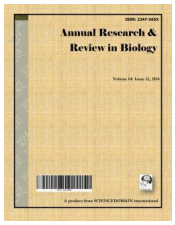

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Sequence Variation in the rDNA Region of Root-Knot Nematodes (*Meloidogyne* spp) Infecting Indigenous Leafy Vegetables in Kisii and Transmara Sub-counties, Kenya

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Authors' contributions

This work was carried out in collaboration amongst all authors. Author JMM designed the carried out field and laboratory experiments and made the write-up and collected the relevant data. Author CG provided financial support for DNA sequencing, made follow ups to the experiments and write –ups. Author WW supervised the experiments and offered financial assistance through the Gatsby Charitable Foundation, UK. Author SR made follow ups to the experiments, write –ups and technical advice in the laboratory. Author PKM assisted in the greenhouse experiments and the write-up of the article. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The aim of the study was to evaluate the utility of DNA sequencing in differentiating Root-Knot Nematodes (RKN) (*Meloidogyne* spp) infecting Indigenous Leafy Vegetables (ILVs) in Kisii and Transmara sub-counties in Kenya.

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Study Design: Cross-sectional study.
Place and Distribution of Study: Nematology Laboratory, Kenyatta University and International Livestock Research Institute (ILRI) Laboratories, Nairobi, between March 2009 to September 2011.
Methodology: Samples were obtained from two sites; Kisii and Trans-mara sub-counties. From each selected site; 10 farms which grew ILVs were randomly sampled. From each farm 10 plants of each ILV infected by Root knot nematodes were randomly selected. They were uprooted together with the surrounding soil and transported to the laboratory. In the laboratory female second-stage juveniles (J2) were extracted and used for genomic DNA isolation and sequencing. PCR amplifications of the extracted DNA was carried out for each isolate using primers: SSU18A (5'-AAAGATTAAAGCCATGCATG-3') and SSU26R (5'-CATTCTTGGCAAATGCTTTCG-3'). Double stranded DNA were sequenced by cycle sequencing with Big Dye 3.0 Terminator cycle sequencing kit and analysed with an ABI 310 Gene Analyser. Sequences were determined on one strand using the M13 forward primer. Sequence information was assembled using sequencer 4.1. Alignments were performed using ClustalW.
Results: All the three major species identified, namely *Meloidogyne arenaria, M. javanica* and *M. incognita* amplified using SSUrRNA produced a single PCR product of 700 bp and 11 sequences obtained that were compared with nucleotide sequences in the Gene bank using the BLAST Software to determine similarities. 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.
Conclusion: The selected primers can be used for determining variation in the rDNA region in RKN infecting ILVs in Kenya.

Keywords: DNA sequencing; indigenous leafy vegetables; root-knot nematodes; PCR.

1. INTRODUCTION

Indigenous leafy vegetables (ILVs) are plants whose leaves or above ground parts have been incorporated in a community's traditions for use as foodstuff over a large period of time [1]. They contribute to food diet, income generation, good nutrition and health to resource-poor farmers. The selected ILVs were *Solanum nigrum* L., *Cleome gynandra* L. and *Amaranthus* spp. The growing awareness in recent years of the health promoting and protecting properties of non-nutrient bioactive compounds found in green vegetables has directed increased attention to vegetables as vital components of daily diets [2]. The ILVs are valuable sources of protein, vitamins especially A, B, C and K and essential minerals like iron, calcium, potassium, magnesium and zinc [2]. The genus *Meloidogyne* comprises all Root-Knot Nematodes (RKN) [3]. It contains over 100 described species [4]. Plant parasitic nematodes are among pests that have been associated with vegetable losses. Root-knot nematodes are the most economically-significant group of plant nematodes attacking roots and tubers of crops [5] causing serious problems on many indigenous leafy vegetables in Kenya. For a long time now, identification has been based on morphological characters and this is a difficult task even to the most qualified taxonomists. Despite the numerous studies about their biology and taxonomy, their identification to the species level still pose a huge challenge to many diagnosticians mostly because of their very small inter-specific morphological variation [6,3].

