Title of PhD thesis : Root-lesion and cyst nematodes in cereals fields in Morocco : Species identification, population diversity and crop resistance

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Abstract : Cereal cyst nematodes (CCN, *Heterodera* spp.) and root-lesion nematodes (RLN, *Pratylenchus* spp.) are important plant-parasitic nematodes of wheat and exist in most of the cereal growing regions of the world. As there was limited information on the distribution of CCN and RLN species in wheat fields in Morocco, a survey was organized in 2011. A total of 75 soil and root samples were collected from fields in Gharb, Saiss, Zaers and Chaouia before the wheat and barley harvest (May to June). Cysts were extracted from soil by flotation and decanting through 200-µm sieves. Vermiform stages were extracted from roots and soil with an automated zonal centrifuge. They were identified up to species level using morphological and molecular methods. The survey revealed that 69% of the samples were infested with four species of *Pratylenchus*, *viz. P. thornei*, *P. penetrans*, *P. pseudocoffeae* and *P. pinguicaudatus.* The most prevalent species was *P. penetrans*, present in the four regions. Cereal cyst nematodes were found in 16% of the soil samples and were represented by two species, *viz*. *H. avenae* and *H. latipons*. *Heterodera avenae* was the most prevalent, occurring in 13% of the fields and associated with wheat in the 3 regions where it was found. *Heterodera latipons* was detected only in one sample, originating from Ain Jmaa (Saiss).

The morphological and molecular characteristics of 11 populations of CCN collected from different wheat growing regions of Morocco were studied. Morphometrics of cysts and second-stage juveniles (J2) were generally within the expected ranges for *H. avenae*; only the isolate from Aïn Jmaa showed morphometrics conforming those of *H. latipons.* When using species-specific primers for *H. avenae* and *H. latipons,* the specific bands of 109 bp and 204 bp, respectively, confirmed the morphological identification. In addition, the internal transcribed spacer (ITS) regions were sequenced to study the diversity of the 11 populations. These sequences were compared with those of *Heterodera* species available in the GenBank database (www.ncbi.nlm.nih.gov) and confirmed again the identity of the species. Ten sequences of the ITS-rDNA were similar (99-100%) to the sequences of *H. avenae* published in GenBank and three sequences, corresponding with one population, were similar (97-99%) to *H. latipons*.

During the survey of the wheat-growing area of Morocco, 17 populations of RLN were collected. They were identified on the basis of their morphological and morphometric characters, and by molecular methods. Microscopic observations of females and males demonstrated the occurrence of *P. penetrans* in 13 of the 17 samples; *P. thornei* and *P. pseudocoffeae* were detected in four samples from Zaers and a single sample from Settat, respectively. A duplex PCR primer set was used to

confirm the presence of *P. penetrans* while the species-specific forward primer PTHO and the common reverse primer D3B were used for *P. thornei*. For the remaining populations, the D2-D3 expansion segments of the 28S rRNA gene were amplified and the obtained sequences were compared with those of *Pratylenchus* species in the GenBank database. This comparison confirmed the morphological identifications and revealed a population of *P. pinguicaudatus*. The study of the phylogenetic relationship of the Moroccan *Pratylenchus* populations showed a high similarity (99-100%) between all *P. penetrans* populations. The population dynamics of six *Pratylenchus* populations from Morocco were evaluated on carrot-disk cultures at 4, 8 and 12 weeks after inoculation, and at 10, 15, 20 and 25°C. The optimum temperature for reproduction of all populations was 20°C. After 8 weeks at this temperature, nematode numbers increased up to 458-fold, 310-fold and 252-fold for the four populations of P. *penetrans*, the *P. thornei* and the *P. pseudocoffeae* populations, respectively.

A real-time quantitative PCR assay was developed for the accurate detection and quantification of another root-lesion nematode, *P. thornei*. A qPCR primer set, including two primers and a probe, was designed based on the sequence of the β-1,4-endoglucanase gene. The assay was optimised by using the primers with SYBR green I dye and setting the qPCR program to different annealing temperatures ranging from 62 to 69°C. Based on the Ct values, the program with an annealing temperature of 69°C was retained. The specificity of the qPCR assay including the probe was confirmed by the lack of amplification of DNA from 47 populations belonging to 15 other *Pratylenchus* species, while DNA from nine isolates from *P. thornei* was amplified. The assay was very sensitive as it was able to detect a single individual of *P. thornei*, even when mixed with up to 80 individuals of *P. penetrans*. DNA was extracted from exactly 80 *P. thornei* individuals. A dilution series from this DNA resulted in a standard curve showing a highly significant linearity between the Ct values and the dilution rates (R^2=0.98; slope=−3.38; E=97.6%). The qPCR assay proved to be specific and sensitive, thus providing a fast and accurate tool for detection and quantification of *P. thornei* during research, as well as for diagnostic labs.

Breeding for resistant varieties is one of the most effective methods to control nematodes. A collection of 14 spring wheat and of 11 winter wheat lines, developed at CIMMYT, for resistance to both nematode species. Individual plants were grown in sand in small tubes (15× 20× 120 mm) placed in a random design with ten replicates in the greenhouse. The resistance level was evaluated based on the numbers of nematodes extracted from both roots and soil of each line. Trials were terminated 9 weeks after nematode inoculation. The numbers of *P. penetrans* and *P. thornei* were determined using a microscope. Three lines (L9, L12 and L13) were found resistant to *P. thornei* and one of these (L9) was also resistant to *P. penetrans*. To investigate the stability of this resistance, J2 of *Heterodera avenae* were simultaneously inoculated. The reproduction of both lesion nematodes *P. penetrans* and *P. thornei*, was assessed both by counting and by using the developed qPCR assays. Our results showed that the wheat lines L9 and L9, L12, L13 remained resistant to *P. penetrans* and *P. thornei*, respectively. The outcome of this study is valuable to wheat breeding programmes in Morocco and the world. However, the resistant lines should be validated under natural field conditions. These findings are important to understand the background of the source(s) of resistance responsible for inhibition of nematode reproductions in promising wheat lines.