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Eco-friendly management of plant parasitic nematodes

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Abstract

Background/Objective: Root-knot nematodes and cyst nematodes are two important plant-parasitic nematodes that cause severe plant diseases in various plant species around the world. They act as obligate and biotrophic parasite within the plant body. The objective of the study is to review for suitable management to keep the nematode population density below the threshold level. Methods: In spite of several nematode control practices such as crop rotation, use of biopesticides or nematicides, each has some limitations of their use but biotechnological applications including RNAi or miRNA represent a potential breakthrough in the application of functional genomics for plant nematode control. Here, a comparison is made between some old and modern nematode management practices but recent data shows that application of RNAi or miRNA has a better option of nematode control in some crop plants. Findings: Efficacy and biotechnological success can be maintained by holistic grasping of several soil biological and ecological factors. Therefore, modern approaches those reviewed herein due to their usefulness in minimizing plant nematode populations and increasing crop yield should be incorporated into management systems. The scientific community has entered into a new era that shows the tools to actually unravel the underlying molecular mechanisms, making this an opportunity for a review of our current knowledge and better understanding. Application: These modern eco-friendly practices may not quickly perform as synthetic chemicals, but they are pest specific, non-toxic to humans or environment, and also serves as a sustainable tool for disease management. Novelty: The present communication identifies plant nematode control approaches with emphasis on modern research. This review article emphasized the importance of modern biotechnological approaches for better crop yield than the common older practices.

Keywords: Root-Knot nematode; biotrophic Parasite; threshold level; nematicides; biopesticide; micro RNA

1 Introduction

Plant parasitic nematodes (PPNs) constitute serious threats on crop yield both in quantity and quality, globally. They are responsible for 10% global crop losses annually which estimates US \$173 billion per year ⁽¹⁾. Many of the PPM acts as pests on a

wide range of several important agricultural crops, exhibit a variety of feeding habits, ranging from migratory browsers to sedentary endoparasites with some specialized host associations.

In agricultural crop production systems, PPN mainly controlled by nematicides and chemical soil fumigants for decades but in recent years, several nematicides have been withdrawn from the market because they cause environmental pollution (2) and contaminate ground water (3). In addition, nematicides often do not provide long-term suppression of the pathogen or reduce infected gall number in the particular plant. Resistant crop varieties acts as molecular breeding approach mainly time taking and R-gene sources are also limited, so transgenic plants with nematode resistance varieties are now developed with the advancement of RNAi technology (4-6) and protease inhibitors (7,8). This is especially timely with the current technological advances and major changes in the external environment, which have positively altered the outlook for modern approaches. The function of small noncoding RNAs in plant nematode control was established in case of root-knot or cyst nematodes of Arabidopsis thaliana mutants disrupted for miRNA or siRNA pathway for increased resiatance (9-11). Nowadays, it proves that miRNAs move not only within an organism, but also across kingdoms and activates expression of genes in evolutionary distant organisms (12-14). In association with RNA binding proteins, the presence of a methyl group on the ribose of the last nucleotide and also expression of exosomes may contribute to the stability and transfer of plant miRNAs across kingdoms (15,16). So, miRNA based strategies which exploits the potential of plant miRNAs to migrate across kingdoms and ultimately silence some specific genes in distantly related organisms. By this way this artificial miRNAs can be regarded as valuable tool in global agricultural challenges (17,18).

The aim of this review is to mainly highlight the contribution of modern practices in inhibiting nematodes in disease suppressive soils. In addition, the review will highlight the importance of the performance of these new technologies in plantnematode interactions.

2 Old Practices of Nematode Control

Management of nematodes must focus on reducing their population number for better crop yield in any country. There are several traditional practices followed by several countries, cultural practice is one of them. Various types of cultural practices as well as old techniques are summarized below.

2.1 Crop rotation

This technique is very useful for management of nematodes in case of annual crops. In this system, their population number decreases when non-host or resistant crop is grown in the field. A two-year rotation is useful in several plants such as maize, sorghum etc. for effective nematode control. However, it is very old practice for reducing several types of different soil-borne organisms, and it also facilitate the increase of population number of other PPN species present in that particular field (19,20).