DNA based techniques provide more reliable and precise information for species identification [7,8] as opposed to morphological identification. DNA based techniques require amplification of a specified region of the genome using polymerase chain reaction (PCR). Useful loci have been identified in both mitochondrial and nuclear genomes. Ribosomal RNA is a multi family gene within the nucleus. Its coding sequences are most conserved gene and have received considerable attention with respect to nematode identification, evolutionary and phylogenetic studies. These sequences consist of several hundred tandemly repeated copies of transcription unit which encodes for 18s, 5.8s, and 26s genes with internal transcribed spacers ITS 1 and ITS 2. Depending on the level of investigation, researchers have chosen different regions; Large sub unit ribosomal DNA (LSUrDNA), small sub unit ribosomal DNA (SSUrDNA), or ITS region. The 18s and 26s genes and spacers differ greatly in the rate of evolution, they can reveal phylogenetic relationships ranging from distantly related organisms to the level of populations [9,10]. It's

commonly accepted that the level of intra-specific sequences variation among ribosomal sequences is low due to their concerted evolution that is single repeats in multigene evolve in concert resulting in homogenization of all repeats in an array. The small sub unit rDNA (SSUrDNA or 18s) sequence dataset is currently unique for the phylum because the sequences are available for large number of identified specimen across known phylogenetic diversity. The 5' third of 1600 base pair SSU gene contains 50% of the nucleotide variability of the whole gene, as it encompasses both conserved stem and highly divergent loop regions [11,12]. The gene is also of relatively constant length and can be aligned with some confidence. The SSU is also present in 50-100 copies per genome and hence more abundant target than single copy gene [13]. This pattern of conservation and divergence favors it for this study.

Management strategies of RKNs have relied chiefly on the use of chemical nematicides [14]. Other management practices such as crop rotation and use of resistant host crops are more environmentally and economically sound. However, because of both variability in the nematode host range and specificity of action of the resistance genes [15], this strategy requires accurate and preliminary detection and identification of the nematodes to optimize the use of selected cultivars to be adapted in the crop rotation systems. There is need therefore, to develop accurate methods for nematode identification. This study therefore was intended to do nucleic-acid based profiling for diagnosing the characters of nematodes infecting ILVs in Kenya. The success of this method would be essential for developing efficient and sustainable integrated pest management (IPM) strategies, especially those that are based on breeding for resistance and crop rotation. Data obtained can be utilized in estimating the diversity and distribution of RKN with a view to devising the best methods and practices for their control.

2. MATERIALS AND METHODS

2.1 Study Design, Study Sites and Sampling

This study was a cross-sectional design. Purposive sampling was done in two sites in Kisii and Trans-mara Sub-couties. From each selected zone, 10 farms which grew ILVs were randomly sampled. From each farm, 10 plants each i.e. *Solanum nigrum*, *Amaranthus hybridus* and *Cleome gynandra* infected by RKNs were randomly selected and carefully uprooted, together with surrounding soil, packaged in paper bags and transported to Kenyatta University, Nematology Laboratory for further processing.

2.2 DNA Extraction and Purification

The female nematodes were handpicked from the root galls and the second-stage juveniles (J2) were extracted through extraction tray method. Genomic DNA was purified from individual RKNs according to a protocol used for cyst nematodes [14] with slight modifications. For the females about 40 of them per isolate were frozen in a pre-cooled mortar and ground to fine powder. A 50 µl lyses buffer comprising (0.1M Tris-HCl pH 8.0, 50mM EDTA, 1%w/v of SDS, 0.17M NaCl and 5 µl of proteinase K (5µg/µl)) was then added. The homogenate was incubated at 37ºC overnight. The liberated DNA was then extracted against an equal volume of chloroform: Isoamyl Alcohol (25:25 µl). The aqueous phase was then transferred to a clean 1.5 ml eppendorf tube using a wide bored pipette. Fifty µl of Isopropanol was then added and incubated at -20ºC for 20 minutes. Centrifuging at 12 000 rpm for 10 minutes followed, and the DNA re-suspended in 50 µl distilled water. Five µl of Ammonium acetate was added followed by 500 µl of ice-cold absolute ethanol. DNA was pelleted by centrifuging at 12000 rpm for 10 minutes; then washed with 100 µl of 70% ethanol, centrifuged at 12000 rpm, air –dried and re-suspended in 50 µl of distilled water. The DNA was then stored at -20ºC waiting further analysis.

