



## Resistance to root-knot nematode (*Meloidogyne enterolobii*) in *Capsicum* spp. accessions

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### ABSTRACT

*Meloidogyne enterolobii* has become one of the major constraints in *Capsicum* field crop, especially because genes conferring resistance to others *Meloidogyne* sp. are not effective against *M. enterolobii*. This study aimed to identify resistant *Capsicum* spp. accessions to nematode *M. enterolobii* and to classify the accessions according to resistance degree. Thirty-nine accessions of the Universidade Estadual do Norte Fluminense Darcy Ribeiro (UENF) gene bank were evaluated, being 12 accessions of *C. annuum*, 11 *C. chinense*, ten *C. baccatum* and six *C. frutescens*. The experiment was carried out in a greenhouse in a completely randomized design with ten replications and one plant per plot. The inoculation was carried out 20 days after germination being inoculated 500 eggs per plant and evaluation conducted after 75 days. Reproduction factor and reproduction index were determined, and accessions were classified according to resistance degree to the nematode. There were significant differences among the accessions tested in relation to the traits evaluated, indicating the existence of variability among and within species of *Capsicum*. Only UENF 1730 (*C. chinense*) accession was considered resistant.

**Key words:** chili pepper, disease resistance, germplasm evaluation, pre-breeding, sweet pepper

### *Resistência a Meloidogyne enterolobii em acessos de Capsicum spp.*

### RESUMO

A identificação de fontes de resistência a *Meloidogyne enterolobii* em plantas do gênero *Capsicum* tornou-se uma necessidade premente para o desenvolvimento de cultivares resistentes a esse nematoide já que genes que conferem resistência à espécie de *Meloidogyne* não são eficazes contra *M. enterolobii*. O presente trabalho teve como objetivo avaliar acessos de diferentes espécies de *Capsicum* quanto à reação ao nematoide *M. enterolobii* e classificá-los quanto ao grau de resistência. Trinta e nove acessos da coleção de germoplasma da Universidade Estadual do Norte-Fluminense Darcy Ribeiro (UENF) foram avaliados, sendo 12 acessos de *C. annuum*, 11 de *C. chinense* dez de *C. baccatum* e seis de *C. frutescens*. O experimento foi conduzido em casa de vegetação no delineamento experimental inteiramente casualizado, com dez repetições e uma planta por parcela. A inoculação foi feita 20 dias após a germinação sendo inoculados 500 ovos viáveis por planta e avaliação conduzida após 75 dias. As variáveis avaliadas foram o fator de reprodução e o índice de reprodução a partir do qual se estabeleceu o grau de resistência ao nematoide. Houve diferença altamente significativa entre os acessos para as variáveis testadas indicando existência de variabilidade entre e dentro das espécies de *Capsicum*; apenas o acesso UENF 1730 (*C. chinense*) foi considerado resistente.

**Palavras-chave:** pimenta, resistência a doenças, avaliação de germoplasma, pré-melhoramento, pimentão

## Introduction

Genetic resistance is considered the most efficient way to control plant diseases and the search for resistant genotypes is fundamental for the development of breeding programs that aim to develop resistant cultivars to the pathogens. An example of successful use of resistance genes in plants is the case of the interaction between *Capsicum* plants, which include chilies and sweet peppers, and nematodes of the genus *Meloidogyne*.

The resistance to species that are considered important from economic standpoint such as *M. incognita* (Kofoid and White) Chitwood, *M. arenaria* (Neal) Chitwood and *M. javanica* (Treub) have been associated with at least nine dominant genes (*N*, *Me1*, *Me2*, *Me3*, *Me4*, *Me5*, *Me6*, *Me7*, *Mech1* e *Mech2*) that are considered independently acting on gene-for-gene basis (Djian-Caporalino et al., 2001; Wang & Bosland, 2006; Wang et al., 2009; Babu et al., 2011; Fazari et al., 2012). Some of these genes, as *Me1*, *Me3* e *Me7* are considered thermostable and effective for a wide range of *Meloidogyne* species, including *M. incognita*, *M. arenaria* and *M. javanica* (Djian-Caporalino et al., 1999).

