Novel Biotechnological Interventions in Plant Nematode Management Technologies

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Mujeebur Rahman Khan, Ziaul Haque, and Rahul Kumar Sharma

Abstract

Plant nematodes are major threat to crop production. They cause significant damage to agricultural crops and suppress their yields. While many methods of control have been proposed for nematode management, only a few have proved effective in the long run. The widespread elimination or restriction on conventional nematicides has compelled the development of new methods of pest and disease control. Biotechnological approaches applied to nematode management show promising and viable options at this juncture. Reduced nematode infection and proliferation in the transgenic host plants have been attributed to the use of nematode resistance genes, protease inhibitors, nematoxic proteins, and chemodisruptive peptides. Furthermore, with the development of RNAi technology, new targets have been discovered that may be exploited for nematode suppression. The present chapter examines the potential of all these biotechnological interventions for their application in commercial nematode management.

Keywords

Heterodera spp. · *Meloidogyne* spp. · Polymerase chain reaction · RNAi technology · Transgenic plants

M. R. Khan (✉) · Z. Haque · R. K. Sharma
Department of Plant Protection, Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh, Uttar Pradesh, India

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7.1 Introduction

Nematodes are pseudocoelomic and the most commonly occurring multicellular animals on the planet, making about 80–90% of all multicellular invertebrates (Khan 2008, 2016). However, only a 5–10% of the world's nematode taxa are known (Haque and Khan 2021). Parasitic and free-living forms of the nematode can be found in any terrestrial or aquatic ecosystem. Free-living species can be classified as bacterivores, mycophagous, algalivores, herbivores, omnivores, or carnivores, and they can be found in both saltwater and freshwater environments, as well as in soil (Mohiddin et al. 2010; Mohiddin and Khan 2013). However, only a small proportion of the known soil nematodes have the ability to parasitize plants, insects, mammals, and humans (Khan 2008, 2023).

Plant-parasitic nematodes have emerged as a serious threat to the world's food supply due to the extensive damage they cause to agricultural and horticultural crops. There are approximately 4100 known species of plant-parasitic nematodes, which inflict crop losses close to US$ 175 billion annually (Haque and Khan 2021). Endoparasitic nematodes (root-knot, root lesion, and cyst-forming nematodes) are major nematode pests of agricultural crops (Mohiddin and Khan 2014; Khan et al. 2022). While many methods of control have been proposed for nematode management, only a few have proved effective in the long run. A concerned grower primarily relies on nematicides to control the disease problem. When infestation level in soil is high, it becomes essential to grow a non-host crop, otherwise soil has to be disinfested with fumigants such as methyl bromide, and metham, etc. Since application of most of the fumigants has been banned or it involves soil covering, etc., granular nematicides such as carbofuran, phorate, thionazin, etc. at a dose of 4–5 kg ai/ha can provide satisfactory decline in the nematode population. These nematicides are relatively safer, hence can also be applied at post-planting stage. In case of transplanting crops, it is always advisable to disinfest the planting materials by root-dip treatment with 100–200 ppm carbofuran solutions. When plants are small and nematode infestation has been detected, a foliar spray with phenamiphos or oxamyl @ 5 l/ha can effectively decrease the level of soil infestation. Khan et al. (2014) reported satisfactory control of root-knot nematode in rice by applying phorate through root dip and soil application. In recent year, some new molecules/chemicals such as fluopyram and fluensulphone have been found effective against soil nematodes (Haque and Khan 2021). The fluensulphone (Nimitz TM) has a novel mode of action by disrupting the nematode feeding and causing paralysis that cumulatively leads to their death. Similarly, Fluopyram selectively blocks cellular energy production in nematodes by inhibiting complex-II system. However, before their wide use, the impact on soil microbial community is needed to be essentially examined. There are other nematicides which also can suppress nematodes. Application of nematicides, however, should be restricted to serious or epidemic situations.

Biotechnological approaches applied to nematode management, however, show promising and viable options. The use of nematode resistance genes, protease inhibitors, nematicidal proteins, chemo-disruptive and elicitor peptides, RNAi

technology, and the development of nematode resistance transgenic plants are recent biotechnological approaches which have substantial potential for nematode management. A number of studies and reviews published over the past few decades have attested the success of these methods for nematode control. This chapter provides an overview of the significant breakthroughs on novel biotechnological interventions for managing plant-parasitic nematodes, in agricultural crops.