2.3 Determination of DNA Concentration

The genomic DNA concentration was determined by mixing 5 µl of the DNA with 495 µl of distilled water in a micro-centrifuge tube. Quantification was done by adding dilute DNA samples to millimeter ultraviolet silica cuvette containing double distilled water that had been set and loaded in Gene Quant Spectrophotometer (Biochrom, Cambridge-UK). Absorbance was calculated automatically by the Gene Quant spectrophotometer (Biochrom, Cambridge-UK) and the DNA concentration printouts generated. Standardization of the DNA was performed by diluting the stock DNA that was above 50 ng/ µl with double distilled water accordingly.

2.4 Molecular Analysis of Nematode Isolates

2.4.1 SSUrRNA PCR Amplification

PCR amplifications of the extracted DNA was carried out for each isolate in a reaction volume of 25 µl containing 13.3 µl of water, 3.5 µl of dNTPs (concentration in mM), 2.5 µl of 10X PCR buffer, 0.5 µl of Taq polymerase, 5 µl of DNA template and 0.1 µl of each primer. The primers that were used are SSU18A (5'-AAAGATTAAAGCCATGCATG-3') and SSU26R (5'-CATTCTTGGCAAATGCTTTCG-3'). Reaction conditions included initial preheating at 94ºC for 5 minutes, 35 cycles of (94ºC for 1 minute; 52ºC for 2 minutes; 68ºC for 2 minutes) and final extension step at 68ºC for 10 minutes.

2.4.2 Gel purification of the PCR product

Quick clean 5 M Gel Extraction kit (Qiagen England) was used to purify the PCR products from the gel according to manufactures instructions.

2.4.3 DNA sequencing

Double stranded DNA was sequenced by cycle sequencing with Big Dye 3.0 Terminator cycle sequencing kit and analysed with an ABI 310 Gene Analyser (Perkin Elmar Applied Biosystems). Sequence was determined on one strand using the M13 forward primer. Sequence information was assembled using sequencer 4.1 (Genecodes Corp.). This was done at ILRI laboratories, Kabete. Alignments were performed using ClustalW [16].

2.4.4 Blast analysis

The sequences obtained were compared with nucleotide sequences in the Gene bank using the BLAST Software programme to determine similarities.

3. RESULTS AND DISCUSSION

3.1 DNA Extraction

The juveniles were hatched from the female eggs and DNA extracted from these juveniles using a standard protocol [17].

3.2 SSUrRNA Analysis

DNA was amplified using the set of primers (SSU18A and SSU26R) for the Small Sub Unit ribosomal Ribonucleic acid and run on 0.8% Agarose gel. All the three species amplified using SSUrRNA produced a single PCR product of around 700 bp (Fig. 1).

Based on this product, species could not be differentiated. The PCR products were then purified to be subjected to DNA sequencing. The DNA sequencing is able to separate very similar organisms even with one base pair difference.The purified PCR products are shown in Fig. 2.

