2$ = 0.99; slope = -3.4 for *H. avenae* and *H. filipjevi*, respectively). A final validation test showed a high correlation between real numbers of J2 in a sample and the numbers detected in that sample by qPCR. The two qPCR provide a sensitive tool for the rapid detection and quantification of both species whether they occur alone or in mixed populations with other *Heterodera* spp.

**Keywords:** Nematode, qPCR, *H. avenae, H. filipjevi*.

## 25 Developing species-specific PCR assays for identification of *Heterodera filipjevi* and *H. avenae*

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*Heterodera avenae* is an economically important cyst nematode that restricts production of cereal crops in the Pacific Northwest USA. *H. filipjevi* also occurs in winter wheat fields in Oregon. Identification of these two species is important for recommending and implementing effective management practices. Primers were designed from the internal transcribed spacer (ITS) regions of *H. avenae* and *H. filipjevi* ribosomal DNA. The primers were specific when tested with DNA from forty isolates belonging to ten *Heterodera* spp., five *Globodera* spp., five *Meloidogyne* spp., four *Pratylenchus* spp., and three other plant-parasitic nematode species. *H. filipjevi*-primers were also predicted to be highly specific using *in silico* analysis and sixty ITS sequences of closely related *Heterodera* spp. from many other countries. PCR reaction and amplification conditions were established, and *H. avenae* and *H. filipjevi* were clearly distinguished by unique PCR amplicons specific to each target species. Robust PCR amplification was achieved with DNA extracted from a single egg or second-stage juvenile (J2) using a laboratory-made worm lysis buffer, and DNA from 0.5 egg or 0.5 J2 using a commercial kit. The PCR assays were successfully employed for the differentiation of *H. filipjevi* and *H. avenae* populations collected from eight locations in three Pacific Northwest states (Oregon, Washington, and Idaho). This is the first report of a species-specific PCR assay to detect and identify *H. filipjevi*. The species-specific end-point PCR assays for *H. filipjevi* and *H. avenae* will enhance the diagnosis of cereal cyst nematode species in infested fields.

## Session V: Genome and parasitism genes
## Chairmen: Prof. Mike JONES, Dr. Deliang PENG

### 26 Gene silencing in root lesion nematodes significantly reduces reproduction in host plants

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Root lesion nematodes (RLNs, *Pratylenchus* species) are major root pests of many crop plants such as wheat and sugarcane. In Australia *P. thornei, P. penetrans* and *P. neglectus* can reduce wheat yields by 7-15%, and *P. zeae* reduces sugarcane yields by a similar amount in fine textured soils. To study the potential of applying gene silencing technology to RLNs, the transcriptome of *P. thornei* has been sequenced using Roche 454FLX technology and annotated (Nicol, P. et al 2012, *Int. J. Parasitol*. 42, 255-237). These data provided information on potential gene targets for silencing. After optimising protocols to deliver dsRNA to P. thornei by 'soaking', the results showed that P. thornei and P. zeae are both highly amenable to gene silencing. Following soaking of RLNs in solutions containing dsRNA to two genes involved in nematode movement, and culture for five week on' mini' carrot discs, there was a 77-81% reduction in nematode replication (Tan, J-A.C.W. et al 2013, Experimental Parasitology 133, 166–178). These results were confirmed and extended by challenging transgenic wheat and sugarcane plants expressing dsRNA to target genes with root lesion nematodes.

## 27 Comparative analysis of complete mitochondrial genome sequences confirms independent origins of plant-parasitic nematodes

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The nematode infraorder Tylenchomorpha (Class Chromadorea) includes plant parasites that are of agricultural and economic importance, as well as insect-associates and fungal feeding species. Among tylenchomorph nematodes, the superfamilies Tylenchoidea and Aphelenchoidea represent the largest assemblages of plant-parasitic chromadorean members. Monophyletic grouping and/or phylogenetic positions of these two superfamilies within chromadorean nematodes have been the topic of debate over many decades. We investigated phylogenetic relationships of the Tylenchoidea and Aphelenchoidea among other members of chromadorean nematodes based on comparative analysis of complete mitochondrial genome data, including three newly sequenced complete genomes from *Bursaphelenchus mucronatus, B. xylophilus* (Aphelenchoidea) and *Pratylenchus vulnus* (Tylenchoidea). Phylogenetic hypotheses for these mitochondrial genomes, based on different tree-building methods, did not support their monophyly: Aphelenchoideawas positioned basal to the Rhabditomrpha+Diplogasteromorpha+Ascaridomorpha+Panagrolaimomorpha clade, and Tylenchoidea was found to be the most basal taxon of the Chromadorean clade. Comparison of gene arrangement data corroborated the phyletic separation of these two groups: Similar gene arrangement patterns are found among the sampled species of the Rhabditomrpha /Diplogasteromorpha/ Ascaridomorpha/ Panagrolaimomorpha clade and aphelench species, with some minor exceptions. In contrast, only a single block (*rrnL-nad3*) is shared between aphelench(*Bursaphelenchus* spp.) and all three tylench (*P. vulnus, Radopholus similis* and *Heterodera glycines*) species. Additional mitochondrial genome sequences from as yet unsampled taxa will provide useful information for better characterizing the deep node phylogeny and mitochondrial genome evolution of nematodes.

**28 Comparison of transcriptome pre- and post-parasitic stages of the nematode *Heterodera avenae***

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As a worldwide plant pathogen, *Heteroderaavenae*, is an obligate parasite in cereal crops. In China, the occurrence of *H. avenae* had distributed in 13 provinces and about 4 million hectares wheat fields were infested, and reduced the grain yield in significant proportions. However, a lack of genomic information and less genes information in the public databases has hindered the comprehensive elucidation of the molecular mechanisms coordinating its parasitism and pathogenicity. Using 454 Flx+ pyrosequencing, we analyzed the transcriptome of *H. avenae* and collected totally 1,066,719 reads including 551,935 from the pre-parasitic stages and 514,784 from the post-parasitic stages. These were assembled into 10,841 contigs with a mean length of 1,440 bp, among which 2892 contigs were differentially expressed between pre- and post-parasitic stages, and remained 71,401 singletons with an average length of 430 bp. Homology searches revealed that 59% of all contigs had significant matches with annotations to NCBI Nr database. In addition, 2855 (26%) and 1856 (17%) of those contigs were functionally classified using GO hierarchy and KEGG pathway respectively. The post-parasitic up-regulated genes mainly enriched in the some metabolism pathways such as Amino Acid Metabolism, Carbohydrate Metabolism, Lipid Metabolism and Biosynthesis of Other Secondary Metabolites, because life cycle of parasitic nematode turned into a more active phase after infection. We also identifiedsomeeffectors in pre-parasitic stages expressed highly such as mimicking plant annexin 4F01,plant cell wall degradation enzymesGHF5 beta-1,4-endoglucanase, pectate lyaseand so on.Furthermore, a lists of new putative effectors were found and under investigation.

