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Chapter 2

Methods and Tools Currently Used for the Identification of Plant Parasitic Nematodes

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Abstract

Plant parasitic nematodes are one of the limiting factors for production of major crops worldwide. Overall, they cause an estimated annual crop loss of $78 billion worldwide and an average 10–15% crop yield losses. This imposes a challenge to sustainable production of food worldwide. Unsustainable cropping production with monocultures, intensive planting, and expansion of crops to newly opened areas has increased problems associated with nematodes. Thus, finding sustainable methods to control these pathogens is in current need. The correct diagnosis of nematode species is essential for choosing proper control methods and meaningful research. Morphology-based nematode taxonomy has been challenging due to intraspecific variation in characters. Alternatively, tools and methods based on biochemical and molecular markers have allowed successful diagnosis for a wide number of nematode species. Although these new methods have been useful due to their practical, fast, accuracy, and cost effective, the use of integrative diagnose, combining morphology, biochemical and molecular data is more appropriate when necessary to strength diagnose, define species boundaries, and to have a more suitable molecular database for nematode species. Here, we report a review on current methods and tools used to identify plant parasitic nematodes.

Keywords: diagnosis, isozyme, integrative, molecular, PCR, plant parasitic nematodes, root-knot nematodes

1. Introduction

Nematodes are diverse, microscopic multicellular animals comprising free living to plant parasitic species. They parasitize a wide range of plant species, including monocots and dicots

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and are one of the most limiting factors for major crops, causing an estimated annual crop loss of $78 billion worldwide and an average crop yield loss of 10–15% [1–3].

Reliable, fast, and proper nematode diagnosis and specimen identification are mandatory for choosing adequate management control strategies and for avoiding spreading of exotic nematodes in quarantine materials [4–7]. Nonetheless, nematodes are one of the most difficult organisms to be identified, either due to their small, microscopic sizes or due to the difficulties in observing key diagnostic characters/features under conventional light microscope [5, 7–10]. In addition, the differences of some of these morphological and morphometric characters are subtle, subjective, and have overlapping characters or show intraspecific variation which compromise proper identification or may lead to erroneous identity of the species [5, 10, 11]. Furthermore, nematode identification using classical morphology requires well trained and experienced nematode taxonomists which are in decline these days due to lack of interest of young scientists in classical taxonomy [10].

Currently, new methods and tools using biochemical and molecular approaches have been successfully used as diagnostic for plant parasitic nematodes [4, 6, 7, 10, 12–16]. Despite the feasibility and accuracy in using biochemical and molecular-based detection tools and methods these days, diagnoses based on morphology are still sufficient or are required in some specific cases. Thus, when possible, the use of integrative diagnostic/taxonomical approaches using morphological, biochemical, and molecular data may be more time consuming but overall may lead to a more accurate diagnosis of nematode species, especially for those cryptic or newly described species.

Diagnostic laboratory that provides testing for plant parasitic nematodes has been increasing in recent years due to increased occurrence, damage, and dissemination of plant parasitic nematodes, lack of proper control management strategies, and high population density of key nematode pests in agricultural systems [17]. The current withdrawal of most chemical nematicides from the market is direct consequence of their toxicity and side effects to environment and human health. Alternative means in controlling plant parasitic nematodes for a sustainable cropping system include the use of resistant cultivars, the use of non and poor hosts, crop rotation, crop succession, and biological control [5]. However, accurate and fast identification of nematodes to species and subspecies levels is mandatory not only to be successful in choosing a proper management strategy but also for studying their genetic and biological variability or to avoid global spread of exotic and quarantine pathogens [4, 6, 7, 18, 19].

The goal of this chapter is to report a literature review of methods and tools to identify the most common genera of plant parasitic nematodes and its use to other nematode species as well.

2. Morphology-based diagnosis of nematodes

Nematode diagnosis and taxonomy have traditionally relied on morphological and anatomical characters using light microscopy. Lately, despite the increased interest in molecular diagnosis, due to its feasibility which allow quick and easy identification of specimens and allow researchers and extensions folks to use these techniques for routine use [10], classical taxonomy

using reliable and nonoverlapping morphological characters is still an important tool for the identification of nematodes mainly for the following reasons: (i) it allows a clear link between function and morphological aspects of the specimen analyzed; (ii) it is still a method that provides fast results; (iii) it is suitable for quantitative evaluations; (iv) it is cheaper; and (v) it is used for population surveys of plant parasitic nematodes with the objective to recommend management control strategies [10].