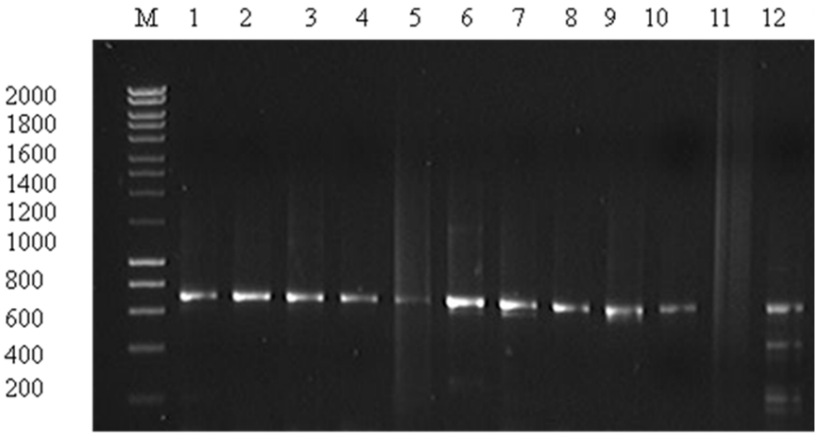

Fig. 1. Agarose gel of amplified PCR products of RKN using SSUrRNA primer
Key: M-2Kb Ladder

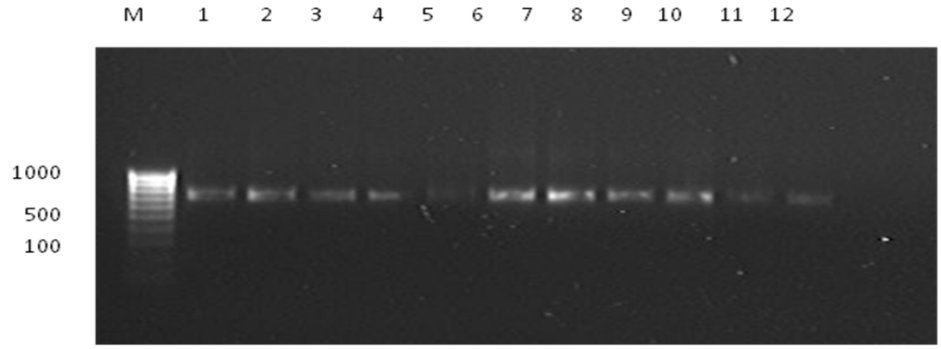

Fig. 2. Agarose gel of amplified and purified PCR product of RKN ready for sequencing
Key: M-1Kb Ladder