**Acknowledgement:** The research was supported by Special Fund for Agro-scientific Research in the Public Interest in China (No.200903040)

**29 Identification of an annexin-like parasitism gene from cereal cyst nematode, *Heterodera avenae***

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Parasitism genes encoding secreted effector proteins of plant parasitic nematodes play important roles in facilitating parasitism. An annexin-like gene was isolated from the cereal cyst nematode *Heterodera avenae* with the most similarity to *annexin 2* which encodes a secreted protein of *Globodera pallida.* Southern blotting revealed that there are at least two copies of *annexin* in *H. avenae.* This identified *Ha-annexin* encodes a predicted 326 amino acid protein containing four conserved annexin domains. The protein has no N-terminal secretion signal peptide predicted by SIGNAL P 4.0, which is the same with ANNEXIN 2of *Globodera pallida*. However, *in situ* hybridization showed that *Ha-annexin* transcripts exclusively expressed in the subventral gland cells of the pre-parasitic second-stage juveniles, which indicated that Ha-ANNEXIN is probably a secreted effector protein as ANNEXIN 2 of *Globodera pallida.* Quantitative real-time RT-PCR analysis confirmed that *Ha-annexin* was up-regulated in the parasitic second-stage juveniles, correlating with the time when feeding cell formation is initiated. When transiently expressed in onion epidermal cells, Ha-ANNEXIN was localized in the whole cell. Together, these results suggest that *Ha-annexin* most likely encodes a secreted effector protein that contributes to the early parasitic-stage process of *H. avenae*. The detail functions of this gene need to be analyzed in future.

**Acknowledgement:** The research was supported by Special Fund for Agro-scientific Research in the Public Interest in China (No.200903040)

## Session VII: Biological control and IPM

## Chairmen: Prof.Joong-Ki PARK， Prof. Jingwu ZHENG

### 30 Control Options against the Cereal Cyst Nematodes *Heteroderafilipjevi* in Turkey

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Soil borne pathogens including the Cereal Cyst Nematode (CCN) caused by *Heterodera* species are a threat facing cereal production systems worldwide and considered one of the major limiting cereal productions in rainfed regions among the plant parasitic nematodes. Damage exerted root system; therefore nematode damage is especially severe under rainfed growing conditions in more arid areas where water stress is regularly present. The most preferable method to control the CCN is through the use of genetic resistance. Resistance is biologically effective, economically acceptable, and environmentally friendly. However, up to now, resistance has only been identified against one of the CCN nematodes, *H. filipjevi* in Turkey and foreign wheat germplasm though this resistance is not yet present in high yielding cultivars. Resistance to the other nematodes in the CCNs complex is still being sought. Therefore, alternative approaches limiting the damage caused by CCN to wheat are needed. The soil borne pathogens program at CIMMYT Turkey established different control options under the umbrella of the Integrated Pest Management strategy to control cereal nematodes including the use of endophytic microorganisms and the use of seed treatment through establishing bilateral project with leading institutes. As a result of screening wheat germplasm against the CCN hundreds of moderately resistant to *H. filipjevi* in winter wheat germplasm are available now and many of them were distributed to international collaborators and they are being implemented in the breeding programs. The preliminary results of using seed treatments showed that seed treatment of wheat susceptible germplasm gave up to 47% reduction in number of *H. filipjevi* cyst per plant but did not reduce the number of cyst in the resistant germplasm since the cyst number was low and no room to decrease it further.

**Keywords**: Endophyte, *Heterodera*, Resistance, Seed treatment

## 31 Occurrence and Development of the Cereal Cyst Nematode (Heterodera avenae) in Shandong, China

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The cereal cyst nematode (CCN) has been found in 16 provinces of China, including the Shandong winter wheat region. This study investigated dynamic changes in *Heterodera avenae* in root and soil for two consecutive years in a field experiment. Wheat roots were sampled during growing season, and *H. avenae* in wheat root and soil were counted. The results determined that the nematode densities in wheat root and soil of the two tested varieties had the same patterns over a two year period. Juvenile numbers in wheat roots were greatest during April when soil temperature was between 13–20.5℃. Cysts in rhizosphere soil increased significantly when new cysts were formed after Zadok47 (Booting Stage) ($P < 0.05$). There was a second-stage juveniles (J2) peak during Zadok 28 and 30 period, the number of J2 at the Zadok 13 (seedling stage) was the lowest. Our results provide important information indicating that the J2 population in root and soil increased after the wheat winter dormancy, which may provided valuable insights into an approach for integrated management of cereal cyst nematode, e.g. applying chemicals to kill J2 with irrigation after wheat winter dormancy.

**Acknowledgement:** The research was supported by Special Fund for Agro-scientific Research in the Public Interest in China (No.200903040)

## 32 The researches of *Heterodera glycines* and resistant soybean germplasm in China

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The soybean cyst nematode *(Heterodera glycines*, SCN) is a destructive pest of Glycine max (soybean). Five SCN samples from different areas were collected from China, the population P1 is race 1, HG-type is 2,5,7 ; the population P2 is race 2, HG-type is 1,2,5,7; the population P3 is race 3, HG-type is 5,7; the population P4 is race 4, HG-type is 1,2,3,5,7； the population P5 is race 5, HG-type is 1,3,4,7. There are more than 15 million hectares of soybean infested by SCN distributed in the provinces of northern of Yangtze River. And the most serious area of SCN is the west of Northeast of China. More than 100 resistant cultivars to SCN race 3 were identified from 15000 varieties. The resistance mechanism underlying *Heterodera glycines* infection is complicated.

Grouped and deducted the resistant genes from the 67 resistant soybean cultivars (Liaodou10 and Lee68 as the susceptible control) by the interorganismal genetics concept and greenhouse identification technique. According to known resistant genes, unknown resistant genes were deducted for the other cultivars. The results suggested that the cultivars tested were classified 7 groups. Resistant genes to at least two races of *H.glycines* were included in each cultivar. Huipizhi black soybean, Wuzhai black soybean, PI437654, Pingdingshan small black soybean and Black soybean (8498) included more than one resistant gene. Those cultivars were better resistant plasmas in the all tested varieties.The results provided to the breeders more information for selecting resistant soybeans and would accelerate breeding procedure for more new resistant varieties to *H.glycines*.

The suppressive subtractive hybridization (SSH) libraries were constructed to identify the differential gene expression profiles. RNAs were extracted from roots tips of resistant-nematode *G.max* Xiaoliheidou at 12h, 24h, 36h, 48h, 72h post inoculation and non-inoculation, respectively. A total of 166 expressed sequence tags (ESTs) spliced by CAP3 software were obtained after differential screening and northern blot. Those annotated genes by BLAST from the forward and reverse libraries were classified into nine functional categories including plant-pathogen interaction, stress and defense related, transcription regulation, signal transduction, transport, metabolism, cell cycle and DNA processing, cell component, protein synthesis and nucleotide binding. There were 16 differentially expressed genes were confirmed by quantitative real- time PCR. The trends of genes expression profiles were elevated in SCN-infected roots compared to uninfected roots including glutathione S-transferase, syringolide-induced protein, glucose-6-phosphate-dehydrogenase, nodulin-26, S-adenosylethionine synthetase, isoliquiritigenin 2'-*O*-methyltransferase-like, cinnamoyl coA reductase-like protein, Ring-H2 finger protein, glutathione peroxidase. The expression of genes like cyclin-dependant kinases and serine hydroxymethyltransferase1 were induced to decrease after infection. Genes encoding lipoxygenase, phenylacetaldehyde reductase, MYB transcription factor, histone showed to be up-regulated or down-regulated at different time point.

