ACTIVITY REPORT

Topic: Identification of Cereal Csyt Nematode Populations Using Molecular Methods
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Host: Prof. Deliang PENG, IPP-CAAS (Chinese Academy of Agriculture Science, Beijing, China)
Period of work: 06 st July 2013 to 25 st August 2013

PURPOSE

The sedentary Cereal Cyst Nematodes (CCN: Heterodera spp.) have a global distribution and cause significant economic yield losses in many countries of the world, in particular where rainfed cereal predominated systems are practiced (Nicol *et al*., 2003). Recent surveys of cereal fields in Southeast Anatolion Plateau (SEA) and Central Anatolian Plateau of Turkey showed that three cereal cysts nematodes species are widely distributed in major wheat and barley cultivating areas, with *H. filipjevi* being common in Central Anatolian Plateau, whereas *H. avenea* and *H. latipons* being dominant in Southeast Anatolia Plateau (Abidou *et al*., 2005; İmren, 2010).

The identification of *Heterodera* species using morphological and morphometrical characteristics is time consuming and requires great skill and training by the observer. However, the analysis of coding and non-coding regions of ribosomal DNA (rDNA) became a favorite way for nematode identification (Vrain *et al*., 1992; Wendt *et al*., 1993; Zijlstra *et al*., 1995). The internal transcribed spacer region (ITS) is variable and therefore useful for nematode identification and phylogenetic studies at species level. Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) or –sequencing based on ITS-regions of the rDNA repeat units has provided to be a reliable tool for quick and precise identification of cyst nematode species and subspecies (Bekal *et al*., 1997; Subbotin *et al*., 1999; Subbotin *et al*., 2000; Rivoal *et al*., 2003; Madani *et al*., 2004; Abidou *et al*., 2005; Smiley *et al*., 2008).

The aim of the course was to identify the Cereal Cyst Nematode populations (*Heterodera* avenae group species) collected from Eastern Anatolia in Turkey using moleculer tools.

WORK DESCRIPTION

During my stay at the IPP-CAAS (Chinese Academy of Agriculture Science, Beijing, China) under the supervision of Prof Deliang Peng I worked on the identification of *Heterodera* populations collected from different regions in Turkey. The below steps where followed:

- Selecting appropriate nematodes cysts for identification
- DNA-extraction by first crushing the cysts in water using a rod placed on a micro-vibro-mixer and subsequently by incubating at 65°C in the presence of ProteinaseK (combination of mechanical and enzymatic DNA-extraction method)
- DNA-extraction by first crushing the cysts in water using a rod, isolating a few juveniles with a fishing needle, cutting the juveniles in a drop of water with a scalpel on a glass-slide under a binocular, and by transferring the pieces of nematodes into a tube for incubation at 65°C in the presence of ProteinaseK

- Amplification of the ITS-rDNA region using the 'Ferris-primers'
- Electrophoresis to be able to select the samples with a positive result
- Amplification of DNA (Using Re-amplification methods)
- Sending the samples for sequencing to a sequencing service at a private company in China.

Results:

The results of the molecular tools for the identification of the 27 cereal cyst nematodes populations demonstrated that 5 cyst populations were found to be *Heterodera latipons* and 22 populations were identified as *H. filipjevi* in Eastern Anatolian region of Turkey.

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