3.3 Sequence Alignment

The eleven sequences were aligned with sequence alignment tools (ClustalW2) to show areas with variability and areas of conserved regions as shown in Fig. 3. Areas of conservation are illustrated with stars (*). These are areas where similar nucleotides have aligned themselves while variable regions are not. Areas of variability are indicated as follows (:) where there is an insertion of nucleotide G or A its indicated with a single dot (.), where the insertion is the nucleotide T, is indicated with two dots (..) while if the insertion is the nucleotide C it's plain.

```
M1          TAAAACAAATTTCGCTGAGGCAAAGTGGGCGTGGATTTCGATG-TTCGCTGTTCGCGGGA 107
M8          TAAAACAAATTTCGCTGAGGCAAAGTGGGCGTGGATTTCGATG-TTCGCTGTTCGCGGGA 109
M2          TAAAACAAATTTCGCTGAGGCAAAGTGGGCGTGGATTTCGATG-TTCGCTGTTCGCGGGA 109
M7          TAAAACAAATTTCGCTGAGGCAAAGTGGGCGTGGATTTCGATG-TTCGCTGTTCGCGGGA 109
M9          TAAAACAAATTTCGCTGAGGCAAAGTGGGCGTGGATTTCGATG-TTCGCTGTTCGCGGGA 106
M12         TAAAACAAATTTCGCTGAGGCAAAGTGGGCGTGGATTTCCATG-TTCGCTGTTCGCGGGA 111
M6          TAAAACAAATTTCGCTGAGGCAAAGTGGGCGTGGATTTCCATG-TTCCCTGGTCTCGGGA 109
M3          TAAAACAAATTTCGCTGAGGCAAAGTGGGCGTGGATTTTCAAGGTTCGCGGTTCGGGGAA 113
M4          TAAAACAAATTTCGCTGAGGCAAAGTGGGCGTGGATTTCCAAG-GTCCCCGGTCCCGGGA 112
M10         TAAAACAAATTTCGCTGAGGCAAAGTGGGCGTGGATTTCCAAG-GTCCCCGGTCCCGGGA 111
M5          TAAAACAAATTTCGCTGAGGCAAAGTGGGCGTGGATTTCCAAG-GTCCCTGGTCCCGGGA 116
            ************************************  *:*  ** * * **  **.*

M1          ATGGTTTAAAGGAAAACTCAAATTGGGCTAATCTAGAAACTCGTGGAGAGAAATAATAGG 167
M8          ATGGTTTAAAGGAAAACTCAAATTGGGCTAATCTAGAAACTCGTGGAGAGAAATAATAGG 169
M2          ATGGTTTAAAGGAAAACTCAAATTGGGCTAATCTAGAAACTCGTGGAGAGAAATAATAGG 169
M7          ATGGTTTAAAGGAAAACTCAAATTGGGCTAATCTAGAAACTCGTGGAGAGAAATAATAGG 169
M9          ATGGTTTAAAGGAAAACTCAAATTGGGCTAATCTAGAAACTCGTGGAGAGAAATAATAGG 166
M12         ATGGTTTAAAGGAAAACTCAAATTGGGCTAATCTAGAAACTCGTGGAGAGAAATAATAGG 171
M6          ATGGTTTAAAGGAGAACTCAAATTGGGCTAATCTAGAAACTCTTGGAGAGAAATAATAGG 169
M3          AGGGTTTAAAGGAAAACTCAAATTGGGCTAATCTAAAAACTCGGGAAAAAAAATAATAGG 173
M4          AAGGGTTAAAAGGAAACTCCAATTGGGCTTAACCAAAAACCCCGGGAAAAAAAATATAAG 172
M10         AAGGGTTAAAAGAAAACTCCAATTGGGCTAAACCAAAAACCCCGGGAAAAAAAAAAAAG 171
M5          ATGGGTTAAAGGAAAACTCCAATTGGGCTAAACCAAAAACCCCGGGAAAAAAATAATAAG 176
            * ** *****.*..*****.*********:*:* *.**** *  *.*.*.***::*:*.*

M1           ATTAAAAAATTTTTTGAAAAATTTAAAGTTTATTATTATAATAAGCTTTGTTTTTTGAAG 227
M8           ATTAAAAAATTTTTTGAAAAATTTAAAGTTTATTATTATAATAAGCTTTGTTTTTTGAAG 229
M2           ATTAAAAAATTTTTTGAAAAATTTAAAGTTTATTATTATAATAAGCTTTGTTTTTTGAAG 229
M7           ATTAAAAAATTTTTTGAAAAATTTAAAGTTTATTATTATAATAAGCTTTGTTTTTTGAAG 229
M9           ATTAAAAAATTTTTTGAAAAATTTAAAGTTTATTATTATAATAAGCTTTGTTTTTTGAAG 226
M12          ATTAAAAAATTTTTTGAAAAATTTAAAGTTTATTATTATAATAAGCTTTGTTTTTTGAAG 231
M6           ATTAAAAAATTTTTTGAAAAATTTAAAGTTTATTATTATAATAAACTTTGTTTTTTGAAG 229
M3           ATTAAAAATTTTTTTGAAAATTTTAAGGTTAATAATAAAAATAACCTTTGTTTTTTAAAA 233
M4           AATAAAAAATTTTTTGAAAAATTTAAAGTTTATTAATAAAATAAGCTTTGTTTTTTGAAA 232
M10          AATAAAAAATTTTTTGAAAAATTTAAAGTTTATTAATAAAAAAAGCTTTGGTTTTTGAAA 231
M5           AATAAAAAATTTTTTGAAAAATTTAAAGTTTATTATTATAATAAGCTTTGTTTTTTGAAA 236
             *:******:**********:*****.***:**:*::*:**:** ***** *****.**.
```