**Acknowledgement:** This research was supported by Special Fund for Agro-scientific Research in the Public Interest (200903040-03).

## 33 Biocontrol of cereal cyst nematode by two bacteria agents

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The cereal cyst nematodes (CCN) are recognized as one of the most important disease on cereals, which has caused significant damage and yield loss in Huanghuai wheat production area of China. With more attention on human health and environment, the problems caused by chemical control have led to increasing interest in the use of biological microorganisms.

In order to seek for biocontrol agents to this disease, 33 strains of bacteria were isolated from the cysts in the diseased fields of Henan province. Their biocontrol abilities to CCN were tested in pots and it indicated that the two isolates (strain 09B18 and strain 09X01) showed good effect to this disease. This study was done to investigate the role of the bacteria 09B18 and 09X01 as biocontrol agent against CCN. They were identified by morphological classification, physiological biochemical characteristic analyses and molecular identification. The bacterialcolony features was observed and gram staining was conducted by both the human eye and scanning electron microscope to make preliminary appraisal. The physiological characteristics were studied according to *Bergey's Manual of Systematic Bacteriology* such as carbon utilization, voges-proskauer test, acid production of xylose. As for molecular identification, DNA was extracted and purified by "Dneasy Tissue Kit" and 16S rRNA gene was amplified by PCR and the primers were 27F(C) AGAGTTTGATCCTGGCTCAG and 1492R(C) TACGGCTACCTTGTTACGACTT. Also, the sequences were submitted on genebank and registration number were obtained. By dressing the seed with the bacteria suspensionof $10^8$ cfu/ml on beef extract peptone medium, the nematicidal potential of the two strain was tested in the greenhouse in 2011, and it was tested in the diseased fields of Xuchang city in Henan in 2012. Their activity was compared with that of 60% Avermectin (6ml/Kg). At the same time, the effect of the treatment on the growth parameters of wheat plant was also examined. The biocontrol mechanism was studied through the mortality of second stage juveniles (J2) under different exposure times (24, 48 and 72 hours) and the inhibation of eggs hatching in the cell suspensionof $10^8$ cfu/ml and filtrates respectively, with sterile water as control.

Based on a comparative 16S rRNA gene sequence analysis, strain 09B18 (sequence number is FJ982658) is closely related to *Bacillus cereus* (99%) and 09X01 (sequence number is HM854372) is close to *Achromobacter xylosoxidans* (99%) respectively. Combined with morphological and physiological characteristics, 09B18 strain was identified as *Bacillus cereus*, which was first report as biocontrol agents of cereal cyst nematode, and strain 09X01 was determined as *Achromobacter xylosoxidans*, which was first report as biocontrol agent of cyst nematode. In 2011, the cysts decreasing rates of the treatment with 09B18 and 09X01 agent were 75.87% and 70.21% in the greenhouse. It was 37.54% and 35.55% respectively in Xuchang diseased fields in 2012. While the cysts decreasing rates of 60% Avermectin (6ml/Kg) in pot and the field was 64.14% and 54.18%, respectively. At the same time, the bacteria 09B18 can increase the fresh weight and the output of wheat plant obviously. The adjusted mortality of 09B18 filtrate on second stage juveniles at 72h reached 100% and 09X01 filtrate reached 99.5%. The filtrates of the two bacteria showed high insecticidalactivity to juveniles, while the suspension of the bacteria cells was rarely to kill second stage juveniles. The percentage of egg hatching with 09B18 filtrate was 20% after 30 days while the rate in sterilized water was 83%. Thereafter, the two bacteria can be supposed as potential biocontrol agents of cereal cyst nematode of wheat by drawing them through integrated pest management programs.

**Acknowledgement:** The research was supported by Special Fund for Agro-scientific Research in the Public Interest in China (No.200903040)

## 34 First report of cystoid nematodes in China with notes on *Cryphodera sinensis* n. sp. (Nematoda: Heteroderidae)

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The family Heteroderidae of plant-parasitic nematodes is a group of important plant parasites containing cyst nematodes (or cyst-forming nematodes) and cystoid nematodes (or non-cystforming nematodes), but less work on Heteroderidae has been done in Chinese tropical and subtropical areas. During the past three years, surveys for the presence of Heteroderidae nematodes in Chinese tropical and subtropical regions have been done. Besides some *Heterodera* species belonging to cystnematodes have been identified, three *Cryphodera* populations belonging to cystoid nematodes were found by using comparative morphological, morphometric and molecular studies. Among these three populations, one from Hunan province has unique morphological characters, SSU, LSU D2D3 and ITS rRNA sequences, and it was identified as a new species, namely *Cryphodera sinensis* n. sp. In addition, the phylogenetic trees based on LSU D2D3 and rDNA-ITS showed that the other two *Cryphodera* populations from Guangxi and Hainan province are in a strong supported monophyletic clade with other *Cryphodera* species. Because only second-stage juveniles were obtained from these two *Cryphodera* populations, it is impossible to identify what kind of species the two *Cryphodera* populations are. In China, cystoid nematodes have never been reported to date. This is the first record of the cystoid nematode *Cryphodera* in China.

**Acknowledgement:** The research was supported by Special Fund for Agro-scientific Research in the Public Interest in China (No.200903040)

## 35 Diagnosis of Cryphodera brinkmaniInterceptedon Juniperus chinensis Imported into China from Thailand

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The non-cyst forming heteroderid nematode *Cryphodera brinkmani*was detected in roots of Chinese juniper (*Juniperus chinensis*) imported from Thailand. Lots of larvae and males were found, fewer females. Morphology and morphometrical traits of the intercepted population on this new host for *C. brinkmani* were in agreement with the original description, except for some minor differences on male morphology. Molecular data for this species were obtained using D2-D3 expansion regions of 28S rDNA. The phylogenetic relationships of this species with other representatives of non-cyst and cyst-forming Heteroderidae using 28S rDNA were presented and indicated that *C. brinkmani* clustered together withother *Cryphodera*sp. Analysis of morphology and molecular biology confirmed and supported the species identifications. According to our knowledge, it is the first time this nematode was intercepted on new host from new country.