Other disadvantage in using classical taxonomy, besides the drawback of obscure morphological characters afore mentioned, the preparation of nematode specimens for classical identification, may result in modifications of the nematode, which may be difficult in its proper identification. For instance, much of the nematode body composition is proteins and fats, which undergo immediate coagulation or other alteration during the processing of the specimens, due to the use of substances such as sucrose, formaldehyde, glycerin, and the heating to which they are normally subjected during fixation [20]. In this way, specimens after being extracted, killed, fixed, and mounted on microscopic preparations, frequently present artifacts that make it difficult to locate external or internal structures of diagnostic value, or even produce characteristics that are not natural [20].

According to Inserra et al. [20], other disadvantages of light microscopy, in relation to other methods (electron microscopy, isozyme electrophoresis, and molecular methods), are as follows: the need for specimens in excellent preservation conditions; some characteristics of diagnostic value show high intraspecific variation, reason for the need for more than safe examination, need for abundant and updated scientific literature, the need for a taxonomist to be deepened in taxonomical studies, who probably would be a specialist in only a few groups of nematodes; several morphological and morphometric characteristics of diagnostic value are modified by environmental factors, such as geographic location, host plant species, host plant mineral nutrition, and light. However, the main disadvantage is that microscopic examination is not sufficient for identifying morphological characters that are extremely difficult to observe [20]. Thus, using integrative diagnostic approaches with more than one diagnostic method is less prone to errors.

3. Morphological and biochemical identification of *Meloidogyne* spp.

Root-knot nematodes (RKNs), *Meloidogyne* spp., are the most aggressive, damaging, and economically important group of plant parasitic nematodes infecting important crops worldwide. Currently, about 97 *Meloidogyne* spp. have been described [5], of which *M. arenaria, M. incognita, M. javanica*, and *M. hapla* represent up to 95% of RKN in cultivated soils.

This group of nematodes is highly diverse, showing a continuum of diversity in terms of cytogenetics (variable chromosomes numbers with aneuploidy and polyploidy states), mode of reproduction (ranging from amphimixis to obligatory-mitotic parthenogenesis), specialization in parasitism, species complexes, cryptic species, interspecific hybridization, and broad host ranges [4, 6, 7, 18, 21]. Overall, this high level of diversity contributes to an extremely complex relationship with their hosts that lead to highly successful parasitism. For example, the three major *Meloidogyne* spp. (i.e., *M. incognita, M. javanica*, and *M. arenaria*) are highly polyphagous, infecting more than 3000 plant species [5].

Diagnosis of *Meloidogyne* spp. has traditionally relied on the characterization of female perineal patterns and morphometrics. However, since these morphological characters overlap in some RKN species (e.g., in *M. paranaensis, M. konaensis*, and *M. enterolobii*), misidentification of species using morphology as the only criteria is often frequent [22, 23].

The morphology of female perineal patterns has been a character most frequently used in several laboratories for the identification of *Meloidogyne* species, a character located in the posterior body region of adult females. This area comprises the vulva-anus area (perineum), tail terminus, phasmids, lateral lines, and surrounding cuticular striae. Preparation of perineal patterns for the observation and identification of *Meloidogyne* spp. has been covered by different authors. A more detailed account on root-knot nematode perineal pattern development was given by Karssen [23]. **Figure 1** summarizes the perineal patterning for 12 major *Meloidogyne* species that are considered important to major crops [5].

For many years, the identification of *Meloidogyne* spp. has been relied upon the characterization of adult female perineal pattern and the use of several morphometric and morphological features of juveniles. To these characters were added features of male (although they are rarely seen), such as the form of the labial region, including annulation, and the form of stylet and basal knobs. However, with increasing numbers of described species, the value of many of these characters, themselves showing often large intraspecific variation, was eroded almost to the point where robust identification tended to involve a fair measure of serendipity. As an example, what may be termed the *incognita*-type of perineal pattern is now known to occur in a substantial number of species, some of which were commonly misidentified as *M. incognita*.

As an alternative to morphological identification of *Meloidogyne* spp., esterase patterning has been used for diagnosing *Meloidogyne* spp. from a wide range of samples and has been proved to be species-specific for a number of species [13, 24, 25]. *Meloidogyne* spp., isozyme electrophoresis patterning has discriminated all of these otherwise cryptic species, however, this technique is restricted to females [24]. Examples of esterase patterning for major *Meloidogyne* spp. are shown in **Figure 2**.