Fig. 3. Aligned sequences showing variable and conserved regions
Key: M1-M12 indicate sequences in this study

3.4 Blast Analysis

BLAST Software programme was used to determine similarities in nucleotides obtained with those in genebank (NCBI). From the blast analysis several sequences of *Meloidogyne* nematodes (5 S ribosomal RNA) were identified with regions that matched with the obtained sequences. The Accession numbers from the Gene bank are gb/GQ395523.1, gb/GQ395513.1 and gb/FJ555690.1 representing 5S ribosomal RNA of *Meloidogyne arenaria, M. javanica* and *M. incognita,* respectively. These sequences showing areas of conservation (with a star) and variable regions (without a star) are shown in Fig. 4.

3.5 rDNA Sequences

Identical rDNA sequences were obtained for isolates M1, M2 and M10 which had a base pair length of 685 base pairs. Isolates M4 had a length of 686 base pairs. Isolates M3,M6 and M8 had a length of 688 base pairs. Isolate M7 was the shortest with 675 base pairs. Isolates M9 had 683 base pairs in length while the longest sequences were isolates M5 and M12 which had 692 base pairs. A total of 11 sequences were aligned to the previously published alignment [18]. The final alignment contained 686 positions with 189 Characters which were phylogenetically informative under parsimony analysis. A+T content ranged from 71.3% in the sequences analysed.

The populations of *M. arenaria, M. incognita ,M. hapla* and *M. javanica* did not give identical 5S rDNA sequences that correspond with the published sequences for the same species (18). Between the two sequences from *M. arenaria* (Isolates M1 and M9) present study versus accession number gb/ GQ395523.1 the differences were related to three deletions of A nucleotide and a substitution of nucleotide A to C. The *M.javanica* (Isolates M7, M8, M2 and M6) present study versus accession number gb/ GQ395513.1 presented insertions of 5A nucleotides and some deletions at the 5' end of the sequence. The *M.incognita* (Isolate M5) present study versus accession gb/ FJ555690.1 presented some insertions and substitutions of nucleotides between them. Finally the two sequences obtained for *M. hapla* (isolates M3 and M4) differed slightly with the published sequence (accession number gb/ GQ 130136.1) (Fig. 5).

3.6 Distance matrix analysis

Distance matrix analysis of 11 sequences based on 5 S rDNA sequences between *Meloidogyne species* isolated was analysed and a phylogenetic tree obtained including the host plants (Fig. 6). The nearly complete 5S rDNA sequences obtained from the 11 sequences varied from 675 to 692 base pairs. The pair wise distances among the *Meloidogyne spp* 5S rDNA sequences is shown in Table 1.

Fig. 4. Sample sequences aligned with those from the Gene bank showing areas of conservation and variable regions

Fig. 5. Aligned sample sequences with those from the gene bank showing gaps, insertions and deletions within the nucleotides

Table 1. Pair wise distance matrix analysis

M1										
M2	1.671									
M3	1.562	1.562								
M4	1.554	1.549	0.274							
M5	1.65	0.039	1.546	1.535						
M6	1.677	0.008	1.552	1.555	0.043					
M7	1.215	1.548	1.287	1.433	1.562	1.564				
M8	1.546	1.44	1.56	1.641	1.384	1.431	1.669			
M9	1.63	0.033	1.575	1.582	0.037	0.073	1.57	1.421		
M10	1.56	1.531	1.135	0.526	1.513	1.546	1.645	1.668	1.467	
M12	1.597	1.654	1.488	0.973	1.63	1.659	1.734	1.644	1.534	0.18

Differences between species varying from 0.008 to 1.669 base pairs. The absence of difference among taxa is due to the fact that some sequences presented character ambiguity which is not counted by the distance analysis. The overall mean distance is 0.026.