However, one *Meloidogyne* species, identified as *M. enterolobii* (Syn. *M. mayaguensis*) (Yang & Eisenback, 1983) have become relevant in the areas of *Capsicum* spp. cultivation because the sources of effective resistance against other nematodes, are ineffective in its control and also to the high virulence development causing small deformations in the root system and consequently a reduction in the quality and quantity of fruit (Carneiro et al., 2006; Brito et al., 2007). Moreover, this nematode has several hosts, including other vegetables, fruit trees, forest trees, ornamentals and weeds, thus hindering their control (Guimarães et al., 2003; Rodriguez et al., 2003; Brito et al., 2007; Bitencourt & Silva, 2010; Melo et al., 2011).

This study aimed to screening a *Capsicum* gene bank to identify resistant genotypes to nematode *M. enterolobii*.

## Material and Methods

Thirty-nine accessions of *Capsicum* germplasm collection were evaluated, being 12 accessions of *C. annuum*, 11 *C. chinense*, ten of *C. baccatum* and six of *C. frutescens*. These accessions were characterized for morphological and agronomic traits by Sudré et al. (2006; 2010) and Bento et al. (2007); for resistance to yellow mosaic (*Pepper yellow mosaic virus* - PepYMV) by Bento et al. (2009) and anthracnose (*Colletotrichum gloeosporioides*) by Silva (2012).

The plantlets were grown in trays of 128 cells with commercial substrate and after the emergence of two pairs of true leaves, seedlings were individually transferred to plastic pots containing substrate mixed and homogenized with the formulation osmocote 17-07-12. The experiment was carried out in a greenhouse in Campos dos Goytacazes, RJ, Brazil, in a completely randomized design with ten replicates and one plant per plot.

The inoculum source used was an isolate of *M. enterolobii* maintained in tomato plants in a greenhouse. This isolate was obtained from a commercial orchard of guava trees in São João da Barra, RJ, Brazil (lat. 21 ° 41'22" S, long. 41 ° 03'20" W).

For inoculum preparation a modification of the methodology proposed by Cotter et al. (2003) was used: parasitized roots were placed into 1 L flasks filled with 500 mL of water and agitated in a commercial shaker (Tecnal model TE240) for four minutes. Subsequently, the eggs of the nematode were obtained by passing the resultant suspension through a sieve of 100 mesh and 500 mesh.

The *Capsicum* plants were inoculated 20 days after germination with the aid of pipettes being inoculated 500 viable eggs per plant. Evaluations started 75 days after inoculation, when the root system of each plant was washed and processed individually according to Cotter et al. (2003) with modifications. The root systems were placed individually in glass jars of 500 mL filled with 300 mL of aqueous bleach 6%. Thereafter, the bottles were agitated in a commercial shaker (Tecnal model TE240) for four minutes, 130 revolutions per minute. The nematode eggs were collected by passing the resulting slurry into sieves of 100 and 500 mesh and three aliquots 1 mL were examined for counting the number of total egg population. The number of eggs per gram of root was calculated by dividing the number of eggs by root fresh weight.

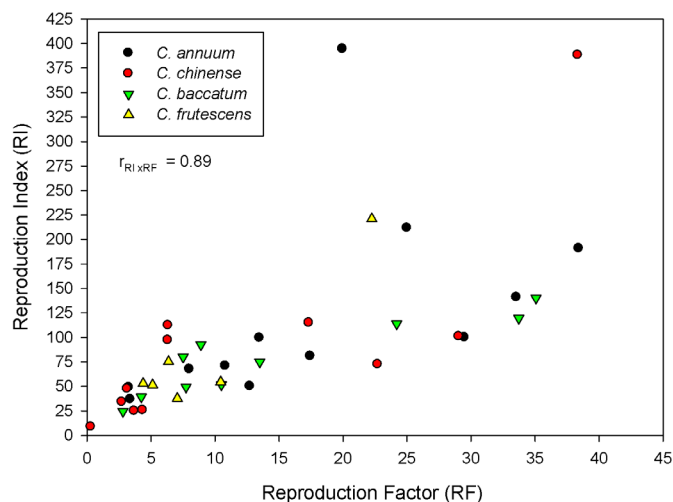
The reproduction factor (RF) was determined by dividing the number of viable eggs from the final population by the number of initial population. The value of the reproduction index (RI) was calculated by the formula: 100 (number of eggs per gram of root of each repetition/average number of eggs per gram of root of susceptible cultivar Ikeda). According to the criterion established by Taylor (1967), the degree of resistance was rated as susceptible (S) - RI greater than 50% of the value obtained for the cultivar Ikeda; slightly resistant (SR) - RI 26-50%; moderately resistant (MR) - RI with 11-25%; very resistant (VR) - RI with 1 to 10%; highly resistant (HR) - RI with less than 1% and immune (I) - when there was no reproduction.