2.1.1 Use of nematode free planting material

It is generally low cost and farmer friendly practice. But due to spreading of nematodes is too high, so chance of contamination in non-infected fields is also too rapid. Therefore, it is more or less complicated strategy to employ in the field.

2.1.2 Flooding and fallowing

Flooding and fallowing have long been used to control nematodes and other soil pests where water level is easily controllable and also the level of water can be maintained for few months. These practices generally used in paddy fields where water supply is much more. Flooding generally kills nematodes by not providing their host plant for too long time. In recent time this practice is restricted because it often resulting in erosion by wind and water, which might hamper physical structure of soil and organic matter⁽²¹⁾.

2.1.3 Crop Root Destruction

In India, Crop root destroying was a traditional method. Apart from plant nematodes, it also kills insects, weeds etc. especially roots of maize, sugarcane, cotton plants but at the same time this technique might destroy soil organic matter and texture. So, it is not a useful technique for proper controlling of nematode.

Other old practices includes heat, solarization, hot water treatment etc. These were moderately effective in plant nematode control because these treatment were applicable only under specific dose and exposure.

3 Modern Practices of Nematode Control

Some of the modern approaches of plant nematode control are discussed below for their sustainable control. These new technologies are more advanced than the old ones which are more effective and accurate with very high promising results. These modern practices are more eco-friendly, yields more crop and also nontoxic as it was found in case of synthetic chemicals. These modern techniques must include computational data in certain crop plants for their better management. Various types of modern practices are discussed below:

3.1 Biological Control

In present days, biological control is a promising and new area of nematological research. Identifying, understanding, and utilizing microorganisms or microbial products to control several types of plant diseases and to enhance crop production are integral parts of sustainable agriculture. Biological control has the potential to control crop diseases while causing no or minimal detrimental environmental impact and it is currently accepted as a key practice in sustainable agriculture because it is based on the management of a natural resource. There are several predators, parasites, nematophagous fungi, endoparasitic fungi, predacious fungi, opportunistic fungi which more or less minimize nematode control but biocontrol with rhizobacteria is more advanced one. These rhizobacteria such as for example Pseudomonas sp., P. fluorescens, Bacillus subtilis, are important in nematode control. The main advantage of these bacteria is that they are easy to culture in vitro and they produce toxins against nematodes which are capable of infecting several crops and they induce suppressive activities against M. incognita by minimizing gall formation, controlling nematode reproduction, and hatching and killing juveniles through the release of toxins (22-25). So, biocontrol of soil borne plant pathogens with bacteria has been studied as an alternative or complementary approach to physical and chemical disease control measures for several years. These bacteria can antagonize soil-borne pathogens through various types of mechanisms: (a) They often reside in the rhizospheric region of the soil and also prevent the growth of pathogenic organisms by their own feeding mechanisms, the secretion of antibiotics, the release of toxins, and several enzymes that interfere with the recognition patterns of plant nematode interactions (26); (b) Several root-associated strains of fluorescent Pseudomonas spp. produce and synthesize several types of metabolites that are mainly inhibitory to soil-borne plant pathogens. 2,4-diacetylphloroglucinol (2,4-DAPG) is one among such metabolites which received attention because of its production by a wide range of Pseudomonas used for the biocontrol of plant root diseases. 2,4-DAPG is a phenolic compound with broad-spectrum antibacterial, antihelminthic, antifungal, phytotoxic and nematicidal activity (27); (c) Chitinases and glucanases lyse microbial cells and these compounds also helps in the reduction of deleterious and pathogenic rhizo-sphere microorganisms, which creates the environment more favourable for root growth; (d) Lastly, production of antibiotics by these bacteria such as pyoluteorin (PLT), pyrrolnitrin, and Phenazine-1-carboxylate, under certain conditions these antibiotics helps to improve the ecological fitness of these bacteria in the rhizosphere, which can further influence long-term biocontrol activity (28) . So, these antagonistic bacteria have been repeatedly shown to be one of the promising microorganisms for the biocontrol of plant pathogens.