**Key word:** intercept; *Cryphodera brinkmani*; *Juniperus chinensis*; Thailand

**36 Temperature-manipulated development of novel cellular and mycelium stages of *Pasteuriapenetrans***

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The development of *Pasteuria penetrans* in root-knot nematodes had been further studied using light and scanning electron microscopy. Some rod-like bacilli were observed when root-knot nematodes infected with *P. penetrans* were cultured for 12 days at 25℃/35℃ 10 h dark/14 h light. Rod-like bacilli were 0.6-0.9 µm in length and about 0.6-0.9 µm in diameter and some of them accumulated to form cellular masses. Numerous of thalli had produced two days later and gathered around the metacopus or the intestine of nematode. At this time, mycelia like zingiberhitherto not documented were observed under scanning electron microscopy. The mycelia like zingibershould be long to vegetative thalli and they were still detected after 700 accumulated degree days. It suggested that vegetative growth and differentiation may simultaneously occur in most thalli. The development of thalliwould be stopped if they were cultured at 16 ± 1℃.This status can remain no less than 30 days. *P.penetrans* can recover to develop and produce mature endospores if they were transferred to normal condition. The development rate after transferred to the natural condition was still the same as the control culture at the same condition all along. Comparing the yield of spores developing from low temperature treated mycelia with the control, and the result indicated there was no significant different. But the sporulation of sporangia would not be stopped, even if they were cultured at 16 ± 1℃.

## 37 QTL analysis of gene *RKN2* in *Gossypium barbadense* which clusters with gene *rkn1* in *G. hirsutum* for transgressive nematode resistance

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A transgressive factor *RKN2* from susceptible *Gossypium barbadense* Pima S-7 to the root-knot nematode (RKN, *Meloidogyne incognita*) interacts with the resistance gene *rkn1* in *G. hirsutum* AcalaNemXto produce higher resistance. The two genes are clustered together linked to SSR marker CIR316, and QTL analysis in an $F_{2:7}$ (Pima S-7 x AcalaNemX) population confirmed marker CIR316 on the telomeric region of chr11 contributed 51% of resistance to both root-galling and nematode egg production. QTL analysis of the test cross AcalaNemX x $F_1$ (Pima S-7 x Acala SJ-2) indicated the allele of marker CIR316 from Pima S-7 on chr11 contributed 29% and 27% of resistance to root-galling and nematode egg production, respectively, in the presence of *rkn1*. When susceptible Pima S-7 was crossed with susceptible Acala SJ-2, transgressive resistant lines were found in $F_2$ progenies. QTL analysis of $F_2$(Pima S-7 x Acala SJ-2) suggested that the allele of marker CIR316 on chr11 contributed 15% and 22% of resistance to root-galling and nematode reproduction, respectively. There was no contribution on homoeologous chr21 with different alleles from those on chr11. BAC sequences from marker CIR316 close to RKN resistance genes contained two copies of resistance gene analogs (RGA), one of the RGA sequences (3148 bp) of CIR316 on chr11 had 83% identity with another RGA (3375 bp) of CIR316 on chr21.These sequence comparisons provided further insight into the organization and molecular evolution of the RKN-resistance gene cluster on chr11 and its homoeolog chr21. Based on these and previous findings, transgressive segregation is common in cotton and efforts to identify novel phenotypes for biotic and abiotic stress resistance traits among progenies developed from stress susceptible or sensitive parent combinations are worthy of increased attention in plant breeding programs.

# General Abstracts

### 38 Pathytope Stability of Heterodera avenae from Shanxi, China and suggestion on revision of Pathotype characterization system

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Cereal cyst nematode, *Heterodera avenae*, is one of the most important limit factors for wheat production in China. The present pathotype characterization system for *H. avenae* was put forward by S. Andersen and K. Andersen in 1981, which based on the reaction of nematode population to 23 cultivars or lines of cereals (International Test Collection, ITC), including 11 of Barley, 7 of Oats, and 5 of wheat. All of which were divided into two group A and group B. In 1997, one cereal cyst nematode population from Taigu, midland of Shanxi, China were characterized based on the reaction to group A. It was found that all the barley and oats cultivars were resistant, only Capa, Festigyay, and Aus 13807 of wheat were susceptible. During 2011-2012, two *H. avenae* populations from Wenxi and Yuncheng, south of Shanxi were characterized for their pathotype based on the reaction to the ITC. All the data are similar to that made in 1997 to Tagu population. It showed that the pathotype of *H. avenae* in Shanxi is relatively stable. Because of the wide distribution in China, and most of ITC are resistant to the Chinese *H. avenae* populations. We do think it is good to add some Chinese resistant cultivars and omit some of foreign resistant cultivars or lines in ITC. That will be useful for monitor the pathotype variation of *H. avenae* in China.

**Key words**: *Heterodera avenae*, Pathotype, International Test Collection.

**Acknowledgement:** The research was supported by Special Fund for Agro-scientific Research in the Public Interest in China (No.200903040)

### 39 Research on infection and development dynamics of *Heterodera avenae* to spring wheat in Qinghai province

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The infection period, development progress, dynamics and generation of *Heterodera avenae* on spring wheat in Qinghai province was carried out by using seedling observation combined by field investigation. The results show that 2 instar larvae hatched out of the overwintering cyst in soil infected the wheat seedling roots by the rise of temperature. Since the middle of April, the larvae completed three stages from two age to four age in wheat root late April to early July and the white female adults exposed on the root surface In July. From August to September the new cyst falled into the soil, and it's for Wintering period from the middle of October to next April. *Heterodera avenae* occurs one generation during the whole growth period of spring wheat.

**Key words:** *Heterodera avenae* Woll.；infection；development；dynamics

**Acknowledgement:** The research was supported by Special Fund for Agro-scientific Research in the Public Interest in China (No.200903040)

## 40 The dynamics of *Heterodera avenae* in winter wheat in Hebei province

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Cereal cyst nematode (*Heterodera avenae*) has become the main disease in wheat production in China and even worldwide. Understanding the dynamic of cereal cyst nematode in the field is important for the control of the disease. Three surveys were conducted in the main winter wheat production area in Hebei from October, 2009 to September, 2012. The results showed that few J2 were detected in soil in wheat sowing period (October 6th), and it reached the peak (12.3-18.6 J2/100ml soil) in late November (23th to 27th, Nov.) before the soil freeze, then the number of J2 dropped to 0.1 J2/100ml soil in 23rd of December and no J2 isolated in soil layers (0-14 cm) in January. Afterwards the number of J2 in soil increased again with the soil temperature increasing, and reached the peak in early April with 52- 65 J2/100ml soil, it decreased to 1.3 J2/100ml soil in May 12th. No J2 isolated from early June. Very low number of J2 with 0.4-3 J2/100ml soil could be extracted in the soil from July to September. J2 could penetrate root from October 15 (0.7 J2/plant) to November 10th (0.3 J2/plant), no obvious peck found before soil freeze and the nematodes stop to penetrate during soil freeze. J2 started to penetrate root again from end of February and the higher number of J2 with 59-102 J2/plant were found from 6th-14th of April, then the number of J2 penetrated to the root decreased. Accordingly, third stage juveniles formed between October and November, and peaked between late April and early May. The white females were detected from mid of May and higher number of cyst formed in late May. The eggs in the cyst from June to end of July were mainly in the embryonic development stage. In summary *H. avenae* occurs one generation per year in wheat in Hebei province and the main damage period is from late March to early April in spring.

