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Host suitability of peppers to the false root-knot nematode *Nacobbus aberrans*

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ABSTRACT

The false root-knot nematode, *Nacobbus aberrans*, causes severe damage to field and greenhouse pepper crops in several localities of the American continent. No commercial peppers (*Capsicum annuum*) resistant to this nematode are available up to the present. Host suitability of 6 experimental and 3 commercial peppers (some of them carrying *Me1* and *Me7* resistance genes to *Meloidogyne* spp.) to two *N. aberrans* populations were evaluated under greenhouse experiments. None of the peppers was found to be resistant to the nematode. The evaluated parameters exhibited significant differences among some peppers tested within a single population and between populations for a single plant material. Some peppers carrying resistance showed higher nematode reproduction than some lines that did not possess resistance genes. These results confirmed that the genes conferring resistance to *Meloidogyne* spp. do not provide protection against this species of root-galling nematode. Host suitability of pepper lines carrying *Me1* or *Me7* resistance genes against *N. aberrans* is evaluated for the first time. Search for resistant genes against this nematode in wild peppers growing in areas where this nematode is indigenous should be promoted.

1. Introduction

Pepper (*Capsicum annuum* L.) is a valuable vegetable for human nutrition due to its content of bioactive nutrients, which are important dietary antioxidants (Navarro et al., 2006). In addition, pepper production is a good source of income for small producers in several developing countries worldwide (Bosland and Votava, 1999). The ban of the use of artificial colours and the rise in production costs has increased the international demand for this crop, resulting in a growing interest in the pepper crop in Argentina (Gonzalez Vera et al., 2002). The pepper cultivated area in the country is currently about 13000 ha in fields, representing a production of 65000 t.

Globally, pepper is cultivated in temperate, subtropical, and tropical areas where the root-knot nematodes (RKNs), *Meloidogyne* spp., complete several life cycles annually (Thies and Fery, 2000a), with *Meloidogyne incognita* being one of the major pests throughout the world. In the American continent, the pepper crop is also seriously affected by *Nacobbus aberrans* (Thorne, 1935) Thorne and Allen, 1944. This nematode is known as the false root-knot nematode because it also induces the formation of galls in the host roots. It is one of the top 10 plant-parasitic nematodes based on economic importance (Jones et al., 2013). The species is native to the Americas (Sher, 1970) and has quarantine importance (EPPO, 2009). This nematode causes severe damage to pepper production in Mexico and South America (Inserra et al., 1985); however, in most pepper production regions where the parasite occurs, losses have not been estimated. In Argentina, *N. aberrans* has been detected affecting the pepper crop in several provinces (Chaves and Sisler, 1980; Costilla and Ojeda, 1985; Del Toro et al., 2004; Doucet and Lax, 2005), with damage being more pronounced in greenhouses than in the field. Pepper cultivars commonly grown in Argentina are susceptible to the nematode. Managing this parasite is especially complex due to the variable behaviour observed

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among populations towards a single host and its wide host range (Costilla, 1990; Castiblanco et al., 1999; Lax et al., 2011).

Control of plant-parasitic nematodes mainly involves pre-plant fumigation with methyl bromide; however, due to increasingly strict policies on methyl bromide use and its inevitable loss of registration for pre-plant agricultural applications, genetically-based resistance is becoming increasingly important in the management of nematodes damaging pepper production (Thies and Ariss, 2009). One of the best alternatives to cope with nematode infestations relies on the deployment of resistance genes (R-genes), which represent an efficient, environmentally safe and economically sustainable control method (Djian-Caporalino et al., 2009). Resistance in *C. annuum* is associated with dominant *N* (Thies and Fery, 2000b), *Mech* (Djian-Caporalino et al., 2007), and *Me* genes (Djian-Caporalino et al., 1999, 2001; Berthou et al., 2003; Pegard et al., 2005). Some genes (*Me4*, *Mech1, and Mech2*) are specific to certain *Meloidogyne* species or populations, whereas others (*Me1*, *Me3*, and *Me7*) are effective against a wide range of *Meloidogyne* species, including *Meloidogyne arenaria*, *Meloidogyne javanica*, and *M. incognita*, the most common species in Mediterranean and tropical areas (Djian-Caporalino et al., 2007). However, nematode populations are able to break down plant resistance, and genetic resources in terms of R-genes are limited; sustainable management of these resources is thus important for R-gene durability (Barbary et al., 2014).