One of the earliest examples of the use of isozyme phenotypes to distinguish *Meloidogyne* spp. was given by Esbenshade and Triantaphyllou [25], who reported esterase patterns for 16 *Meloidogyne* species, with the most common phenotypes being A2 and A3 (*M. arenaria*), H1 (*M. hapla*), I1 (*M. incognita*), and J3 (*M. javanica*). In landmark surveys for *Meloidogyne* spp. using isozyme [12, 25] study, approximately 300 populations originate from 65 countries and several continents. In later surveys, Carneiro et al. [22] found 18 esterase phenotypes among 111 populations of *Meloidogyne* spp. from Brazil and other South American countries. Isozymes continue to be widely used for diagnosis of *Meloidogyne* spp. despite some limitations. Nonetheless, isozyme phenotyping has been used for a large number of species [6]. Schematic diagrams of isozyme patterns based on surveys, including those conducted in the international *Meloidogyne* project have been published [8, 12, 22, 25] and provide important references.

Several isozyme systems have been used, nonetheless, carboxylesterase/esterase EST proved to be the most useful in discriminating *Meloidogyne* species. Others, such as malate dehydrogenase (MDH), are also often included to confirm species identification [25]. Enzyme phenotypes

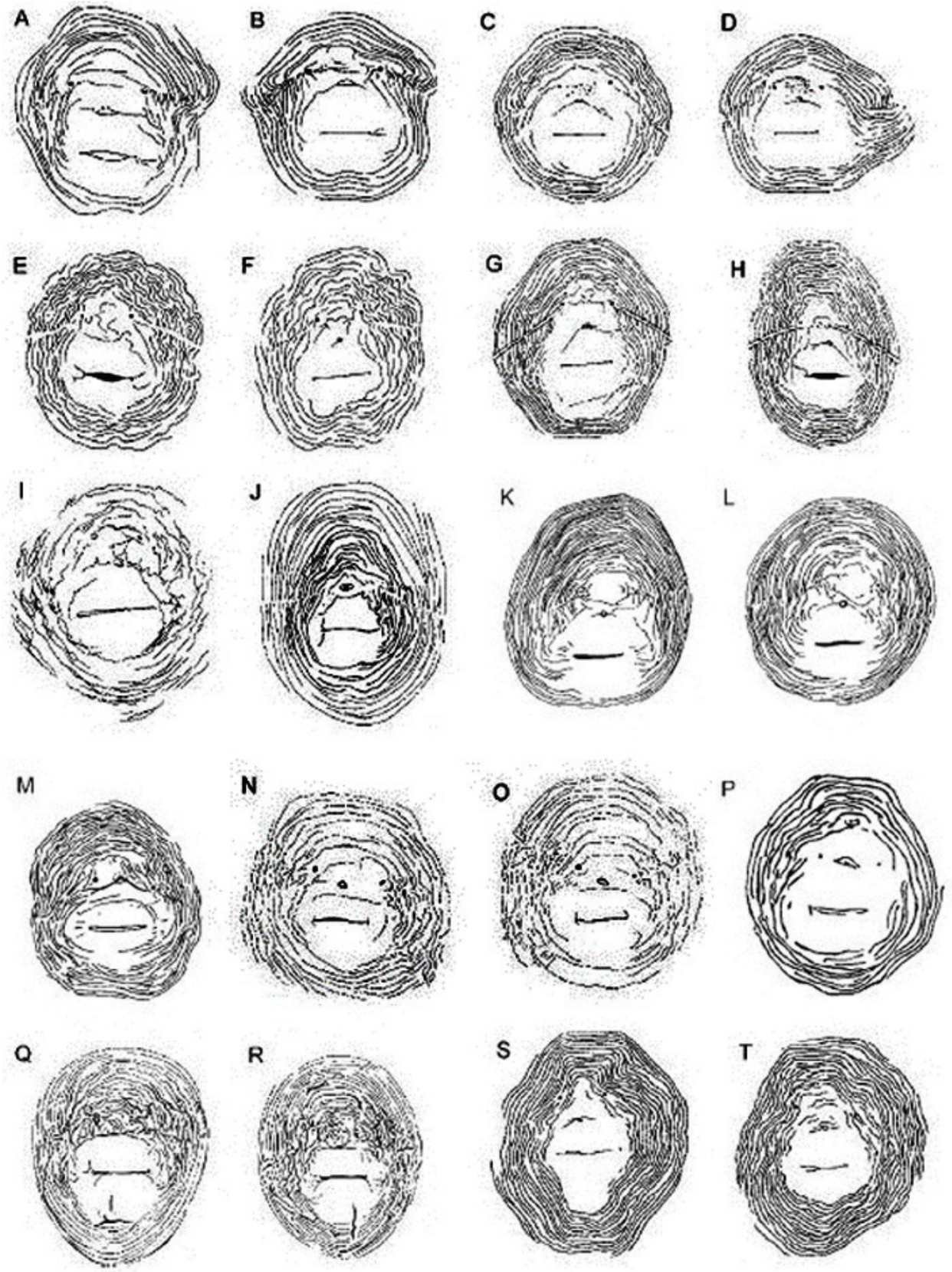

Figure 1. Comparison of perineal patterns for 12 major *Meloidogyne* spp. A, B: *M. arenaria*; C, D: *M. hapla*; E, F: *M. incognita*; G, H: *M. javanica*; I: *M. acronea*; J: *M. chitwoodi*; K, L: *M. enterolobii*; M: *M. ethiopica*; N, O: *M. exigua*; P: *M. fallax*; Q, R: *M. graminicola*; S, T: *M. paranaensis*. Drawings not to scale [5].

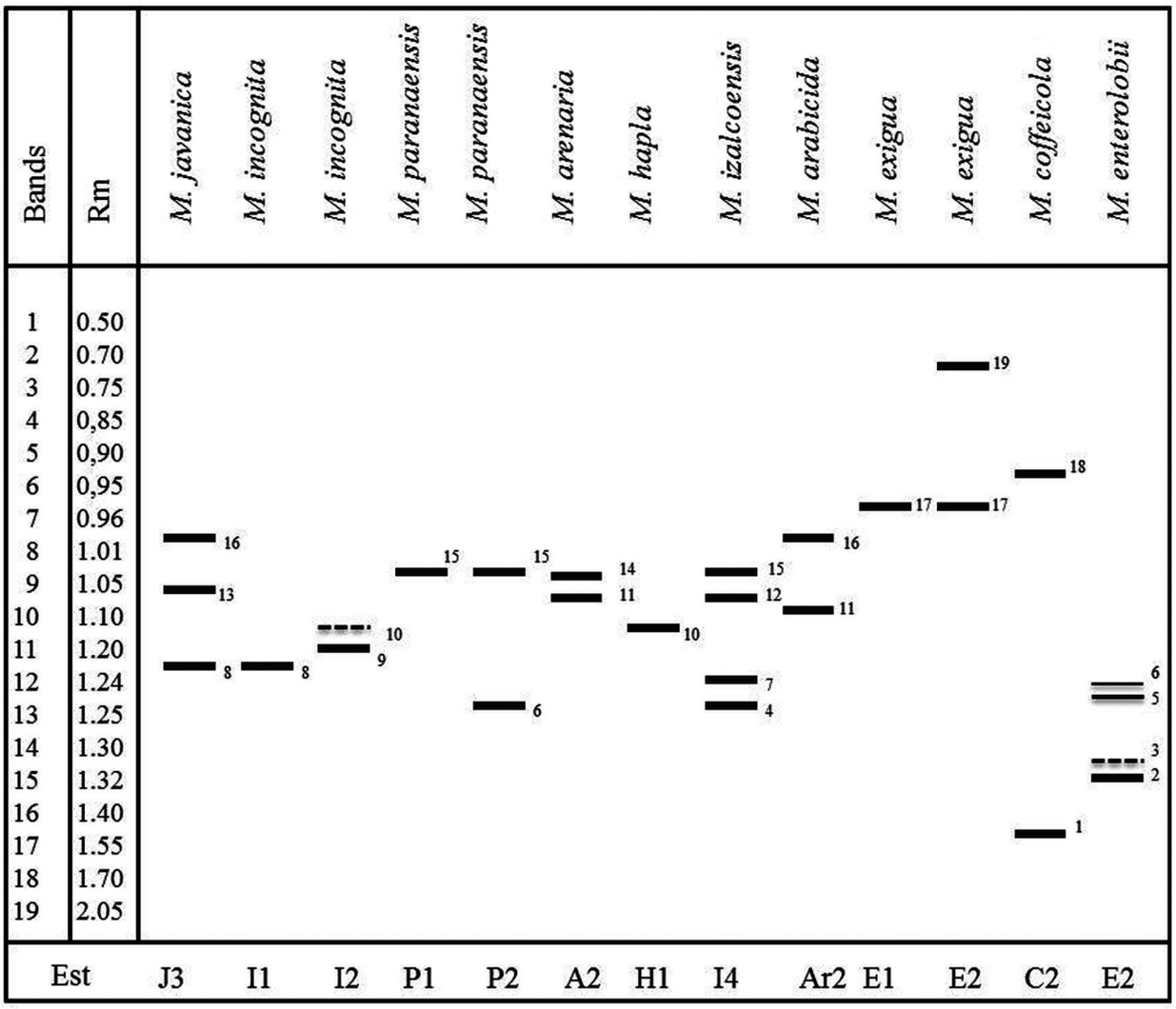

Figure 2. Esterase phenotypes (Est) of major *Meloidogyne* spp. associated with coffee. Rm = ratio of migration in relation to the fast band of *M. javanica*. Dotted lines indicate weak bands [24].