3.1.1 Use of Biopesticides

In recent days, biopesticide are used mainly as preventive measures. They are generally pest-specific, non-toxic to humans, biodegradable and serve as a tool for nematode management. Neem, several plant extract, Bacillus thuringiensis etc acts as a biopesticide in recent days. Neem leaf, neem oil, neem cake etc acts as a controlling agent in plant-parasitic nematode (29,30). Several plant-extract such as thiophenics, alkaloids, glucosides, phenolics, fatty acids (31) etc helpful in nematode control as they are generally safe for environment and human health (32). *Pseudomonas spp.* acts as a biopesticide, this bacteria generally present roots of different plants, Pseudomonas fluorescens contains delta endotoxin gene of Bascillus thuriengiensis, produced four times more toxin protein product and has the more potentiality to kill nematodes (33). Different *Pseudomonas spp.* producing different beneficial compounds such as plant hormones, exoenzymes, antibiotics, nutrient chelators and signal molecules (34) these compounds suppress plant nematodes via antibiosis and at the same time trigger plant host defences (35).

3.1.2 Use of Bacteriocin

Bacteriocins, a ribosomally synthesized, antimicrobial substance which is extracellularly released, have now a days major application as food preservatives⁽³⁶⁾, growth regulator⁽³⁷⁾ and livestock management⁽³⁸⁾. Bacteriocin and Pseudomonas fluorescens have the role in reducing nematode population, gall formation in tomato plants and also enhance crop yield. So, it is a new technique of biological control in case of plant parasitic nematode (Figure 1).



Fig 1. Arrrow shows root-gall formation by plant parasitic nematodes in Tomato-plant

3.1.3 Use of natural pr oducts

Due to several environmental problems regarding the use of chemical nematicides or pesticides for control of plant parasitic nematodes, use of plant essential oils provide some important clues. These plant essential oils are now used as natural products in pest management system $^{(39,40)}$. It was also proved that two important plant essential oil such as citral and menthol increase shoot length, shoot weight, crop yield and also increase phenylalanine ammonia lyase activity in case of infected Okra plant $^{(41)}$ (Figures 2 and 3). So these essential oils are used as alternatives in plant nematode management.

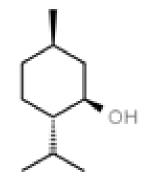


Fig 2. Chemical structure of menthol

Fig 3. Chemical structure of citral

3.1.4 Biotechnological approach of plant-nematode control

Recent research on modern biotechnological methods of nematode control aims either to exploit natural resistance present in gene pool of particular crop plant and their relatives or to employ synthetic forms of disease resistance, such as those based

on expression of specific pathogenesis related proteins, disruption of feeding cells, on gene silencing (RNAi) technology. Like eukaryotic system by inducing posttranscriptional silencing of protein coding genes, the discovery of RNAi in nematodes, the infected plants produce double stranded RNA (dsRNA) to nematode target genes and also silence their activity. Fire et al. showed that addition of neurostimulants to the 'soaking' solution for H. glycines J₂s they could be induced for taking maximum amount of dsRNA for the induction of RNAi (42-44). The technique of RNAi was first reported in Caenorhabditis elegans by Guo and Kemphues (45). Controlling of nematodes by RNAi technology, is performed partially in planta and partially within the nematodes. When plants are infected with nematodes, the plant derived dsRNAs or siRNAs are generally taken up by nematodes through their oral stylets and RISC will bind to siRNA and induces degradation of nematode genes. The number of siRNAs are then multiplies in plant nematodes with the help of RNA-dependent RNA polymerase (46). Kimber et al (47) confirmed that in case of potato plants flp gene disruption CN G pallida initiates motor disruption and unusual neuronal sensitivity to RNAi. This RNAi technology has some important benefits than other old practices. Tuschi and colleagues suggested that only a single base mismatching between a siRNA and its target mRNA prevented gene silencing (48). It is also a long-term nematode protection strategy and has no interactions with non-target organisms. To achieve this greenhouse experimental studies will be necessary to verify its duration period. Table 1 shows the control of some plant parasitic nematodes by RNAi gene silencing technology.