**Acknowledgement:** The research was supported by Special Fund for Agro-scientific Research in the Public Interest in China (No.200903040)

## 41 Pathotype characterization of the cereal cyst nematode *Heterodera avenae* in Beijing, China

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The cereal cyst nematode *(Heterodera avenae)* has caused huge yield loss on wheat crops. Pathotype diversity study could offer support for wheat planting, diseases control, breeding for disease resistance and choices of varieties. The pathotype of *H. avenae* in Beijing, China was identified using 23 standard international differentials and 4 local cultivars Wenmai19, Aikang58, Taikong6, Zhongyu in 2011 and 2012, respectively. Three differential barleys La Estanzuela, Dalmatische and Varde were susceptible. The six oats were resistant to the tested nematode population. The wheats Iskamisch K-2-light and Psathias were resistant while the Capa, Loros, AUS 10894 and 4 local cultivars were all susceptible. The results showed the population avirulent to Ortolan (Ha 1), Hetar, Sun II. According to the character item, which showed the Caiyu CCN population virulence pathotype classified to be group Ha 31.

**Acknowledgments:** This study was supported by the Special Fund for Agro-scientific Research in the Public Interest (No. 200903040)

## 42 Chemotaxis of *Ditylenchus destructor* to extracts from sweet potato

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*Ditylenchus destructor* Thorne could cause severe damage to sweet potato, potato and other host plants in China. Host plant phytochemicals play important roles in the nematode behavior, thus identification of the compounds that attract the nematodes to the plant is important for the nematode control. In the present study, experiments were carried out to investigate chemotaxis of *D. destructor* to different host plant and determine the attraction intensity of different host plants. Sweet potato was consistently and significantly much more attractive than potato and carrot in all these assays. Compared with the stems, leaves and storage roots of sweet potato, stems showed the significantly strongest attraction ability to nematodes and our result showed that the attractant from sweet potato was stable to heat. Differences in the relative attraction to *D. destructor* among sweet potato cultivars were also studied, the results showed that the stems of susceptible cultivars Lizixiang, Jishu 98, Shangshu 19 were significantly more attractive than the resistant cultivars Xushu 25, Xushu18, Jishu 17-52; in contrast, there were no significant difference of the storage roots among different sweet potato cultivars. Furthermore, the compounds of sweet potato were extracted and studied in relation to the host-finding behavior of the nematode. The fractions extracted with butyl alcohol from stems were significantly more attractive than extractions by water, alcohol, petroleum ether and ethyl acetate. The results of this study suggest that extracts from sweet potato can potentially be used as baits in a trap for the control of *D. destructor* in the field.

**Key words:** *Ditylenchus destructor,* compounds, attraction, chemotaxis

## 43 The detection of cyst-forming nematodes on Poaceae in China

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Members of cyst nematodes are among the most important plant-parasitic nematodes in agriculture, which can infect plant hosts from Poaceae. During a survey from 2010 to 2012, some populations of cyst nematodes parasitized Poaceae were detected by the sieving-decanting method on the roots and in rhizosphere soil in China. Based on both morphological and molecular identification, four species including *Heteroderaavenae*, *H.filipjevi*, *H.elachista* and *Cactoderaestonica* were indentified. *H.filipjevi* from Henan provinceand *H.avenae* from Anhui, Hebei, Henan, Shandong and Shanxi provinces infected wheat, which belong to 'Avenae' group. *H.elachista* on rice from Hunan province belongs to the 'Cyperi' group, while two populations of *Cactoderaestonica* from Liaoning provincewere recovered on *Setaira viridis* (L.) Beauv, one of grass from Poaceae. This work was supported by the Special Fund for Agro-scientific Research in the Public Interest 200903040-03.

**Acknowledgement:** The research was supported by Special Fund for Agro-scientific Research in the Public Interest in China (No.200903040)

## 44 Biological characteristics of *Heterodera elachista* on rice in Hunan, China

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The rice cyst nematode (*Heterodera elachista*) is an important pest in hilly rice fields of Hunan Province, China, and has been previously identified from rice fields in Japan and Iran. *H. elachista* can decrease yield by 7–19%, and has the most severe impact during the later stages of plant growth. Biological characteristics of *Heterodera elachista* were investigated by using artificial inoculations with second-stage juveniles in laboratory and periodical sampling in rice field. The optimum temperature range for hatching of J2 from cysts of *H. elachista* was 28 to 32℃, and root diffusate of rice, leachates of rice soil and twenty-time rice root juice stimulated emergence of J2 from cyst. The shortest life cycle at 30℃ from the infective juvenile to the emergence of second-stage juvenile is 18 days while from egg to egg stage is 22 days. The invasion and development of *H.elachista* were favored by relatively high temperatures, e.g. 28~35℃. Root infection and yield loss caused by *H. elachista* on rice can be greatly reduced with appropriate management of irrigation water. *H. elachista* is unable to penetrate rice roots under anaerobic soil conditions.

**Acknowledgement:** This research was supported by Chinese Special R & D Fund for Pubic Benefit Agriculture (200903040).

## 45 Effect of Abamectin on cereal cyst nematode of wheat

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Cereal cyst nematode (CCN) is globally and economically important in wheat production systems, and distributed widely in main wheat growing areas in China. In order to investigate the effect of Abamectin against CCN and its application method, the plot trials were conducted by soil treatment, seed processing and root irrigation with Abamectin emulsifiable concentrate (EC), controlled releasing Abamectin capsule suspension (CS) and Abamectin granules (GR), respectively. Three groups of results were obtained as follows: (i) the control efficacies of Abamectin against CCN by root irrigation were 22.3% and 31.4% with Abamectin EC (225 g a.i./ha) and Abamectin CS (225 g a.i./ha) before overwintering, respectively, and 73.6% and 70.4% during regreening stage of wheat, respectively. (ii) the control efficacies against CCN were 21.8%, 16.6% and 27.8% by soil treatment with Abamectin G, Abamectin EC and Abamectin CS before sowing, respectively. (iii) the efficacies against CCN were 23.4% and 52.4% by seed dressing with Abamectin EC and Abamectin CS at the ratio of 2% of seed weight, respectively. The results suggested that Abamectin was an available nematicide to control CCN, and taken together, seed processing with the controlled releasing Abamectin CS must be the most effective method considering the ease of operation and efficacy based on our study.

**Acknowledgement:** This study was sponsored by the special fund for Agro-scientific Research in the Public Interest (200903040).