patterning are designated, indicating the *Meloidogyne* species that each specifies and the number of bands detected. Phenotypes with the same number of bands are differentiated by small letters [12, 25]. Enzyme patterns are usually compared with a known standard, with *M. javanica* being frequently used to determine migration distances among bands. Isozymes are used primarily with female egg-laying stage, using single individuals. Miniaturization and automation of the electrophoresis systems and the use of precast polyacrylamide gels (i.e., PhastSytem, Pharmacia Ltd, Uppsala, Sweden) have made isozyme phenotyping a widely used technique in most labs [22, 23, 25]. Classical electrophoresis methods using vertical and horizontal systems were also described in details in Refs. [13, 25], respectively.

Aside from the initial equipment cost, the consumables required are relatively inexpensive and isozymes have been often used for field surveys, diagnosis, as well as with routine screening of glasshouse cultures to assure species stability and pure cultures. The relative stability of isozyme phenotypes within *Meloidogyne* species makes them an attractive system,

although there are some drawbacks. For instance, the occurrence of intraspecific variants and the difficulty in resolving the same esterase phenotype between species (e.g., *M. exigua* vs. *M. naasi*) have required the use of an additional enzyme system (e.g., MDH) to confirm species identity. In addition, weak bands on the polyacrylamide gel may need to use a larger number of females per well (e.g., *M. exigua*) [22]. For some species, there are more than one esterase phenotype for a same species—e.g., *M. javanica* (J3, J2, and J2a), *M. incognita* (I1, I2, and S2), *M. arenaria* (A2 and A1), *M. exigua* (E1, E2, and E3), and *M. paranaensis* (P1 and P2) [8, 9, 26].

In surveys with the objective to study *Meloidogyne* biodiversity and nature conservancy, isozymes are a convenient first stage in species identification and have enabled the study of species diversity and frequency of a particular species, as well as their abundance. Females recovered after allowing multiplication of field samples on a generally susceptible host such as tomato (*Solanum lycopersicum*) can be tested for their isozyme phenotypes and the associated egg mass reserved for further characterization, if necessary [22]. Thus, novel isozyme phenotypes have been frequently found in these surveys in conserved areas, overall adding to the understanding of species ecology and biogeography of *Meloidogyne* spp. The Esbenshade and Triantaphyllou [25] listed Est F1 as an undescribed phenotype from Brazil; Later, *M. paranaensis* was described showing this phenotype [27]. In addition, Carneiro et al. [22] listed the patterns Est K3, Est Y3, and Est L3 as atypical esterase phenotypes; later, *M. ethiopica* and *M. inornata* were identified showing these new esterase patterns [8–9]. Recently, *M. luci* (Est L3) was described as a new species [28]. The phenotype Est Sa4 (Rm 73.5, 78.0, 53.0, 59.0), a new esterase phenotype from coffee in Central America, was later described as *M. izalcoensis* [29].

Isozyme electrophoretic profiles, often using esterase (EST) and malate dehydrogenase (MDH), have been established for a number of species [6] and can provide a useful routine diagnostic test particularly for morphologically variable species, such as *M. arenaria*. This species showed different profiles and high intraspecific variability, it may be an indication of the existence of species swam. Recently, the Est phenotype A3 of *M. arenaria* was identified as *M. morocciensis* [9].

Although isozyme electrophoresis is currently one of the best methods for *Meloidogyne* spp. diagnosis, it seems likely that DNA-based methods and tools will soon usurp this method for many applications where finer resolution, particularly of intraspecific variation, is paramount [6]. Nonetheless, the use of an integrative diagnosis, combining more than one approach, such as morphology, morphometrics, biochemical, and molecular data is less prone to error and could be used when possible.

4. Molecular diagnosis of plant parasitic nematodes

Since the development of polymerase chain reaction (PCR) and the vast amount of genetic data generated with DNA sequencing, molecular-based detection tools have been widely developed and successfully used for the diagnosis of plant parasitic nematodes. Molecular-based detection tools have the following advantages as compared with other methods, (i) can be used in a high throughput manner, (ii) DNA information can be acquired easily with the vast amount of databases and sequencing information, (iii) are cheap, fast, and accurate, (iv) DNA markers are independent of phenotypic variation and developmental stage of the nematode [14].