7.2 Biotechnological Interventions

The advent of biotechnology has opened the door to the exploration of new methods of nematode control. The term "biotechnology" is the result of collaboration between the biological and technological sciences. Technical chemistry and chemical engineering are integral parts of this field, which combines biochemistry and microbiology with an emphasis on practical applications. Managing nematodes is important for food production because nematodes cause substantial losses to agricultural crops. Plant nematodes like other pests and pathogens are controlled with chemicals or natural remedies. Chemicals, besides being costly, create a serious risk of food and environmental contamination. However, the use of non-chemical approaches is a better option in totality. However, traditional farming methods such as cultural practices are slow in action as well as give lower productivity (Rao and Phani 2019). So, to combat the devastating effects of nematodes on agricultural and horticultural crops, the best option is to cultivate resistant varieties. Conventional plant breeding used to create a resistant variety, but this process is much slower. The use of biotechnology presents a viable and efficient option for creating a resistant cultivar. Besides application in host resistance, biotechnology can be applied in various other ways to achieve sustainable nematode management in crops, which are summarized in the following.

7.2.1 Application of Plant Natural Resistance Genes

Host resistance is widely regarded as an eco-friendly and economically viable alternative to chemical treatments. Many R-genes (resistance genes) have been isolated and characterized especially from wild hosts which confer resistance primarily against sedentary endoparasitic nematodes (Table 7.1, Williamson and Kumar 2006; Rao and Phani 2019). Generally, R-genes constitutively occur in plants, albeit at low levels. These genes encode surveillance proteins which detect the effector molecules (pathogen origin) and trigger an efficient defence reaction. The plant R-genes so far identified are part of multigene families with anywhere from a few to over 30 homologs, all of which may contribute to the evolution of resistance specificity (Hulbert et al. 2001).

Natural resistance genes (R-gene) have been isolated from a wide variety of plants and introduced into different economically important crop species, suggesting that resistance or tolerance to nematodes may also be conferred upon other plant

Table 7.1 The list of nematode-resistant genes (Rao and Phani 2019)

Gene	Plant	Nematode
Cre1, Cre3	Wheat	*Heterodera avenae*
Gpa2	Potato	*Globodera pallida* specific populations
Gro1–4	Potato	*Globodera rostochiensis* pathotype Ro1
H1	Potato	*G. Rostochiensis* pathotypes Ro1 and Ro4
Has-1^{Og}	Rice	*Heterodera sacchari*
Hero A	Tomato	*Globodera pallida* pathotypes Pa2 and Pa3 *Globodera rostochiensis* pathotypes Ro1, Ro3, and Ro5;
Hs1^{pro1}	Sugar beet	*Heterodera schachtii*
Ma	Plum	Root-knot nematodes
Me3	Pepper	*Meloidogyne incognita, M. javanica, M. arenaria,* and some *M. hapla* isolates
Mi-1	Tomato	*Meloidogyne incognita, M. javanica, M. arenaria*
Mi-3	Tomato	*Meloidogyne incognita, M. javanica, M. arenaria*
Mi-9	Tomato	*Meloidogyne incognita*
Rhg1, Rhg4	Soybean	*Heterodera glycines* type 0
Rmc1	Potato	*Meloidogyne chitwoodi, M. fallax,* and some *M. hapla* isolates

species through this method. The R-genes occur in monogenic as well as polygenic manner. The nematode single dominant resistance genes encounter with the corresponding avirulence genes (Avr), leading to 'gene-for-gene' interaction. Isolating nematode resistance genes has the practical implication of transferring that resistance to economically significant crop species where it is currently unavailable. Transgenic techniques have proven effective for intraspecific transfer of nematode-resistance genes. However, interspecific transfer has been met with only moderate success. Tomatoes that had been transferred with the *Mi*-1 gene became resistant to the tomato root-knot nematode, but the same gene did not confer resistance to the nematode in tobacco or *Arabidopsis* (Williamson and Kumar 2006). Genotype differences among tomato cultivars were found to impact effectiveness of *Mi*-1 gene even within the cultivated tomato species (Jacquet et al. 2005). It was also determined that map-based cloning and marker-assisted selection methods worked well for nematode resistance breeding. A major quantitative trait locus, *Rhg*4, which imparts resistance against *H. glycines* in soybean was identified and induced through map-based cloning (Liu et al. 2012). The resistant chemical, serine hydroxyl-methyl transferase, was found to be encoded in the *Rhg*4 soybean mutants. For the purpose of marker-assisted selection for root-knot nematode resistance in pepper cultivars, several PCR-based markers closely linked to the *Me*1 gene were developed and demonstrated to be useful (Wang et al. 2018). Moreover, a significant reduction in the adult females population in soybean roots was recorded after over expression of a number of other candidate resistance genes encoding dehydrogenase, ascorbate peroxidase, lipase, ß-1,4-endoglucanase, calmodulin, etc. (Liu et al. 2012). Panella and Lewellen (2007) achieved resistance against *H. schachtii* through transgenic expression of *Hs*1*ro*1, a resistance gene from *Beta*