3.7 Disparity Index Analysis

The overall mean of disparity divergence between sequences was 0.026 (Table 2). The number of base substitutions per site from between sequences is shown in Table 2. Analyses were conducted using the Maximum Composite Likelihood model [19]. The analysis involved 11 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 675 positions in the final dataset. Evolutionary analyses were conducted in MEGA5 [20].

The objective of this study was to estimate the genetic variation in the rDNA region of RKN in ILVs collected from Kisii and Transmara sub-counties, using DNA Sequencing. Two specific primers for plant parasitic nematodes were used. Using DNA sequencing analysis each nematode genotype (i.e isolate) tested could be

differentiated from all the others which showed that the DNA sequencing technique can be successfully applied to the genus *Meloidogyne* for DNA fingerprinting. Similar findings were observed by [21] and [14]. At the Intraspecific level a high degree of polymorphism was

Table 2. Estimates of evolutionary divergence between sequences

	1	2	3	4	5	6	7	8	9	10	11
1											
2	0.025										
3	2.659	2.470									
4	3.572	3.250	0.176								
5	0.237	0.156	2.944	3.607							
6	0.007	0.024	2.404	3.286	0.233						
7	0.031	0.004	2.636	3.418	0.161	0.040					
8	2.730	2.390	0.372	0.197	2.458	2.484	2.530				
9	0.006	0.010	2.689	3.551	0.196	0.016	0.010	2.680			
10	4.274	3.919	0.321	0.037	4.333	3.966	4.095	0.361	4.247		
11	0.019	0.019	2.644	3.498	0.126	0.019	0.030	2.578	0.016	4.207	