Screening and breeding for resistance to *N. aberrans* have focused mainly on potato, pepper, bean and tomato (Manzanilla-López et al., 2002). In pepper, some degree of resistance to the nematode has been detected in *Capsicum baccatum* L. var. *pendulum* (Willd.) (Brunner de Magar, 1967) and in three *C. annuum* cultivars (Sisler and Casaurang, 1983), but it was not associated with a specific gene. For this nematode species, only one study has evaluated the response of commercial and experimental pepper lines carrying *N* and *Me3* R-genes, showing that neither gene conferred resistance to *N. aberrans* populations (Lax et al., 2006). Screening of new germplasm, as well as of commercial cultivars commonly used in the country, and whose response to the nematode is still unknown, is of great importance for managing this parasite. The objective of this work was to evaluate the host suitability of commercial and experimental peppers (some of them carrying resistance to RKNs conferred by the *Me1* and *Me7* R-genes) under greenhouse, to *N. aberrans* populations.

2. Materials and methods

2.1. Nematode populations

Studies were conducted using two Argentine populations of *N. aberrans* from the localities of Río Cuarto (RC) (department of Río Cuarto, province of Córdoba) and Lisandro Olmos (LO) (La Plata district, province of Buenos Aires). The populations were selected based on their known aggressiveness to pepper (Lax et al., 2011). The nematodes were maintained on tomato cv. Platense. Egg masses were removed from infected roots and placed in Petri dishes containing water and maintained at room temperature (25 ± 2 °C) to allow egg hatching. After three days, active second-stage juveniles (J2) were collected and immediately used to inoculate pepper plants.

2.2. Plant material

The characteristics of the peppers evaluated are indicated in Table 1. California Wonder was used as a control because of its susceptibility to the nematode. Sakata Seed Sudamerica Ltd. (Brazil) provided the inbred experimental lines of *C. annuum*, some of them carrying *Me1* (AF 8724 and AF 8765) and *Me7* R-genes (AF 2739) to *M. arenaria, M. incognita* and *M. javanica* (Djian-Caporalino et al., 2007). Commercial peppers [Fyuco INTA, Fenomeno RZ (35–615), Yatasto] commonly used in Argentina and of unknown response to the nematode were also considered.

Seeds were germinated in trays with sterile soil. Four-leaf stage seedlings were transplanted individually into plastic pots (20 cm long × 4 cm wide) containing approximately 80 g of a mixture of sterile soil and sand (3:1). Plant roots were placed on this substrate, immediately inoculated with 1.5 mL of water containing 100 active J2 (Initial population = Pi), and covered with a similar amount of substrate. Pi density was selected based on previous experiments (Lax et al., 2006, 2011). A completely randomized experimental design was used with 8 plants for each pepper. Plants were watered as needed to maintain a soil moisture level similar to that in the field. The experiment was conducted twice under greenhouse at a mean temperature of 25 ± 2 °C and a 14-h photoperiod. Plants were watered as needed and were uprooted 60 days after inoculation. Roots were carefully washed to remove soil particles and observed under stereoscopic microscope; the number of galls and egg masses present in the entire root system were counted. To count the number of eggs per plant (Final population = Pf), egg masses were extracted and immersed in a 1% NaClO solution for 4 min (Hussey and Barker, 1973). Reproduction Factor (RF) was calculated for the different plant material (RF = Pf/Pi). Based on this parameter, a pepper was considered resistant at RF = 0, of intermediate resistance at RF < 1, and susceptible at RF ≥ 1 (Charchar et al., 2003). Reproduction index (RI) was used to assess the degree of resistance/susceptibility of the different germplasm; the index was calculated as the Pf on a plant material divided by the Pf on the susceptible cultivar × 100. Plants in which RI was more than 50% were considered susceptible; 25 ≤ RI ≤ 50%, slightly resistant; 10 ≤ RI ≤ 25%, moderately resistant; 1 ≤ RI ≤ 10%, very resistant; RI ≤ 1%, highly resistant; or immune when nematodes did not reproduce (Hadisoeganda and Sasser, 1982).

2.3. Statistical analyses

Normality and homogeneity assumptions were confirmed for all variables before statistical analyses. Data were normalized by transforming them into log_{10} (x + 1) for the analysis. Mean values were statistically compared using a Scott–Knott test ($p = 0.05$) with the aim of evaluating possible differences among plant materials for a single population and between populations for a single pepper. Data from each experiment were combined for analysis using InfoStat (2002). Host suitability of individual peppers was compared with that of the susceptible control California Wonder by the Dunnett's *t*-test (Dunnett, 1955), using XLSTAT version 2015; this test was performed with the RF and the RI values.

3. Results

Both populations of *N. aberrans* reproduced on all the pepper genotypes. The values of the assessed parameters differed among treatments (Tables 2 and 3). Differences in the behaviour of a single population on the different plant materials were observed; behaviour also differed among populations on a single pepper, especially in Fyuco INTA, AF 10742 and AF 10743, which do not possess resistance genes.

For both populations, the number of galls of all the peppers carrying resistance did not show significant differences from the susceptible control; the same result was obtained with non-resistant peppers AF 10744 (LO population), Fyuco INTA and Yatasto RZ (RC population). The number of galls in AF 10744 (RC), Yatasto RZ and Fyuco INTA (LO) was lower than in the control; the