## 46 Identification of two pharyngeal gland specific pectate lyases from the cereal cyst nematode*Heterodera avenae*

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Parasitism genes encoding secretory proteins expressed in the pharyngeal glands of plant-parasitic nematodes playcrucial roles in nematode parasitism of plants. Two pectate lyases genes (*Ha-pel-1* and *Ha-pel-2*) expressed in the pharyngeal glands of the sedentary cyst nematode, *Heterodera avenae*, were cloned from a Pre-parasitic second stage juveniles cDNA library, and the corresponding genomic DNAs were subsequently cloned.*Ha-pel-1* and *Ha-pel-2* consist of 1717 nucleotides encoding 521 amino acids and 1000 nucleotidesencoding328aminoacids,respectively.DNA gel blotting confirmed that these two genes were of nematode origin and present as members of a small multigene family. The deduced protein sequences HA-PEL-1 and HA-PEL-2 share only27% identity and 46% similarity. Phylogenetic analysis revealed that they cluster in two different clades. Both of the predicted proteins have a putative signal peptide for secretion. *In situ* hybridization showed that the transcripts of*Ha-pel-1* and *Ha-pel-2*accumulated specifically in the two subventral gland cells of *H. avenae*. Moreover, RT-PCR showed that both genes were expressed in the migratory preparasitic stage although the level of expression between the two genes was different. These results indicatide that HA-PEL-1 and HA-PEL-2 may be important enzymes early in the migration process.

**Acknowledgments:** This research was support by the National Natural Science Foundation of China (No. 31201493) and by the Special Fund for Agro-scientific Research in the Public Interest (No. 200903040).

## 47 The distribution and rDNA-ITS analysis of Cereal Cyst Nematode（*Heterodera avenae*） in Shandong Province, China

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The cereal cyst nematode（*Heterodera avenae*） is the most important nematode on wheat and cereal crops in China. The occurrance and distribution of *Heterodera* spp. were investigated by random sampling method from 19 counties of Shandong province. The specie was identified as *Heterodera avenae* with the morphological and rDNA-ITS analysis..The cereal cyst nematode (*Heterodra avenae*) was detected from 84.2 percent samples collected from Linyi、Laiwu、Zibo、weifang、dongying、weihai、yangtai. The highest cyst and egg number existed in Dongying city and Weifang city, the lowest cyst and egg number existed in Linyi city and Yantai city. This survey results will beneficial for making suitable management strategy and control measures.

**Acknowledgement:** This Research was supported by Chinese Special R & D Fund for Public Benefit Agriculture（200903040）and Ministry of Science and Technology (2009DFB30230).

**48 Molecular characterization and functional analysis of a new acid phosphatase gene *(Ha-acp1*) from *Heterodera avenae***

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For sedentary endo-parasitic nematodes, parasitism genes encoding secretory protein expressed in the subventral glands cells always play an important role during the early parasitic process. A new acid phosphatase gene (*Ha-acp1)* expressed in the subventral glands of the cereal cyst nematode (*Heterodera avenae*) was cloned and the characteristics of the gene were analyzed. Results showed that the gene had a putative signal peptide for secretion and *in situ* hybridization showed that the transcripts of *Ha-acp1*accumulated specifically in the subventral gland cells of *H. avenae*. Southern blot analysis suggested that *Ha-acp1* belonged to a multigene family. RT-PCR analysis indicated that this transcription was strong at the pre-parasitic juveniles. Knocking down *Ha-acp1* using RNA interference technology could reduce nematode infectivity by 50%, and suppressed the development of cyst. Results indicated that *Ha-acp1* could play an important role in destroying the defense system of host plants.

**Keyword:** *Heterodera avenae*, acid phosphatase gene, Ha-acp1,RNA interference

**Acknowledgments:** This research was support the National Natural Science Foundation of China (No. 31201493) and by the Special Fund for Agro-scientific Research in the Public Interest (No. 200903040).

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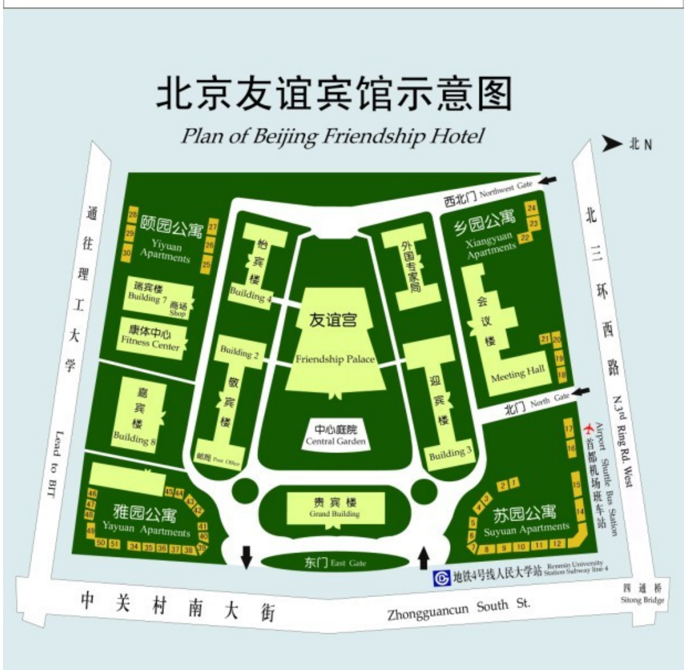
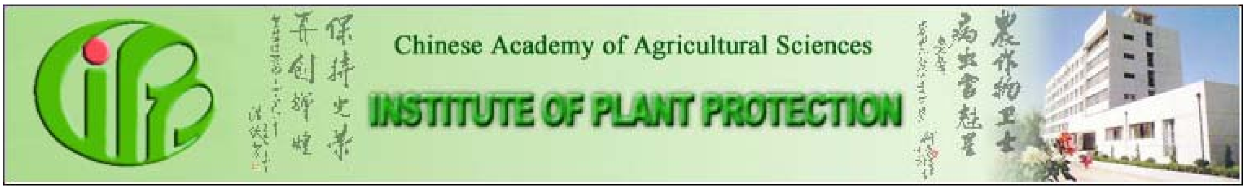
# Map of Friendship Hotel and Beijing




# Introduction of IPP, CAAS

The Institute of Plant Protection (IPP), Chinese Academy of Agricultural Sciences (CAAS), which was founded in 1957, is a non-profit national institute dedicated to both basic and applied sciences of plant protection in China. The mission of the institute is to study and seek the resolution to the main problems theoretically and economically important in plant protection of the country. IPP is committed to develop and extend the research achievements in plant protection, promote the agro-ecosystem and environmental protection, and develop the international collaboration and exchange.

In the institute there are three research departments of plant diseases, insect pests and pesticide, three administration sectors for scientific research management and international cooperation, general affairs, and human resources, a supporting sector of service center, an affiliated experimental station located in Langfang, Hebei Province. The national key laboratory for biology of plant diseases and pests, the key laboratory of pesticide chemistry and application technology of Ministry of Agriculture (MOA), biosafety research center (MOA), supervision and test for plant disease and pest resistance (MOA), and Chinese Society of Plant Protection are all based in the institute. IPP currently has 210 employees. IPP is one of the institutes certified by the Academic Degree Committee of the National Council to authority to grant doctoral degrees in plant pathology, agricultural entomology, pesticide science, weed science, agricultural microbiology, monitoring and forecasting of plant pests and biosafety.