In the statistical analysis, the resistance components were first tested for homogeneity of variances and errors normality, respectively, by Bartlett and Kolmogorov-Smirnov tests at 5% level of probability. Since these assumptions were not met, the data were processed by the equation  $\log(x + 1)$ . After analysis of variance, the mean were clustered by Scott-Knott (1974) test. All analyses were performed by the R program ([www.r-project.org](http://www.r-project.org)).

## Results and Discussion

The genotypes effect for reproduction factor (RF) and reproduction index (RI) was significant by the F test (Table 1), indicating the existence of wide variability among and within *Capsicum* species for resistance to *M. enterolobii*. RF values ranged from 0.30 (UENF 173) to 38.42 (UENF 1627), with an average of 14.19, while RI ranged from 8.79 (UENF 1730) to 394.70 (UENF 1623), with a mean value of 97.47, indicating a high severity of the pathogen on the accessions evaluated (Figure 2; Table 2). The value obtained for RF was superior to those obtained by Oliveira (2007) and Melo et al. (2011) who obtained values of 5.64 and 2.51, respectively.

For *C. annuum* accessions, the mean values of RF and IR were 17.95 and 124.46 respectively, and only UENF 1717 and UENF 1750 as slightly resistant (Table 1). The cultivar



**Figure 1.** Relation between reproduction factor and reproduction index in 39 *Capsicum* spp. accessions inoculated with *Meloidogyne enterolobii*

Ikeda presented a reproduction factor of 29.50, superior to that obtained by Melo et al. (2011) that was 2.6. UENF 1381 and Criollo de Morellos 334 (CM334) are used in *Capsicum* breeding programs, respectively, as resistance sources to bacterial spot (*Xanthomonas* sp.) (Riva et al., 2009) and to different nematodes (*M. incognita*, *M. arenaria* and *M. javanica*). Also, CM334 is resistance source for Potyvirus (Djan-Caporalino et al., 1999, 2001; Janzac et al., 2009). In the present study, UENF 1381 and CM 334 showed, respectively, RF 17.44 and 12.72, and RI of 80.86 and 50.21, indicating high susceptibility to *M. enterolobii*. This result demonstrates the ineffectiveness of gene *Me7*, present in CM334, against the *M. enterolobii* action. Brito et al. (2007) also verified the inefficiency of genes *Mi-1*, *N* and *Tabasco* on infection and reproduction of *M. enterolobii* on tomato and pepper genotypes.

In relation to *C. chinense* accessions, the mean values of RF and RI were 12.30 and 93.43, respectively, being UENF 1554, UENF 1706 and UENF 1780 accessions slightly resistant (RF: 3.14, 2.72 and 4.35, respectively); UENF 1706 moderately resistant (FR: 3.68), and UENF 1730 resistant (RF: 0.30) (Table 1). The UENF 1730 was also identified as resistant PepYMV (Bento et al., 2009) which can provide a more useful resistant source to different pathogens.

For *C. baccatum* accessions mean values for RF and RI were 97.47 and 14.19, respectively. The UENF 1635 and UENF 1718 accessions were considered slightly resistant (FR: 4.24, RI 7.73, respectively) and UENF 1714 moderately resistant (FR 2.80) (Table 1). Considering *C. frutescens* accessions, mean values for RF and RI were of 9.26 and 82.13, respectively, and only UENF 1747 was considered slightly resistant (RF: 7.05).