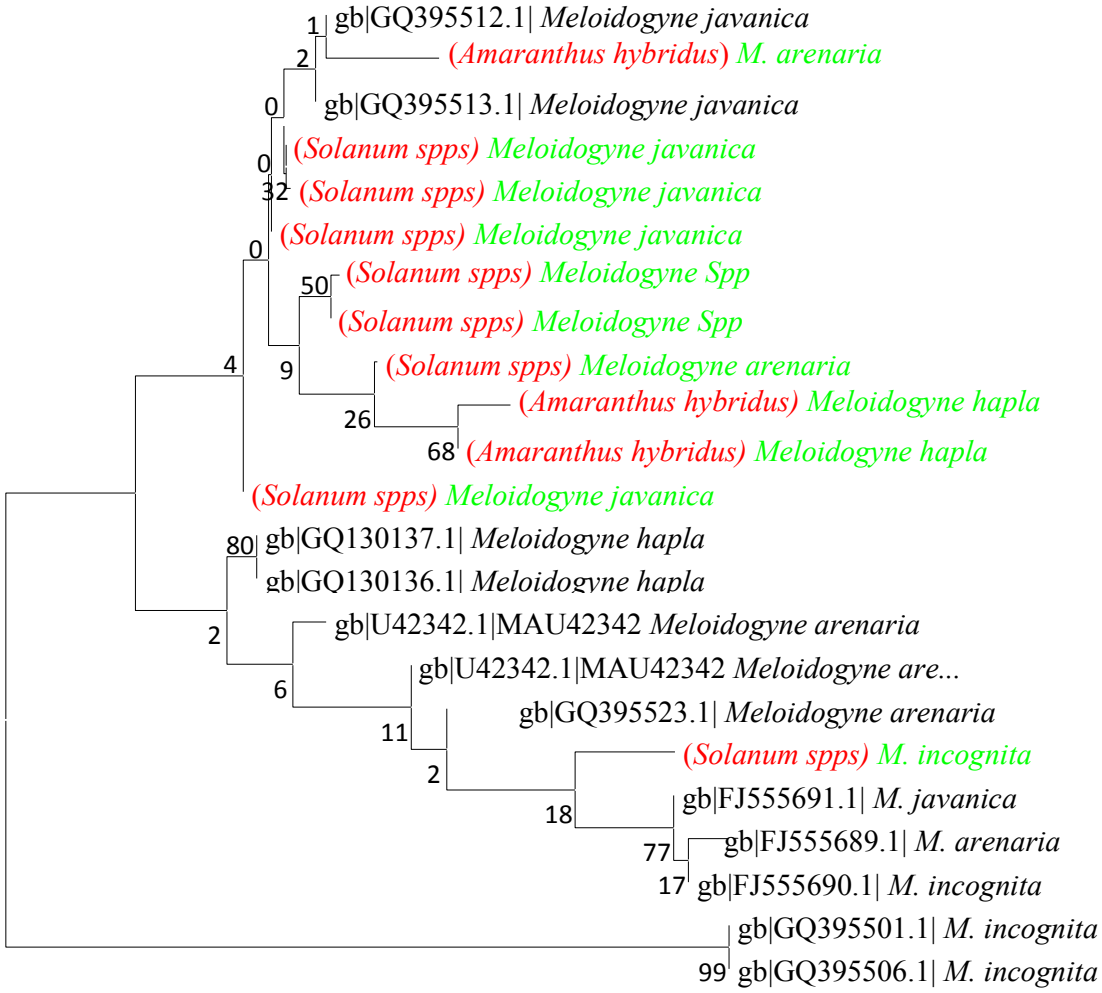

Fig. 6. Neighbour joining Phylogenetic tree of root-knot nematodes (*Meloidogyne* spp) infecting ILVs based on 5s ribosomal RNA. Numbers near nodes represent posterior probabilities

detected within *M. arenaria* isolates compared to the other species analysed. This highest level of genetic variability in *M. arenaria* was not unexpected, since this result was in agreement with previous molecular analysis [14].

These three RKN, *M. javanica, M. arenaria* and *M. incognita* species are homogenously encountered from temperate to tropical regions. In contrast to most *Meloidogyne species*, which are generally parasitic against a specific botanical family only, they are extremely polyphagous pests, with a wide host range of up to 3000 plant species including most of the commercial crops. Of particular concern is also the mode of reproduction of these three species, which is mitotic parthenogenesis. Considering the fact that RKN are probably extremely ancient species, it seems likely that they are indigenous to most of the areas where they have been found. However, recent molecular data demonstrated a low level of intraspecific polymorphism within these three parthenogenetic species [21], which is not in agreement with the hypothesis of their indigenous origin.

In the present study comparisons of corresponding rDNA coding sequences from *M. javanica* and *M. incognita* have indicated a very low nucleotide divergence (0.138), suggesting that their establishment as distinct species was a relatively recent event [22]. Therefore, an alternative scenario could be both recent evolution and spread from a few centers of origin around most of the world through agricultural practices (e.g. infested plant materials). This has been demonstrated for potato cyst nematodes [23]. Considering the enormous host range of RKN, such an explanation for their very large geographical distribution has already been speculated [24]. However current knowledge of evolutionary relationships both between and within amphimictic and parthenogenetic species is too fragmentary to help infer the origin of (putative) ancestral *Meloidogyne*.

The use of molecular markers certainly brings its own problems. Nevertheless the mode of evolution of DNA sequences is better understood than that of morphological traits and can be modeled with some confidence. This allows alternative analytical tools to be used and permits calculation of statistical support for the phylogenies produced. An important consideration is that the rates of phylesis (the generation of taxa speciation) and fixation of molecular change must be of the same order [20]. Thus, a rapidly evolving DNA segment should be used to examine the relationships between species in a genus and a much conserved segment for interordinal, or interphylum relationships.

4. CONCLUSION

From this study it was shown that ILVs are infected by the three major *Meloidogyne* nematodes notably *M. javanica, M. arenaria,* and *M. incognita* The main reason for this might be that in the fields where sampling was done the ILVs vegetables were planted in the same fields with other crops and because *Meloidogyne* has a very high host range hence the attack. The value of PCR markers for developing nematode identification techniques that are uncomplicated, reproducible, simple and safe is clear. At this stage validation and testing of techniques is in process and routine use is increasing. The potential for applying PCR to previously intractable areas of nematode identification and population biology is just beginning to be explored.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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