procumbens, introduced into sugar beet; however, this was associated with other genes that reduced the yield. Additionally, most R-genes are only effective against a single nematode species or pathotype (Ali et al. 2017). The evolution of novel nematode pathotypes with undetectable effectors (avr genes) due to the R-genes is another major drawback of this approach (Jung et al. 1998). In-depth familiarity with plant and nematode genetics is crucial in this regard. This will pave the way for the creation of new strategies for long-term resistance in crop plants by shedding light on the potential mechanisms by which a resistant phenotype is attained.

7.2.2 Utilizing Genes Encoding Proteinase Inhibitor

Proteinase inhibitors are molecules that are synthesized within plants to counteract the effects of proteases and lysases, two types of enzymes that are commonly secreted by pathogens. Aspartic-, cysteine-, serine-, and metallo-proteinases are the four types of proteinases discovered in nematodes. Hepher and Atkinson (1992) first described the potential of plant-derived proteinase inhibitors to combat nematodes; their study focused on transgenic potatoes expressing a cowpea trypsin inhibitor, which conferred protection against the potato cyst nematode, *G. pallida*. Later, it was discovered that *H. schachtii* and *M. incognita* growth and reproduction could be inhibited by *Arabidopsis* plants that overexpressed cystatin Oc-IΔD86 (Urwin et al. 1997). A transgenic eggplant (*Solanum melongena*) that expresses a modified rice cystatin (Oc-IΔD86) gene under the control of the root-specific promoter, TUB-1, has shown resistance against *M. incognita* and also increased the crop yield (Papolu et al. 2016). Positive results against lesion nematode *Pratylenchus penetrans* infecting lily cv. Nellie White has been observed after treatment with this rice cystatin (Vieira et al. 2015). Bananas that had an overabundance of maize cystatin (CC-II) also showed a significant decrease in *Radopholus similis* and *Helicotylenchus multicinctus* infections (Roderick et al. 2012). *Triticum durum* PDW215, a transgenic wheat line, was able to withstand invasion by the cereal cyst nematode, *Heterodera avenae*, because of the serine proteinase inhibitor (PIN2) gene (Vishnudasan et al. 2005).

Rice, potatoes, tomatoes, alfalfa, bananas, and sweet potatoes are engineered to be resistant to a wide variety of nematodes including *M. incognita*, *M. hapla*, *H. schachtii*, *G. pallida*, *Rotylenchulus reniformis*, *Ditylenchus destructor,* and *Pratylenchus penetrans* and showed significant resistance to multiple species of these pests (Chan et al. 2015; Papolu et al. 2016). Additionally, 60–80% less galling and reproduction of *M. incognita* were observed in tomato transgenic lines expressing the hairpin construct of cathepsin L cysteine proteinase (*Mi*-cpl-1) (Dutta et al. 2015). Tobacco transgenic lines expressing dsRNA for the *Mi*-cpl-1 gene also showed partial resistance to *M. incognita* race 3 (de Souza Júnior et al. 2013). By interfering with the nematode's capacity for sex determination and gall formation, heterologous expression of a taro cystatin conferred significant resistance to tomato against *M. incognita* (Chan et al. 2010). Multiple proteinase inhibitor combinations have been shown to increase resistance to nematodes. Hepher and

Atkinson (1992) and Urwin et al. (1998) reported that the resistance to *G. pallida,* and *H. schachtii* is conferred on transgenic *Arabidopsis* lines expressing a translational fusion protein of CpTI and Oc-IΔD86. Chan et al. (2015) observed that the overexpression of the taro cysteine proteinase inhibitor (CeCPI) and the fungal chitinase (PjCHI-1) regulated by a synthetic promoter, pMSPOA, had a detrimental impact on the egg-laying of *M. incognita* females. Given these results, gene pyramiding becomes a viable strategy for enhancing plant defences against nematodes (Tripathi et al. 2017). Accordingly, proteinase inhibitors are a promising candidate for inducing resistance in crop plants against serious nematode species.