Table 1. Control of some	plant parasitic nematodes by	y RNAi gene silencing technology

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Method delivery	of Gene name	Functions of target genes	Nematode species	Percentage of infection reduction	References
Soaking	Mi-gsts-1, EL784458	Gluathione-S trans- ferase	Meliodogyne incog- nita	52%-71%decrease in fecundity	Dubreuil et al., 2007 ⁽⁴⁹⁾
Soaking	Flp	FMRFamide-like pep- tides	Globodera pallida	Inhibition of motility	Kimber et al., 2007 ⁽⁴⁷⁾
Soaking	Bx-myo-3	Myosin heavy chain,tropomyosin	Bursaphelenchus xylophilus	abnormal locomotion, J2-J3 viability reduced at high temperature	Park et al.,2008 ⁽⁵⁰⁾

3.1.5 micro RNA (miRNA) technology

A number of microRNAs which initiates changes in root cells in response to plant nematode infection have recently identified in several plant species. This strategy is an alternative for gene silencing method. Plant miRNAs are noncoding RNAs of 20-22-nucleotide $\log^{(51)}$ which acts by posttranscriptional gene silencing method. To create mature miRNA duplex, plant miRNA precursors which are produced from MIR gene and processed by Dicer-like 1 (DCL1) protein helps in gene silencing by sequence complementarity (52). Complementarity between miRNA/mRNA induces cleavage of mRNA at the nucleotide position 10 or $11^{(53)}$. This plant miRNA may enhance the plant defense mechanism during plant-nematode interaction (54). In in silico prediction study the genes targeted by miRNAs have been identified (55) or by another method of 5'RNA-ligase mediated (RLM)-rapid amplification of cDNA ends (RACE) sequencing (56). So, in recent days application of miRNAs in plant nematode control creates a variety of physiological and morphological processes through the application of modern computational study and also cloning applications (Figure 4). Table 2 shows the list of some recent functionally expressed miRNAs in different plant species.

Table 2. List of some recent functionally expressed miRNAs in different plant species

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Host Plant	Infected part	Nematode species	miRNA	References
Cotton	Roots	Meliodogyne incognita	miR159	Pan et al.,2019 ⁽⁵⁷⁾
Tomato	Galls	Meliodogyne incognita	miR390	Diaz-Manzano et al.,2018 (58)
Soybean	Roots	Heterodera glycines	miR396	Noon et al.,2019 (59)
Cotton	Roots	Meliodogyne incognita	miR827	Pan et al.,2019 ⁽⁵⁷⁾

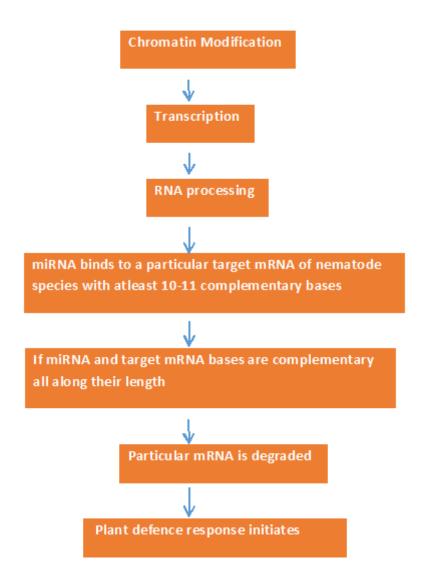


Fig 4. Mechanism of miRNA in plant defense response

4 Conclusion

Although some of the traditional practices of plant nematode control are still used around the world but biotechnological approach plays a great role in recent days. A number of nucleic acid-based approaches for the identification and quantification of genetic variation have been identified and also successfully adapted in nematology. On the other hand, biological control practices also help to maintain ecological balance, eco-friendly and pollution free environment, reducing health hazards but there are some problems about their standardization and mass multiplications, lack of awareness etc. So, strategy involves with RNAi and miRNA which have been developed by many researchers as an efficient tool for proper identification of gene functions in plant parasitic nematodes. Many studies till date focused on miRNAs, but few investigation on siRNAs expressed in roots infected with plant parasitic nematodes in Arabidopsis (60) have revealed maximum galls of 24nt siRNAs known to be relation with RNA-directed DNA methylation. In future, combined studies of iRNAs and miRNAs will be a novel approach to control plant parasitic nematodes that will be environmentally friendly but which gene will be targeted is an essential part to minimize or prevent off-target interactions (61). Nematologist with the use of genome sequencing or microarray study used to evaluate

more suitable gene targets for silencing by RNAi. These modern technological approaches are an effective, economical and a promising approach for improving the plant growth and productivity under nematode infection. However, further research is needed to elucidate the mechanisms underlying the nematicidal activities of modern technologies.

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