Molecular characterization and genetic diversity of tomato root-knot nematodes (*Meloidogyne spp.)* from selected localities of Kenya

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ABSTRACT

Distinguishing pathogenic nematodes on the basis of morphological characters is difficult because differences in characters may be absent or difficult to observe. Hence identification to the species level is increasingly reliant on molecular based techniques. Polymerase Chain Reaction (PCR) based techniques offer possibilities for precision, sensitivity and quantification. Tomato root-knot nematodes from the three selected areas in Kenya were characterized by analysis of isozyme phenotypes and Restriction Fragment Length Polymorphism (RFLP) of the PCR products of mitochondrial cytochrome oxidase subunit II (COII) and the nuclear small subunit ribosomal DNA (SSUrDNA) region.

Enzyme phenotypes, Esterases (EST) and malate dehydrogenase (MDH) were used to characterize different species of *Meloidogyne*, from selected tomato production areas in Kenya. Esterase activity had high polymorphism and was the most useful in the identification of the different species. *Meloidogyne incognita*, *M. javanica* and *M. arenaria* had different isozyme phenotypes. *Meloidogyne incognita* had two phenotypes (I1 and I2) and was the most predominant accounting for 57% of all the specimens studied. *Meloidogyne javanica* (Esterase phenotype J3) occurred in 28% of all the specimens while *M. arenaria* (Esterase phenotype A1 and A2) was found in only 15%.

Primers C2F3 and MRH 106 were utilized to amplify the intergenic region between cytochrome oxidase subunit II (COII) and Large Subunit ribosomal RNA (LSUrRNA) genes of mtDNA of 101 *Meloidogyne* specimens. Specific amplified fragments were about 1.8 kb for 90 *Meloidogyne* specimens, including 57 *M. incognita* populations, 28 *M. javanica* and 6 *M. arenaria* of Esterase phenotype (A1) and about 1.2 kb for 10 *M. arenaria* esterase phenotype (A2).

PCR results gained from DNA of the second stage juveniles from the same female specimens were the same. The amplified products were digested with the restriction enzyme *Hin*fI, and the results showed that all specimens of *M. incognita* could be digested into three restriction fragments of about 1.3, 0.4 and 0.1 kb except for one specimen of *M. incognita* (MIM18) that showed an additional restriction site in the 1300 bp fragment; hence 4 bands at 900, 420, 380, 100 bp were observed. No restriction site on the 1800 bp fragment occurred for *M. javanica* specimens. *M. arenaria* (Esterase phenotype A1) PCR product was digested into two restriction fragments of about 1700 and 100 bp while *M. arenaria* (Esterase phenotype A2) had two restriction fragments at 1.1 and 0.1 kb.

The PCR products obtained using the SSUrDNA primers and DNA from the second stage juveniles produced a single PCR product of 1.0 kb. Digestion of the fragment using *Bam*HI yielded no polymorphism in all the species studied. Thus, isozyme phenotypes and mtDNA cytochrome oxidase subunit II are a rapid and reliable approach for molecular identification of common *Meloidogyne* species in Kenya.