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LEARNING FROM OLIVE EVOLUTION AND CULTIVATION TO UNDERSTAND THE DIVERSITY OF ASSOCIATED PLANT-PARASITIC NEMATODES COMMUNITIES IN MOROCCO

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Abstract

Plant-parasitic nematodes (PPN) significantly contribute to economic losses in the top-ten olive producing countries, especially in the Mediterranean basin. Instead of controlling the main pathogenic nematode species as usual, one innovative strategy to control PPN would be to manage diversity in communities in order to lead them to be less pathogenic. Then, knowing assemblage mechanisms in communities due to evolution and environmental forces is a prerequisite. This study was conducted in Morocco, because (i) information about PPN diversity is lacking, (ii) different forms of olive occur as wild (including two sub species *O. europaea* subsp. *europaea* and subsp. *Maroccana*), feral and typical cropping systems as traditional and high density and as irrigated or not) are present. Morphobiometric observations revealed a very diverse parasite nematofauna (117 species), seven new taxa being recorded for the first time on olive. Tylenchidae, Hoplolaimidae and Telotylenchidae nematodes were dominant (80% of the samples), whereas root-knot nematodes (*Meloidogyne* spp.) were detected in 40% of the samples. Multivariate analyzes showed that the development of Heteroderidae and Longidoridae nematodes was favored in PPN communities on wild olive, while lesion (Pratylenchidae) and root-knot nematodes multiply in orchards. Three *Meloidogyne* species were identified: *M. javanica* on feral and cultivated olive in southern and center Morocco; *M. arenaria* and *M. hapla* on wild olive in the north. Cytochrome oxidase I (COI) and Internal transcribed spacer-2 (ITS2) genes were good markers for species differentiation, but they were not able to distinguish *M. javanica* and *M. arenaria* populations and were not adapted for intraspecific differentiation. However, a significant morphological variability was observed between the *Meloidogyne* species, and within and between *M. javanica* populations. The response of the diversity of PPN communities as well as of *Meloidogyne* populations to olive genotype, geo-climatic zones and soil physico-chemical characteristics, and diversity of plants associated with olive trees is discussed.

Keywords: Diversity, communities, Morocco, olive, plant-parasitic nematodes.

Introduction

Growth and production of olive trees (*Olea europaea*) are affected by several pests. Among soil-borne pests, plant-parasitic nematodes (PPN) are able to feed on roots, leading to

disruption of physiological root processes of plant root (Karssen and Moens, 2006) inducing significant economic losses, especially in nurseries. A high number of PPN species (153 species belonging to 56 genera) have been recorded on olive trees in the world (Ali *et al.*, 2014). They are also able to make gateways for other soil-borne pathogens (bacteria, fungi) such as *Verticillium dahlia* (Lamberti *et al.*, 2001). Nematode control remains difficult to achieve because of the weakening of the available methods, mainly due to a lack of knowledge about the diversity of PPN communities (species co-existence in communities; responses to environmental and evolutive forces. Olive is a good host-plant model that offers the opportunity to assess the human impact on PPN communities from wild to cultivated olive and from traditional to high density cultivation, as it is observed in Morocco. Furthermore, no data were available on PPN associated with olive trees in Morocco.

Thus, this study aims at: (i) describing the diversity of PPN communities associated with olive trees in Morocco; (ii) defining the environmental factors structuring these communities; (iii) studying the diversity of the root-knot nematodes (*Meloidogyne* spp.) detected in the communities.

Materials and Methods

1. Soil sampling and nematode analyses

A survey was conducted in 2012 in 93 sites located in several regions in Morocco. Different olive modalities were included in this sampling: wild (distinguished as *Olea europaea* subsp. *europaea* and subsp. *maroccana*), feral, and cultivated (traditional and high density cultivation, irrigated or not). PPN were extracted from 213 soil samples (Seinhorst, 1962). They were enumerated (Merny and Luc, 1969) under a stereomicroscope (×60 magnification), and identified to family and genus levels (Mai and Mullin, 1996; Siddiqi, 2000). PPN suspensions were kept in glycerol (De Grisse, 1969) for morphological identification of species.

2. Species identification of *Meloidogyne* populations

Tomato plants were reared in soil samples where *Meloidogyne* spp. populations were detected. The specific identification of each population was performed by: (i) a biochemical process based on esterase electrophoresis carried out on the nematode females (Esbenshade and Triantaphyllou, 1985); (ii) molecular process based on PCR-SCARS carried out on juveniles (Zijlstra, 2000; Zijlstra *et al.*, 2000).

3. Genetic diversity of *Meloidogyne* populations

Molecular markers: COX1 (Lazarova *et al.*, 2006) and ITS2 (Clapp *et al.*, 2000) sequences performed on 15 juveniles per population and analyzed with Geneious R7.1.2. Their alignment was carried out with known *Meloidogyne* GenBank sequences. The phylogenetic analyzes were performed with FigTree.

