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Molecular identification of root knot nematodes (*Meloidogyne* species) in Sri Lanka

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Root-knot nematodes (RKNs) from the genus *Meloidogyne* are major obligate endoparasitic pathogens of a multitude of economically important host plants worldwide. Correct identification of RKN species is becoming increasingly important for the design of effective nematode management practices. To our knowledge there were no literature records about molecular identification of RKNs in Sri Lanka. The main objective of this study was to develop a fast, accurate and sensitive Polymerase Chain Reaction (PCR) based molecular assay to accurately identify RKNs parasitizing important vegetable and fruit crops in Sri Lanka. Root knot nematode infected root samples were collected from 4 different localities in Sri Lanka during the years 2013-2014 and labeled as 1-Horana/Tomato, 2- Horana/Spinach, 3-Anuradhapura/Guava, 4-Kalpitiya/Tomato, 5-Colombo/Cherry Tomato, 6- Kalptiya/ Guava. DNA extracted from juvenile stages of *Meloidogyne* was used in PCR. Amplification of ribosomal DNA with MF/MR universal primers yielded a 500 bp fragment specific for genus *Meloidogyne* for samples belonging to all localities. The sequence of 500 bp PCR product was found to be 100 % identical to the most common *Meloidogyne* species available in Genbank. C2F3/1108 primers that result in species-specific PCR products based on size were used to identify and differentiate infected *Meloidogyne* species in the collected samples. Amplification of mitochondrial DNA with C_2F_3/1108 primers yielded an 1100 bp product specific for *M. arenaria* for only sample 1, a 705 bp size products specific for *M. enterolobii* for sample 1, 3 and 6, a 520 bp products specific for *M. chitwoodi* or *M. hapla* for sample 2, 4, 5 and 6. Sample 2, 4, 5 and 6 were further analyzed with 194/195 primers and the exact RKN infected was identified as *M. hapla* by the amplification of specific ~ 700 bp size PCR product. This study demonstrated the occurrence of *Meloidogyne* species in Sri Lanka either alone or in mixed populations. Even though the species *M. arenaria, M. incognita, M. javanica*, and *M. hapla* are generally considered the most widespread, *M. enterolobii and M. hapla* were found to be more widely distributed in the studied areas of Sri Lanka. The protocols optimized in this study would be useful in the future to analyze RKN infected samples collected across Sri Lanka to evaluate the prevalence, incidence and diversity of RKNs more comprehensively.

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