DNA-based markers have been proved reliable and have allowed diagnosis and description of new species for several groups of nematodes, including key genera such as *Meloidogyne, Pratylenchus, Globodera*, and *Heterodera* [4, 6, 7, 10, 18, 30–32]. DNA-based detection tools make excellent methods of nematode diagnosis since they are simple, accurate, and fast [6, 7] and can be used with a wide range of sample types, including host tissue, eggs, egg masses, soil extracts, and fixed samples [16].

Nowadays, most labs worldwide are commonly using molecular methods to diagnose nematodes since cost associated with reagents and equipment are affordable and there has been a crescent interest in molecular taxonomy by young scientists [10, 16]. These methods have been used ordinary and are sensitive enough to detect individual nematodes from complex types of samples, including soil samples and species mixtures in the field [21, 33–35]. Some limitations of molecular-based detection tools include problems associated with optimization and validation of tools and methods, DNA extraction protocols, conditions of samples (i.e., quarantine specimens), amount of target DNA in a sample, cross contamination, false positive and negative results, which overall should be used carefully as to not compromise the ultimate result of diagnosis [16].

4.1. Ribosomal DNA

A vast amount of examples of nematode diagnosis has mostly been based on amplification of target DNA by PCR using species-specific primers. PCR-based detection methods have revolutionized the area of diagnostics of nematodes and have been used due to improved sensitivity, specificity, speed, relatively ease to perform, and cost effectiveness compared with other diagnostic procedures [4, 6, 7, 10, 18]. One of the approaches to design DNA-based markers that can aided diagnosis of nematodes has been based usually on conserved regions in the ribosomal DNA (rDNA) cistron, i.e., the external transcribed spacer (ETS), internal transcribed spacers 1 and 2 (ITS1 and ITS2), and the intergenic spacer regions 1 and 2 (IGS1 and IGS2) [7]. Schematic representation of these genetic regions is shown in **Figure 3**.

In this way, sequences that are divergent among nematode species and conserved within several isolates of a same species make ideal target for designing species-specific primers [7]. Ribosomal DNA regions have been very suitable for choosing a target marker since they are

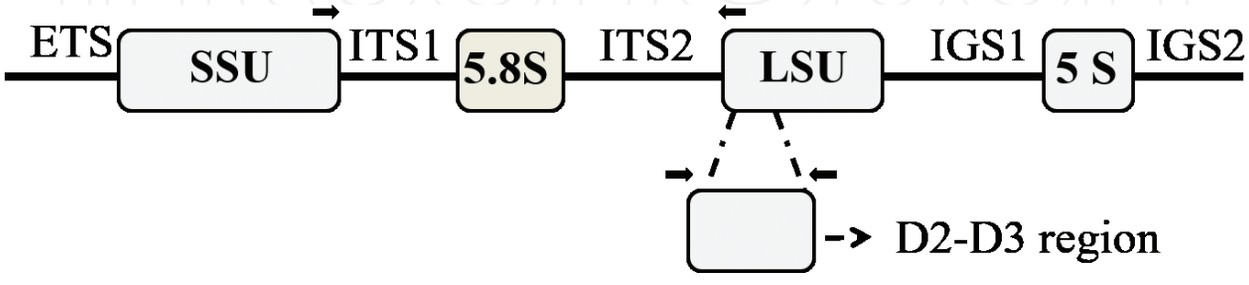

Figure 3. Schematic representation of nuclear rRNA genes in eukaryotic cells. SSU = 18S—small subunit; LSU = 28S—large subunit; ETS—external transcribed spacer region; ITS1 and ITS2—internal transcribed spacers; IGS1 and IGS2—intergenic spacer regions; arrows indicate possible starting point for primer amplification. Open box indicates the D2-D3 expansion segments for the 28S rRNA.

multicopy genes and provide sequences with enough variation that can be used for diagnosis and phylogenetic relationships among species [7].

4.2. Mitochondrial and satellite DNA

Diagnostics of nematodes have also been based on other genomic target regions such as mitochondrial DNA (mtDNA). Mitochondrial DNA genomes are relatively small circular molecules ranging from 12 to 20 kilobases [7, 36]. Divergences in mtDNA sequences due to insertions, deletions, and accelerated ratio of mutations compared with nuclear DNA [7] have provided target markers suitable for discriminating nematode species [37–39].

Satellite DNAs (satDNAs) are highly repeated tandem arrays of short sequences ranging from 70 to 2000 bp. It has different signature sequences, copy numbers, length, and polymorphic regions that can be explored to find species-specific markers [6, 7]. Such PCR-based detection using satDNA markers in nematode diagnosis has been reported by several labs [7, 40, 41] and represents a target option for designing diagnostic primers.