7.2.3 Use of Nematicidal Proteins

The development of nematodes in plants is impeded in part by nematicidal proteins. Some examples of such proteins include lectins, specific antibodies, and Bt Cry proteins and a few reports of their use on a commercial scale are available. Non-immune proteins called lectins have a wide range of biological effects, including anti-inflammatory, antiparasitic, insecticidal, ovicidal, and larvicidal (de Medeiros et al. 2018). The ability of lectin proteins to impede intestinal function in organisms that exhibit or ingest them is a hallmark of their toxicity (Vasconcelos and Oliveira 2004). Concanavalin A, a lectin extracted from the jack bean (*Canavalia ensiformis*), was found to significantly reduce *M. incognita* populations on tomato after being applied (Marban-Mendoza et al. 1987). Soybean agglutinin, wheat germ agglutinin, and Concanavalin A were all used to induce hypersensitivity to *M. incognita* infection in infective juveniles (Davis et al. 1989). The lectin from Snowdrop (*Galanthus nivalis*) expressed in transgenic plants such as potato and rapeseed offered moderate resistance against *G. pallida*, *H. schachtii,* and *P. neglectus* (Burrows et al. 1998; Ripoll et al. 2003). *Moringa oleifera* lectin, recently isolated from *M. oleifera* seeds, has been found to be highly effective against animal nematodes such as gastrointestinal nematodes (de Medeiros et al. 2018). Protein fractionation revealed that lectins were a major determinant of the nematicidal activity of crude protein extracts from *M. oleifera* seeds against *M. incognita* (El-Ansary and Al-Saman 2018).

The Bt toxins from *Bacillus thuringiensis* are suppressive to plant pathogens (Khan and Tarannum 1999; Shahid and Khan, 2019; Khan et al. 2022) and have the potential to impart resistance in plants against nematodes. Marroquin et al. (2000) used Bt toxin as a nematode suppressive protein by exposing *C. elegans* to Cry5B and Cry6A, causing a decrease in nematode reproduction and survival. The reproduction of *M. incognita* was adversely influenced by expressing 54 kDa Cry6A and Cry5B proteins of the hairy roots in tomato (Li et al. 2008). However, the cyst nematode, *H. schachtii,* lacked the digestive capacity to consume this protein due to the limited size of its feeding tube (Urwin et al. 1998). This restriction has prevented the widespread implementation and utilization of this poison. Cheng et al. (2018) reported transformation of the Bt nematicidal cry5Ba3 gene into *Botrytis cinerea* to altered the mycophagous feeding by *Bursaphelenchus xylophilus* and decreased the

nematode fitness. Toxin delivery by the fungus to sites where the nematode forages is a promising avenue of research towards the management of pine wood nematodes by using this "sweet poisoning" tactic to interrupt the nematode's life cycle.

7.2.4 Use of Plantibodies

Plantibodies, which are essentially the antibodies expressed in plants, are another candidates for nematode resistance development. The sedentary endoparasites (*Meloidogyne, Heterodera, Globodera*, etc.) use a number of enzymes and effectors secreted from their pharyngeal glands to trick host plants into changing their cells into feeding sites. It may be possible to dampen the nematode's parasitic ability by directing plantibodies in the opposite direction of the active proteins from these secretions (Ali et al. 2017). The movement and invasion of *G. pallida* in potato roots are affected by amphidial and cuticular secretions. Fioretti et al. (2002) reported that this effect can be blocked by using monoclonal antibodies. Polyclonal and monoclonal antibodies that bound to the cuticular surface of *M. javanica* J_2 altered their behaviour and pattern of movements (Sharon et al. 2002). Because of this, it may be useful to characterize surface antigens from various nematodes to aid in the creation of novel nematode control strategies.

