Molecular characterization sequence variation in the rDNA region of root-knot nematode (Meloidogyne sp.,) in indigenous lefy vegetables

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Indigenous leafy vegetables (IL V s) are plants whose leaves or aerial parts have been integrated in a community's culture for use as food over a large span of time. In the past, traditional societies have exploited edible wild plant resources to obtain their nutritional requirements. Solanum nigrum, Amaranthus hybridus, and Cleome gynandra are the most used ILVs. Root-Knot Nematodes (RKN) belonging to the genus Meloidogyne are the most economically important nematode pests affecting IL V production and have a diverse host range. Plant-parasitic nematodes are responsible for global agricultural losses' amounting to an estimated $157 billion annually. Most IL V crops produced are susceptible to nematode injury, particularly by root knot nematode and sting nematodes. Plant symptoms and yield reductions are often directly related to pre plant infestation levels in soil and to other environmental stresses imposed upon the plant during crop growth. As infestation levels increase so then do the amount of damage and yield loss. Most previous studies on the diversity of Meloidogyne spp. have focused on morphology (for example: perineal patterns, stylet structure, body length), and the response of the populations to differential host test. Morphological differences may be absent or difficult to observe. Identification by these procedures is difficult even for qualified taxonomists. Isoenzyme electrophoresis has also been used to characterize RKN populations. The esterase phenotypes are species-specific and are a good tool for identifying RKN. PCR-based diagnostics offer possibilities for precision, sensitivity and quantification. Ribosomal DNA distinguishes many species of nematodes, investigates intraspecific variation and examines evolutionary relationships. In this study esterase phenotypes (EST), Malate dehydrogenase (MDH) was used. Four esterase phenotypes were recognized on the basis of single bands or combinations of several bands. One Mdh phenotypes (i.e. Nl) and four EST phenotypes (i.e. 12, 13, Al and A2) were detected. Three species were identified from the populations, of which 20 were M incognita (Mdh-Est phenotype NI-I2), 10 were Mjavanica (NI-B), 10 were M arenaria (Nl- A2). All the three species amplified using SSUrRNA produced a single PCR product of 700 bp. The PCR products were then purified and subjected to sequencing. Sequences were aligned with sequence alignment tools (ClustaIW2) to show areas of variability and areas of conserved regions. The obtained sequences were compared with nucleotide sequences in the Gene bank using the BLAST Software to determine similarities. From the blast analysis several sequences of Meloidogyne nematodes (5 S ribosomal RNA) were identified with regions that matched with the obtained sequences. The nearly complete 5S rDNA sequences obtained from the 11 sequences varied from 675 to 692 base pairs. The sequenced products were aligned to investigate sequence diversity and infer phylogenetic relationship among the species. Phylogenetic analysis was done by MEGA (Molecular Evolutionary Genetic Analysis) computer program. The

dendrogram of the phylogenetic tree was drawn using a web based program which confirmed the sequences of the most four common Meloidogyne species. The results of the study will be used to design best IPM programs and better understand taxonomy.