IPP has been undertaking lots of research projects from the national program of plant protection, serving as a nationwide coordinator for the research collaboration in plant protection nationwide. Approximately 50 national and international research projects are under implementation, including the National Basic Research and Development Program, the National High-Tech Research and Development Program, the National Key Technological Research and Development Program, the projects of Natural Science Foundation of China, the ministerial and provincial research projects and international collaboration program.

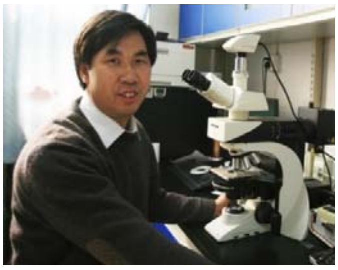
In IPP, more than 2500 scientific research papers and over 100 books have been published, including "Crop Diseases and Pests in China" and "Study on Helicoverpa armigera". The institute has won more than 210 awards for scientific achievement, including 2 natural sciences awards, 2 national invention awards, 23 national advanced science awards, and 125 awards of the ministerial or provincial levels. Most of the researches have already been well recognized worldwide. The monographs "Epidemic systems of wheat stripe rust in China" and "Migration regularity and forecasting of armyworm moth" won second and third prizes in the National Natural Science Award respectively, and "Development of transgenic wheat resistant to barley yellow dwarf virus using genetic engineering approach" is one of the top ten achievements for science and technology in China in 1995. IPP hosts the 15th International Plant Protection Congress in 2004.

The institute won the first place for the contribution to scientific achievements in the comprehensive evaluation of nationwide agricultural institutions organized by MOA in 1992 and 1996. The national key laboratory for biology of plant diseases and pests was placed the top ten national laboratories in the 1$^{st}$ round evaluation of NSFC entrusted by National Planning Committee in 1996. The laboratory ranked 14$^{th}$ place among 56 key laboratories in life science in the 2$^{nd}$ round evaluation organized by NSFC entrusted by Ministry of Science and Technology in 2001. The ministerial key laboratory of pesticide chemistry and application technology was placed the top ten laboratories in the evaluation organized by MOA in 2002.

With the increasing progress of scientific research, improvement of working conditions and development of international collaboration, IPP will certainly be one of the national institutes with the majors covering main research fields in plant protection, the excellent capability to undertake the research projects and make the outstanding research progress. IPP will make fundamental, strategic and profound contributions to plant protection, agricultural production and food safety of the country.

For more information, please visit the website: http://www.ippcaas.cn/sites/IPP/ippc/ippcaas

# Introduction of Nematology Laboratory, IPPCAAS



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## Research Areas

We pay more attention to the important agricultural nematodes, such as cyst nematodes (*H. avenae*, *H. filipjevi*, *H. glycines*, *H.elachista*), root-knot nematodes, root lesion nematodes, stem nematodes and quarantine nematodes. The research areas mainly focus on:

1. Nematode detection, early warning and risk analysis
2. Identification and functional analysis of parasitism/pathogenic effectors genes
3. Germplasm resistant identification and evaluation
4. Molecular interaction between nematode and host
5. Biological control and integrated nematode management
6. Environments biosafety assessment of transgenic soybean

## Selected Publications

1. Peng H, Gao BL, Kong LA, Yu Q, Huang WK, He XF, Long HB, Peng DL*. Exploring the host parasitism of the migratory plant-parasitic nematode *Ditylenchus destuctor* by expressed sequence tags analysis. PLoS ONE 2013 8(7): e69579 (*corresponding author)
2. Long H, Peng DL*, Huang WK, Peng H, Wang GF. Molecular characterization and functional analysis of two new b-1,4-endoglucanase genes (*Ha-eng-2*, *Ha-eng-3*) from the cereal cyst nematode *Heterodera avenae*. Plant Pathology 2012,62:953- 960
3. Long HB, Peng H, Huang WK, Wang GF, Gao BL, Mones M, Peng DL*. Identification and molecular characterization of a new β-1,4-endoglucanase gene (*Ha-eng-1a*) in the cereal cyst nematode *Heterodera avenae*. European Journal of Plant Pathology 2012,134:391–400

## Fundings

1. 973 program (Grant No. 2013 CB127502) from the Ministry of Sciences and Technology, PI：De-Liang Peng
2. Special Fund for Agro-scientific Research in the Public Interest (Grant No. 200903040), PI：De-Liang Peng
3. National Natural Science Foundation (6 individual funds)
4. Environments biosafety assessment for Transgenic crops

# 基因组学 Workflow

Promega

## 纯化RNA

| Product | Cat. NO. | 包装量 |
|---|---|---|
| 新鲜或冰冻样本纯化总RNA | | |
| SV Total RNA Isolation System | Z3101 | 10次 |
| | Z3100 | 50次 |
| | Z3105 | 250次 |
| ReliaPrep™ RNA Cell Miniprep System | Z6010 | 10次 |
| | Z6011 | 50次 |
| | Z6012 | 250次 |
| ReliaPrep™ RNA Tissue Miniprep System | Z6110 | 10次 |
| | Z6111 | 50次 |
| | Z6112 | 250次 |
| FFPE样品纯化总RNA | | |
| ReliaPrep™ FFPE Total RNA Miniprep | Z1001 | 10次 |
| | Z1002 | 100次 |
| 从总RNA中纯化mRNA | | |
| PolyATract® mRNA Isolation Systems | Z5210 | 3次 |
| | Z5200 | 3次 |
| | Z5310 | 15次 |
| | Z5300 | 15次 |
| 从组织/细胞中纯化mRNA | | |
| PolyATract® System 1000 | Z5400 | |
| | Z5410 | |
| | Z5420 | |

## 测浓度

| Product | Cat.NO. | 包装量 |
|---|---|---|
| 染料法测RNA浓度 | | |
| QuantiFluor™ RNA Dye System | E3310 | 1 mL |

## 逆转录

| Product | Cat. NO. | 包装量 |
|---|---|---|
| 试剂盒 | | |
| GoScript™Reverse Transcription System | A5000 | 50次 |
| | A5001 | 100次 |
| Reverse Transcription System | A3500 | 100次 |
| 组分：逆转录酶、RNasin、dNTP等 | | |
| GoScript™ Reverse Transcriptase | A5003 | 100次 |
| | A5004 | 500次 |
| M-MLV Reverse Transcriptase | M1701 | 10,000u |
| | M1705 | 50,000u |
| RNasin® Ribonuclease Inhibitor | N2111 | 2,500u |
| | N2115 | 10,000u |
| dNTP Mix | U1511 | 200μl |
| | U1515 | 1000μl |
| Oligo(dT)$_{15}$Primer | C1101 | 20μg |
| Random Primers | C1181 | 20μg |