Genetic resistance to nematodes is one of the most efficient, economical and causes less environmental impact in controlling this endoparasitic. The use of UENF 1730 accession in breeding programs is promising to develop new cultivars of chili and sweet pepper resistant to *M. enterolobii*. In addition, this *C. chinense* accession is also resistant to PepYMV, an important virus in the *Capsicum* producing regions in Brazil, and it belongs to the same gene pool complex that *C. annuum*, being possible hybridization among these two species.

**Table 1.** Reproduction factor (RF), reproduction index (RI) and degree of resistance (DR) of the *Meloidogyne enterolobii* in 39 *Capsicum* spp. accessions

| Acessos                     | FR      | IR(%)    | GR <sup>1/</sup> |
|-----------------------------|---------|----------|------------------|
| <b><i>C. annuum</i></b>     |         |          |                  |
| UENF 1421 'Ikeda'           | 29.50 a | 100.00 b | S                |
| UENF 1381                   | 17.44 b | 80.86 b  | S                |
| 'Criollo de Morellos'       | 12.72 b | 50.21 b  | S                |
| UENF 1622                   | 10.80 b | 70.91 b  | S                |
| UENF 1623                   | 19.96 b | 394.70 a | S                |
| UENF 1626                   | 13.47 b | 99.55 b  | S                |
| UENF 1627                   | 38.42 a | 190.96 a | S                |
| UENF 1717                   |         |          |                  |
| UENF 1740                   | 3.26 c  | 49.03 b  | SR               |
| UENF 1740                   | 33.55 a | 140.98 a | S                |
| UENF 1741                   | 8.00 b  | 67.61 b  | S                |
| UENF 1750                   | 3.38 c  | 36.90 c  | SR               |
| UENF 1799                   | 25.00 a | 211.81 a | S                |
| <b><i>C. chinense</i></b>   |         |          |                  |
| UENF 1554                   | 3.14 c  | 47.56 c  | SR               |
| UENF 1703                   | 3.68 c  | 24.92 c  | MR               |
| UENF 1706                   | 2.72 c  | 34.09 b  | SR               |
| UENF 1730                   | 0.30 c  | 8.79 c   | I                |
| UENF 1764                   | 22.71 a | 72.53 b  | S                |
| UENF 1765                   | 38.35 a | 388.30 a | S                |
| UENF 1770                   | 17.33 b | 115.00 b | S                |
| UENF 1772                   | 29.05 a | 101.12 b | S                |
| UENF 1780                   | 4.35 c  | 25.79 c  | SR               |
| UENF 1792                   | 6.33 b  | 112.32 b | S                |
| UENF 1798                   | 6.31 b  | 97.38 b  | S                |
| <b><i>C. baccatum</i></b>   |         |          |                  |
| UENF 1490                   | 13.49 b | 75.03 b  | S                |
| UENF 1624                   | 7.50 b  | 80.03 b  | S                |
| UENF 1628                   | 33.73 a | 119.71 b | S                |
| UENF 1635                   | 4.24 c  | 39.43 b  | SR               |
| UENF 1714                   | 2.80 c  | 24.69 c  | MR               |
| UENF 1718                   | 7.73 b  | 49.54 b  | SR               |
| UENF 1732                   | 10.49 b | 51.77 b  | S                |
| UENF 1733                   | 24.19 a | 113.99 b | S                |
| UENF 1737                   | 35.07 a | 140.23 a | S                |
| UENF 1797                   | 8.89 b  | 92.69 b  | S                |
| <b><i>C. frutescens</i></b> |         |          |                  |
| UENF 1731                   | 6.36 b  | 75.33 b  | S                |
| UENF 1747                   | 7.05 b  | 37.74 b  | SR               |
| UENF 1766                   | 22.24 a | 220.98 a | S                |
| UENF 1775                   | 10.44 b | 54.50 b  | S                |
| UENF 1776                   | 5.12 b  | 51.52 b  | S                |
| UENF 1790                   | 4.38 c  | 53.02 b  | S                |

<sup>1/</sup>S: Susceptible; SR: Slightly Resistant; MR: Moderately resistant; e I: Immune.

## Conclusions

UENF 1730 (*C. chinense*) accession was considered resistant to nematode *M. enterolobii* and should be used to incorporate resistance genes in chili and sweet pepper cultivars.

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