

Identification of different root-knot nematodes and detection of intraspecific and intrapopulation genetic variabilities between different nematode samples using RAPD technique

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ABSTRACT

Root knot nematodes (*Meloidogyne* species) are parasites of a multitude of host plants. Estimated yearly crop losses are approximately 5% worldwide, although the damage inflicted in certain regions of developing countries exceeds this level. The amplification of mitochondrial DNA (mtDNA) method was used to distinguish different genera and species of root knot nematodes. Single juveniles were used in a PCR reaction mixture. Primer annealing sites were located in the 3' portion of the mitochondrial gene coding for cytochrome oxidase subunit II and the 16S rRNA gene. Following PCR amplification, fragment of size 1700 bp specific for genus *Meloidogyne* was produced. Digestion of the amplified product with restriction endonucleases allowed discrimination among species with identically sized amplification products. *Hinf* I digestion of the 1700 bp fragment produced a two banded pattern in *M. javanica*, versus a three banded pattern in *M. incognita*. The random amplified polymorphic DNA (RAPD) method was used to investigate the genetic diversity in *Meloidogyne* species isolates and to identify molecular markers characteristic of this species, in order to develop PCR-based markers, which can be used to detect these nematode species in the field. Populations of each of *M. incognita* and *M. javanica* were easily distinguished by differences in fragment patterns with any of 10 RAPD primers. These distinctive RAPD fragments are candidates for intraspecific and intrapopulation identifications. Some primers, e.g., OPA-3, OPA-5, OPB-3 and OPB-5, yielded only a few species-specific bands that enabled differentiation of *M. incognita* and *M. javanica*. Other primers yielded more complex patterns, which were less easily interpreted.

Key words: Molecular markers, plant parasitic nematodes, RAPD-PCR.

INTRODUCTION

Nematodes constitute one of the largest animal phyla, consisting of over a half million species of worms. Four out of

five living animals on this planet are nematodes (Platt, 1994). Nematodes can live as obligatory parasites of plants and animals; they can alternate a parasitic with a free-living life style or be strictly free-living. Despite their