## 纯化gDNA

| Product | Cat. NO. | 包装量 |
|---|---|---|
| 新鲜或冰冻样本 | | |
| Wizard® Genomic DNA Purification Kit | A1120 | 100×0.3ml |
| | A1125 | 500×0.3ml |
| | A1620 | 100×10ml |
| ReliaPrep™ Blood gDNA Miniprep System | A5081 | 100次 |
| | A5082 | 250次 |
| ReliaPrep™ gDNA Tissue Miniprep System | A2051 | 100次 |
| | A2052 | 250次 |
| 甲醛固定石蜡包埋(FFPE)样品 | | |
| ReliaPrep™ FFPE gDNA Miniprep System | A2351 | 10次 |
| | A2352 | 100次 |

## 测浓度

| Product | Cat. NO. | 包装量 |
|---|---|---|
| 染料法测DNA浓度 | | |
| QuantiFluor™ dsDNA Dye System | E2670 | 1 mL |
| QuantiFluor™ ssDNA System | E3190 | 1 mL |

## PCR 扩增

## 普通PCR

| Product | Cat. NO. | 包装量 |
|---|---|---|
| 普通Taq酶预混液 | | |
| GoTaq® Green Master Mix | M7122 | 100次 |
| | M7123 | 1000次 |
| GoTaq® Colorless Master Mix | M7132 | 100次 |
| | M7133 | 1000次 |
| 热启动Taq酶预混液 | | |
| GoTaq® Hot Start Green Master Mix | M5122 | 100次 |
| | M5123 | 1000次 |
| GoTaq® Hot Start Colorless Master Mix | M5132 | 100次 |
| | M5133 | 1000次 |
| 热启动Taq酶，独立组分，方便优化条件 | | |
| GoTaq® MDx Hot Start Polymerase | D6001 | 100U |
| | D6005 | 500U |
| | D6006 | 2500U |
| | D6008 | 10000U |

## 检测表达水平

## 定量PCR

| Product | Cat. NO. | 包装量 |
|---|---|---|
| 染料法 | | |
| GoTaq® qPCR Master Mix | A6001 | 200次 |
| | A6002 | 1000次 |
| GoTaq® 2-Step RT-qPCR System | A6010 | 200次 |
| GoTaq® 1-Step RT-qPCR System | A6020 | 200次 |
| 探针法 | | |
| GoTaq® Probe qPCR Master Mix | A6100 | 100次 |
| | A6101 | 200次 |
| | A6102 | 1000次 |
| GoTaq Probe 2-Step RT-qPCR System | A6110 | 200次 |
| GoTaq Probe 1-Step RT-qPCR System | A6120 | 200次 |

## 基因克隆

| Product | Cat. NO. | 包装量 |
|---|---|---|
| 高保真扩增 | | |
| Pfu DNA Polymerase | M7741 | 100U |
| | M7745 | 500U |
| GoTaq® Long PCR Master Mix | M4021 | 100次 |
| T载体 | | |
| pGEM®-T Vector System I | A3600 | 20次 |
| pGEM®-T Easy Vector System I | A1360 | 20次 |
| 胶回收 | | |
| Wizard® SV Gel & PCR Clean-Up System | A9281 | 50次 |
| | A9282 | 250次 |
| | A9285 | 1000次 |
| T4连接酶 | | |
| LigaFast™ Rapid DNA Ligation System | M8221 | 30次 |
| | M8225 | 150次 |
| T4 DNA Ligase | M1801 | 100U |
| | M1804 | 500U |
| T4 DNA Ligase (HC) | M1794 | 500U |
| 限制性内切酶、琼脂糖、凝胶染料…… | | |

## 转化细菌

| Product | Cat. NO. | 包装量 |
|---|---|---|
| 感受态细胞 | | |
| JM109 | L2001 | 5 × 200μl |
| | L2005 | 20 × 50μl |
| Pro 5-alpha | L1221 | 20 × 50μl |
| BL21 | L1191 | 5 × 200μl |
| | L1195 | 20 × 50μl |
| 蓝白筛选 | | |
| X-Gal | V3941 | 100mg |
| IPTG | V3955 | 1g |
| | V3951 | 5g |
| | V3953 | 50g |

## 质粒纯化

| Product | Cat. NO. | 包装量 |
|---|---|---|
| 高纯度质粒纯化 | | |
| PureYield™ Plasmid Miniprep System | A1223 | 100次 |
| | A1222 | 250次 |
| PureYield™ Plasmid Midiprep System | A2492 | 25次 |
| | A2495 | 100次 |
| | A2496 | 300次 |
| PureYield™ Plasmid Maxiprep System | A2392 | 10次 |
| | A2393 | 25次 |

## 转染细胞

| Product | Cat.NO. | 包装量 |
|---|---|---|
| 高效、低毒转染试剂 | | |
| FuGENE® 6 | E2691 | 1ml |
| | E2692 | 5×1ml |
| FuGENE® HD | E2311 | 1ml |
| | E2312 | 5×1ml |

## 检测

| Product | Cat. NO. | 包装量 |
|---|---|---|
| 双报告基因检测 | | |
| Dual-Luciferase® Reporter Assay System | | |
| Dual-Glo® Luciferase Assay System | | |
| 单报告基因检测 | | |
| Bright-Glo™ Luciferase Assay System | | |
| Steady-Glo® Luciferase Assay System | | |
| ONE-Glo™ Luciferase Assay System | | |

# 卡尤迪生物科技（北京）有限公司

## 公司简介：

卡尤迪生物科技（北京）有限公司是一家致力于研发、生产世界一流品牌的小型便携化的分子生物实验室仪器的高科技生物企业。卡尤迪也是中国第一家、世界第二家致力于研发手持式通用荧光定量PCR仪的生物公司。本公司具有仪器、试剂交叉开发能力，可以进行核酸检测颠覆性的系统设计。

公司目前主要有PCR仪系列、金属浴系列、组织研磨器系列和便携核酸分析系列这四大产品线。

超薄型PCR仪是现在市场上最小巧，最轻薄的PCR仪。机身厚度仅有4厘米，极大的节省了实验空间；人机友好的触摸屏设计，真正实现了傻瓜式操作；12V直流电源供电，可车载供电，是您室外实验的最佳助手。

制冷/加热恒温金属浴系列是一种先进的温控仪器，是市场中体积最小巧，价格计，方便实用的多样化模块，安全环保的低能耗电源等产品优点让卡尤迪金属浴成为了北京各大科研院校老师学生喜爱的实验室常规仪器之一。

G10/G20是市场上动力最强劲的手持式电动组织研磨器，充电后可连续使用最长达10小时。加强版G50提供更强劲的动力，LCD屏幕观测实时转速，并可通过按钮轻松调节所需转速，是各种DNA\RNA提取试剂盒的最佳伴侣，在各种生物样品组织（包括动物、植物）的研磨提取中为您提供愉悦轻松且高效的研磨体验。

BATT-3蓄电池盒可为所有的卡尤迪产品提供外接移动电源，使得野外和移动使用成为可能。

超薄型PCR仪

BATT-3蓄电池盒

G50组织研磨器

金属浴

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