4.3. RFLP, AFLP, RAPD, SCAR

One of the first methods used to differentiate nematode species was restriction fragment length polymorphism (RFLP), a method that uses restriction enzymes to digest whole genomic DNA or an amplified segment of it to generate DNA banding patterns according to divergences in sequences among isolates [7, 42]. This technique can also be coupled with DNA hybridization with radioactive or nonradioactive labeled probes [7]. Although being effective in differentiating nematode isolates, this method is less used nowadays due to technical complexity and the need for a large amount of target DNA, which usually requires preculturing of nematode populations [6, 7].

Alternatively, species-specific primers have been designed from sequences randomly scattered throughout nematode genomes, e.g., DNA band obtained from random amplified polymorphic DNA (RAPD) or amplified fragment length polymorphism (AFLP) gels, with posterior cloning and sequencing of bands differential across related species and their conversion into species-specific sequence characterized amplified region (SCAR) markers [21, 43–46]. SCAR-based markers and rDNA-based specific primers have been used to diagnose nematodes with either conventional or real-time PCR (q-PCR) [6, 7, 10].

Numerous primers and approaches used for diagnosis of nematodes using conventional and quantitative PCR were designed based on several target regions in the nematode genome (e.g., SCAR, rDNA, ITS, D2-D3 segment, IGS, among others). **Table 1** summarizes some of these strategies used in some main studies.

In particular, successful SCAR markers have been developed for diagnosing some of the major tropical *Meloidogyne* spp. associated with important crops such as coffee, guava, and grapevine, including *M. arenaria* [43], *M. incognita* [21], *M. paranaensis, M. exigua* [21], *M. enterolobii* [44], *M. arabicida, M. izalcoensis* [45], and *M. ethiopica* [46] (see a complete list of references for species-specific primers in **Table 1**). These primers were validated in several population studies,

Nematode species	Target region	Method	References
***Meloidogyne* spp.**			
M. arabicida and *M. izalcoensis*	SCAR*	PCR	[45]
M. arenaria	SCAR	PCR	[43]
M. chitwoodi	IGS	PCR	[47]
	SCAR	PCR	[48]
M. exigua	SCAR	PCR	[21]
M. enterolobii	mtDNA	PCR	[49]
	SCAR	PCR	[44]
M. ethiopica	SCAR	PCR	[46]
M. fallax	IGS	PCR	[47]
	SCAR	PCR	[48]
M. graminis	ITS	PCR	[11]
M. hapla	satDNA	PCR	[50]
M. hapla	SCAR	PCR	[48]
	IGS	PCR	[51]
M. incognita	SCAR	PCR	[43]
	SCAR	PCR	[21]
M. javanica	SCAR	PCR	[43]
	SCAR	PCR	[52]
M. marylandi	28S D2-D3	PCR	[11]
M. naasi	ITS	PCR	[52]
M. naasi	28S D2-D3	PCR	[11]
M. paranaensis	SCAR	PCR	[21]
Other parasitic nematodes			
Bursaphelencus xylophilus	satDNA	PCR	[53]
B. xylophilus	satDNA	qPCR	[54]
B. xylophilus	heat shock protein	qPCR	[55]
Ditylenchus destructor D. dipsaci	rDNA	PCR/qPCR	[56]
H. glycines	rDNA	qPCR	[57]
H. schachtii	ITS	PCR	[58]
H. glycines	SCAR	qPCR	[59]
Pratylenchus penetrans	rDNA	qPCR	[60]

*SCAR—sequence characterized amplified region; IGS—intergenic spacer region; ITS—internal transcribed spacer; mtDNA—mitochondrial DNA; satDNA—satellite DNA; PCR—polymerase chain reaction; qPCR—quantitative real-time PCR.

Table 1. Species-specific primers for diagnosis of selected plant parasitic nematodes.

using DNA from a single juvenile (J2), or in multiplex PCR reactions containing mixtures of species, and have become an excellent practical diagnostic kit for certain crops-associated *Meloidogyne* spp. [8, 21, 45, 46]. Interestingly, [61] established a diagnostic key for the identification of seven RKN species, i.e., *M. incognita, M. javanica, M. arenaria, M. enterolobii, M. hapla, M. chitwoodi*, and *M. fallax* using a combination of IGS PCR, SCAR markers, and RAPD profiling.