Morphological markers: 30 juveniles per population were fixed (De Grisse, 1969) and mounted on slides. Four characters were measured under a microscope: the body length, the stylet length, the maximum width of the body (at 25% of approximately from the head), the width at the median bulb (Jipson, 1987). Data were analyzed within and between populations by ANOVA using the R software.

Results

1. Diversity of PPN communities

Taxonomic diversity: Morphobiometric observations demonstrated a high diversity of PPN communities associated with olive in Morocco. These PPN belong to orders Aphelenchia, Dorylaimida and Tylenchida. 117 species distributed in 47 genera and 18 families have been recorded.

Dominance of taxa: Both abundance and frequency of PPN (at family level) showed that Tylenchidae, Telotylenchidae and Hoplolaimidae nematodes were dominant because they were recorded in more than 80% of samples (Fig. 1). On the contrary, Trichodoridae, Criconematidae and Aphelenchidae nematodes were less abundant and less frequent (found in less than 20% of the samples). Paratylenchidae, Longidoridae and Pratylenchidae nematodes were present in 40 to 60% of the samples. *Meloidogyne* species were recorded in less than 40% of the samples.

Community patterns: PCA analyzes have been used in order to explore PPN community patterns according to the different types of olive surveyed. Communities were significantly different in natural olive (wild and feral) and in cultivated olive (traditional and high density). Nematodes such as *Heterodera* spp., *Xiphinema* spp. and *Longidorus* spp. were more abundant in natural olive while the development of other nematode populations such as Pratylenchidae (*Pratylenchus* spp., *Pratylenchoides* spp.) was enhanced under cultivated olive (Fig. 2).

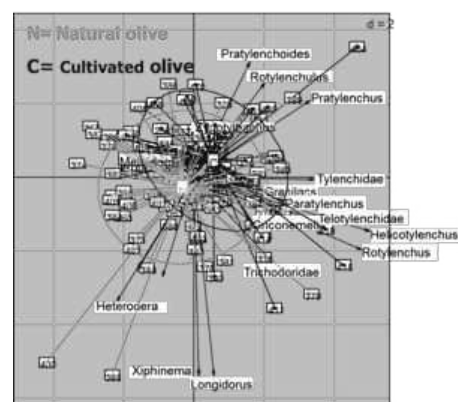
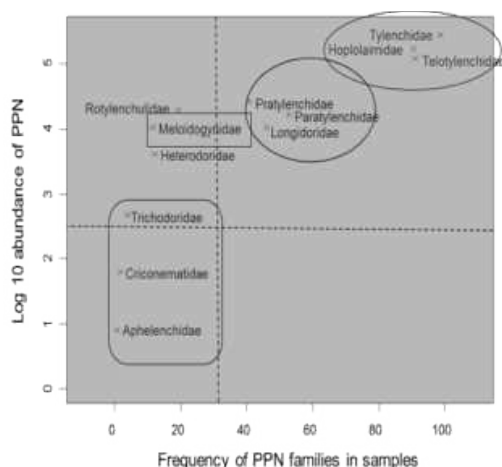


Fig. 1: Dominance of PPN families in samples

Fig. 2: Community patterns in natural and cultivated olive

2. Diversity of *Meloidogyne* species

Species characterization and spatial distribution: the biochemical analysis revealed the presence of five esterase patterns: J2, J3, and J3a corresponded to *M. javanica*, and A2 and H1 corresponding to *M. arenaria* and *M. hapla* respectively. That was confirmed by the PCR-SCARS studies. Two *Meloidogyne* populations did not correspond to any pattern. Their description as new species is in progress. *M. javanica* was associated with feral and cultivated olive (both traditional and high density) in southern and center Morocco, while *M. arenaria*, *M. hapla* and one new species were associated to wild olive in the north. The other new species was also detected on wild olive in the south (Fig. 3).

Genetic diversity of *Meloidogyne* species:

Genetic diversity: The phylogenetic analysis based on COX1 and ITS2 sequences revealed three groups: one with all the *M. javanica* and *M. arenaria* populations, one with the *M. hapla* population, and one with the new *Meloidogyne* species (Fig. 4).



