Identification and characterization of *Meloidogyne* spp. on Musa in Martinique, Guadeloupe and French Guiana.

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INTRODUCTION

In the recent years, the root-knot nematodes (RKN), *Meloidogyne* spp., have been found more and more frequently during routine nematode analysis of banana roots in Martinique and Guadeloupe islands (French West Indies), instead of the formerly omnipresent burrowing nematode *Radopholus similis* (Cobb, 1893). The specific identification of these root-knot nematodes is the first requirement for devising better control methods (appropriate fallow, weed management, use of non-host and/or antagonistic plants, use of tolerant or resistant cultivars) in an integrated pest management program for bananas and other cultivated crops. The objective of the present work was to characterize and identify the species of *Meloidogyne* on banana fields in Martinique, Guadeloupe and French Guiana, and to know which is the predominant species. It also presents RAPD-PCR analyses performed to examine the genetic variation and relationships between the purified isolates belonging to the main *Meloidogyne* species.

MATERIALS AND METHODS

55 banana fields in Martinique, 28 in Guadeloupe and 13 in French Guiana, representative of different ecological situations in terms of soil type, climate, and field history, were selected for their known infestation with RKN. In each selected field, root samples were collected, examined for the presence of Meloidogyne spp. and 10 young females from separated roots were directly analysed by electrophoresis to obtain enzymatic profiles of esterase and malate dehydrogenase. Due to limitations in the biological material available, the RAPD and morphological analyses was performed on only 16 isolates which were purified using only 1 egg-masses and carried to Brazil (Embrapa Recursos Genéticos e Biotecnologia, Brasília, DF) to complete the analysis. (2 isolates of M. javanica, 2 M. cruciani, 7 M. arenaria, 3 M. incognita, 1 M. hispanica, and 3 isolates of unidentified Meloidogyne species). For the morphological studies, at least 30 perineal patterns were examined.

For DNA analysis, total genomic DNA was extracted from nematode eggs of each available isolate. 41 random 10-mer primers, were used in RAPD experiments. Amplification was performed on a PTC-100MJ Research thermal cycler (MJ Research Inc., Waltham, USA). Bands were scored as present or absent directly from the gels. For each isolate, 2 independent PCR reactions were electrophoresed in the same gel, only DNA fragments consistently present or absent between repeats being recorded and considered as binary characters. Moreover, experiments were repeated at least once. DNA fingerprints from each isolate were converted to a 0-1 matrix and a phylogenetic analysis was conducted. Distance analysis was performed, according to the UPGMA method and a consensus dendrogram was computed.

RESULTS

This study, the most detailed and extensive RKN survey in the French Overseas Departments, provided additional and useful informations about the diversity of species and populations on bananas in the genus *Meloidogyne*.

- In Martinique, *M. arenaria*, *M. incognita* and *Meloidogyne* spp., were detected in 58.8%, 40.1% and 1.1%, respectively. Of the 55 analysed areas, 34.6% presented mixed species and the other 65.4 % pure species.
- In Guadeloupe, *M. arenaria*, *M. incognita* and *Meloidogyne* spp. were detected in 80.9, 13.4 and 5.7%, respectively. Of the 28 analysed areas, 39.3 % presented mixed species and the other 60.7%, pure species.
- In French Guiana, *M. arenaria*, *M. incognita* and *Meloidogyne* spp. were detected in 46.0, 49.4, and 4.6%, respectively. Of the 13 analysed areas, 61.5% presented mixed species and the other 38.5% pure species, with *M. arenaria* and *M. incognita* prevailing.

Results showed that RAPD markers produced are consistent with other approaches (enzyme phenotypes and morphology) for identification of species and estimating genetic relationship and diversity among isolates.

M. arenaria was the most important species on banana in Martinique, Guadeloupe and French Guiana. The phenotypes A3, A2 and A1 are species-specific to *M. arenaria*, 61.6% of the populations identified as *M. arenaria* presented the A2N1 phenotype, showing a very high intraspecific variability (61,6 %) based on DNA analysis and congruent with isozyme phenotypes. This genetic diversity of *M. arenaria* may indicate multiple origins for populations classified as *M. arenaria* or more than one species inside the same group. Alternatively, these variations may indicate that *M. arenaria* is an old species that has diverged considerably through multiple mutations and adaptations to different environments.

The Caribbean populations of *M. incognita* had two esterase phenotypes: I2, frequently and I1, occasionally. The phenotype I1 is not so many frequent in this study or in other papers recently written in Brazil in soybean (Castro *et al*., 2003) or banana (Cofcewicz *et al*., 2004). It was related with *M. incognita* var. *acrita*, comparing the perineal patterns with the description made by Chitwoodi, 1949. Although the isolates of *M. incognita* analysed here came from different French Overseas Departments, they were very close in RAPD analysis (100% of bootstrap support). At intraspecific level, only 14.9 % of polymorphism was detected.

M. brasiliensis, characterized with the species-specific Est phenotype Br2. In the dendogram deduced from RAPD data, *M. brasiliensis* clustered with *M. incognita* isolates with 100% of bootstrap support, but with 62. 4 % of polymorphim, showing it is not close to *M. incognita*. Considering the morphological features (perineal pattern, males) and morphometrical data, *M. brasiliensis* is completely different from *M. incognita*.

During this survey, *M. javanica* is a minor species on bananas only in French Guiana. Most of the *M. javanica* populations have a single esterase phenotype, J3, not found in any other *Meloidogyne* species. In our molecular and morphological analyses the isolates J3 and J2 were very close and clustered with 100 % of bootstrap support and presented only 16.6% of polymorphism and typical *M. javanica* perineal patterns.

M. cruciani occurred in French Guiana and Guadeloupe and is also a minor species presenting a species-specific esterase phenotype (Cr3). In the dendrogram analysis deduced from the RAPD data, *M. javanica* and *M. cruciani* are seen to be the most related species, presenting an interspecific polymorphism of 57.4%. In the morphological features (similarities in perineal patterns), this last species seems also to be related to *M. javanica*.

Considering the analysed populations of RKN from these geographical areas, 61.5% of them were found mixed with *M. arenaria* and *M. incognita* prevailing. Similar results were observed in banana plantations from Brazil, where 84% of populations are composed of *M. javanica* and *M. incognita* in mixed infestations (Cofcewicz *et al*., 2004). These data are important and should be considered to apply better control methods (appropriate fallow, weed management, use of non-host and/or antagonistic plants, use of tolerant or resistant cultivars) in an integrated pest management.

References

Castro, J. M. C. E., R. D. Lima, and R. M. D. G. Carneiro. 2003. Variabilidade isoenzimática de populações de *Meloidogyne* spp. provenientes de regiões brasileiras produtoras de soja. Nematologia Brasileira 27: 1-12.

Cofcewicz, E. T., Carneiro, R. M. D. G. ,Castagnone-Sereno, P. and Quénéhervé, P. 2003. Enzyme phenotypes and genetic diversity of root-knot nematodes parasitising Musa in Brazil. Nematology 6: 85-95.