4.4. qPCR and barcoding

Quantitative PCR (q-PCR) is a technique that amplifies and quantifies nucleic acids simultaneously. Its advantage over conventional PCR is that it is fast, sensitive and does not need post-amplification processing of samples normally seen in conventional PCR, which can lead to false results in some cases, reviewed in Refs. [7, 10]. As new genomic sequences become available for plant parasitic nematodes, there have been a vast number of examples of this approach to detect and quantify nematodes from a wide range of samples, including greenhouse, field experiments, ecological studies, experiments with plant-nematode interactions, and virus load in nematode vector and are being used as the advantage to be fast, accurate and to be applicable in high throughput processing systems of large number of samples, reviewed in Ref. [10]. Application of q-PCR in nematode diagnosis using rDNA target or other marker has been showed for major nematode species, including *M. incognita, M. chitwoodi, M. fallax, M. javanica, Bursaphelenchus xylophilus, Globodera rostochiensis*, and *G. pallida*. For a complete list of nematodes, see Ref. [10].

The concept of DNA barcode for nematode taxonomy has been employed in Ref. [62] and is related to a DNA sequence of a particular region in the genome as a mean to give unique signature (barcode) for the identification of nematode species [7]. Although theoretically sounds, this approach has not been widely accepted since there has not been an unique DNA locus that can define the limits of species boundary and be used as universal identification of nematodes, besides the missing link of DNA barcode approach with classical taxonomy which has shown clear methods for species-level resolution [7, 14].

4.5. Soil PCR

Lately, there has been increased interest of labs to perform molecular diagnosis of nematodes directly from soil samples without the need to extract the target nematode species, a strategy commonly used for communities of bacteria and fungi [7]. There are available commercial kits for the direct extraction of nematode DNA from soil extracts and has been successfully employed in some labs. Alternatively, nematodes can be extracted from soil samples using conventional methods such as Baerman funnel, Whitehead tray or other method can be pooled for DNA extraction using commercial kits or other ordinary DNA extraction method. This strategy has been used by several authors and has been proved reliable and time saving [33, 34]. Nonetheless, there are drawbacks associated with this strategy, including underestimation of nematodes due to their uneven distribution in the soil, the limited amount of soil sample used for DNA extraction in commercial kits and the cost associated with sample processing [7].

Alternatively, a method to enrich nematode from soil extract using antibody-based capture was proposed by Chen et al. [63]; however, its use as routine diagnosis in labs needs to be analyzed.

5. Concluding remarks

The accurate identification of nematodes to species and subspecies levels is essential for their control and is a prerequisite to meaningful research. Many nematode species are easily identified based on distinct morphological characters and restricted host ranges. Several species are difficult to identify due to their similarity to other species or poor taxonomic descriptions. The difficulty in identifying nematodes species may result from morphological variations within and between populations from a same species.

Problems in the morphological identification of nematodes species, such as large number of described species within a specific group, e.g., as to compare several observed characters seen by light or scanning microscopy, and lack of apparent differences in a certain feature between species, e.g., perineal patterns, have encouraged much interest in the use of biochemical and molecular techniques as routine methods for the identification of nematodes. Biochemical and molecular methods used for the identification of nematodes are now widely used and are essential for diagnosis of a wide range of plant parasitic nematodes.

A clear understanding of species boundaries and adequate sampling of known species across their geographic areas are lacking in several regions and important crops. The future prospects in nematode taxonomy and diagnostics are dependent on molecular-based methods and tools that will discriminate not only at the species level but also at the level of host races, thereby opening up opportunities for more focused management strategies. Such techniques offer the possibility of rapid, unequivocal diagnostics and should help resolve the present problems associated with relatively morphologically conserved organisms that reproduce, for the most part, parthenogenetically, as is the case of *Meloidogyne* spp.

As for the case of taxonomy for *Meloidogyne* spp., once such molecular techniques are widely employed, no doubt the number of current nominal species will be shown to be junior synonyms, while others, conversely, will be shown to be species complexes, possibly of sibling species. It seems likely that molecular methods will replace isozymes as the preferred diagnostic tool for *Meloidogyne* spp. because of their inherently higher resolution and the opportunity to develop DNA chips for rapid and reliable field identification.

Molecular tools will also enhance our understanding of phylogenetic relatedness of nematodes and its relationship with other plant parasitic nematodes. Although nucleic acids-based detection techniques have been useful in diagnosing nematode species due to their high throughput characteristics, fast, sensitivity and cost effectiveness, the use of integrative diagnose approaches for nematode identification, combining morphology, biochemical and molecular data are more appropriate when necessary to strength nematode identification, define species boundaries, and to have a more suitable molecular database for nematode species.

A vast amount of genetic data are becoming available with nematode genome sequencing, which provides tools to perform comparative genomes and finds target DNA regions that can be used as diagnostic marker.

Molecular-based detection tools and methods are aimed to aid nematode taxonomy and should not totally exclude classical taxonomy approaches since in some cases, they must be complementary for accurate diagnosis.

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