Fig. 3: Spatial distribution of *Meloidogyne* populations associated with olive in Morocco

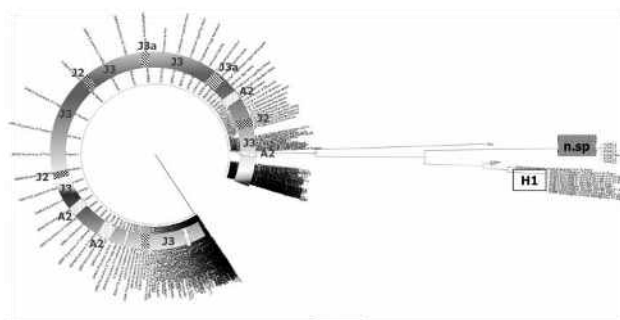


Fig. 4: CO1 gene phylogeny of *Meloidogyne* populations associated to olive in Morocco (code for *Meloidogyne* populations listed in Fig. 3)

Morphological diversity: The morphological variability was analyzed on juveniles from the same population (within population), and on populations from the same species. All the characters measured varied significantly between juveniles from the same population (intra-population variability) and between populations from the same species (inter populations = intra species variability). Variations of the stylet of *M. javanica* juveniles are given as an example (Fig. 5).

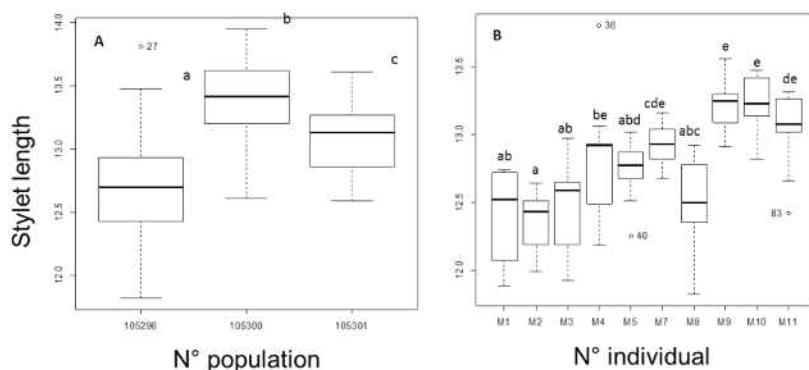


Fig.

5. Stylet length variation of *M. javanica* juveniles between populations

(A) and between juveniles within one population (B).

Discussion

This study is the first report about PPN associated with olive trees in Morocco; it reveals a high diversity of these parasites on olive with 47 genera and 117 species. These data seems to be very important if compared with the list of PPN recorded on olive trees in the world (Ali et

al., 2014). Furthermore, it reports the new detection of 58 species belonging to 7 genera on olive trees.

Modelling the PPN frequency and abundance demonstrated that Tylenchidae, Telotylenchidae and Hoplolaimidae nematodes were dominant in olive orchards regardless of their cultivation modality. These nematodes are not known to induce damages on olive. The high occurrence of Pratylenchidae and Longidoridae nematodes should be kept in attention because they are known to induce damages on olive trees especially in nurseries (e.g. Pratylenchidae) and to be plant virus vectors (e.g. Longidoridae). Root-knot nematodes (*Meloidogyne* spp.) which were detected in less than 40% of the samples are mainly considered among the less frequent species in olive orchards (Inserra *et al.*, 1976; Hashim, 1979; 1983).

Community patterns significantly differed between natural and cultivated olive systems, Heteroderidae and Longidoridae nematodes being dominant in wild systems while root-lesion nematodes (*Pratylenchus* spp.) and root-knot nematodes (*Meloidogyne* spp.) were dominant in cultivated systems. High population levels of *Meloidogyne* spp. in high-density and irrigated orchards would attract attention especially in olive development programs such as in Morocco.

Among *Meloidogyne* species, *M. javanica* was the most frequent all over the olive orchards and on feral olive. The other *Meloidogyne* species (*M. arenaria*, *M. hapla* and two new species) were detected on wild olive. So we suspect that *M. javanica* would be introduced from nurseries (Aït Hamza *et al.*, 2014) where it induces high damages (Hashim, 1983) by reducing plant growth (Nico *et al.*, 2002). The new species detected on wild olive would have co-evolved with these olive trees. This hypothesis would lead to study co-evolution between PPN and olive from the last glaciation and during olive domestication all around the Mediterranean Basin (Besnard *et al.*, 2002).

Molecular markers such as COX1 and ITS2 appeared as good markers to differentiate *Meloidogyne* species but *M. javanica* and *M. arenaria* remains undistinguishable certainly because they belong to the same phylogenetic clade (De Ley *et al.*, 2002). Unfortunately, these markers cannot be used for intraspecific genetic analyses. Other genes must be used such as 63R (Besnard *et al.*, 2014). A significant morphological variability was detected between the *M. javanica* populations recovered, describing an intraspecific variability. The inter-species variability is not yet analyzed.

In order to understand the contribution of intraspecific variability of nematodes to the structuration of PPN communities, the PPN diversity (between and within communities / between and within *Meloidogyne* species and populations) will be explored according to olive genetic diversity and environmental constraints (soil physicochemical factors and climatic parameters).

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