7.2.5 Utilization of Peptide Elicitors and Chemodisruptors

Nematodes that parasitize plants use their wide variety of chemoreceptive neurons to detect and enter the host plant. An alternative method to reduce the number of infectious juveniles entering a plant is the application of chemo-disruptive peptides. The chemoreception and locomotion of *H. glycines* and *G. pallida* were found to be disrupted by peptides mimicking the effects of the pesticides aldicarb and levamisole (Winter et al. 2002). Transgenic potato variety was developed in which only a few females of *G. pallida* were able to develop due to the expression of peptide which inhibited the acetylcholinesterase (Liu et al. 2005). Similarly, resistance to *H. schachtii* and *G. pallida* were observed in *Arabidopsis* and potato plants that expressed a chemo-disruptive peptide for acetylcholinesterase controlled by of root tip-specific promoter and CaMV35S (Lilley et al. 2011b). This method was used to create transgenic potatoes resistant to potato cyst nematodes and expressed rice cystatin (Green et al. 2012). Roderick et al. (2012) and Tripathi et al. (2013) developed nematode-resistant transgenic plantain based on protease inhibitor cystatin. Combining cystatins and a chemo-disruptive peptide with a gene pyramiding strategy, transgenic variety of tomato, banana, etc. resistant to *Meloidogyne* spp. has been developed (Chan et al. 2015; Tripathi et al. 2017).

Lee et al. (2018) discovered that treating soybean seeds with exogenous peptides from plant elicitors (specifically GmPep1, GmPep2, and GmPep3) greatly reduced the reproduction of *M. incognita* and *H. glycines*. Additionally, the peptide treatment prevented the root-knot nematodes from damaging the roots and increased the

expression of nematode-responsive defence genes. While this method has been shown to be successful in combating insect and fungal pests (Lee et al. 2018), the plant nematodes have received very limited attention.

7.3 Application of RNA Interference

Using biotechnology, scientists have been able to use in vitro silencing of parasitism genes to pinpoint the nematode genes that will be most useful for a host-delivered RNA interference (RNAi) strategy by causing the degradation of messenger RNA (mRNA). The double-stranded RNA (dsRNA) mediates gene silencing in a specific target gene or genes. Resistance to *M. incognita* was first achieved through host-delivered RNAi by Yadav et al. 2006, who used tobacco transgenics to express the dsRNA of integrase and splicing factor genes. Reproduction of *H. glycines* was also significantly reduced in transgenic soybeans expressing PRP17 dsRNA (Li et al. 2010a). The gene Mj-far-1 for fatty acid and retinol-binding protein for *M. javanica* were expressed in tomato hairy roots; it reduced its transcript abundance by about 80% (Iberkleid et al. 2013). Transgenic soybeans expressing dsRNA of the major sperm protein coding gene were shown to reduce *H. glycines* fecundity by roughly 68% (Steeves et al. 2006). The soybean transgenic varieties expressing tyrosine phosphatase gene (RNA hairpin) developed significantly fewer root galls of *M. incognita* (Ibrahim et al. 2011). Similarly, potato cvs. Desiree, Russet, and Burbank for expressing an RNAi construct targeting the effector gene (Mc16D10L) became resistant to *M. chitwoodi* (Dinh et al. 2014).

The expression of cell wall degrading enzyme coding genes was altered after in vitro silencing of five esophageal gland genes expressed either in subventral or dorsal glands of *M. incognita,* resulting in decreased penetration of infective juveniles (Shivakumara et al. 2016). This demonstrates the existence of genetic communication between parasitism-related species. Additionally, transgenic brinjal plants had roughly 70% less *M. incognita* multiplication as a result of host-delivered RNAi silencing of msp-18 and msp-20, the pharyngeal gland-specific genes (Shivakumara et al. 2017). Furthermore, it was found that cell wall modifying enzymes (CWMEs) undergo transcriptional oscillation in both developing and penetrating nematodes, indicating a complex interaction between CWMEs and pioneer genes during parasitism (Shivakumara et al. 2017). *Arabidopsis* has shown significant resistance to a variety of nematodes, prompting the identification and subsequent targeting several candidate genes involved in the resistance (Atkinson et al. 2012; Dutta et al. 2014). For plant-parasitic nematodes, host-delivered RNAi transgenics present a novel and potentially useful management tool; however, RNAi-based management is not without the risk of unintended side effects (Danchin et al. 2013). In addition, the RNAi-engineered plants did not exhibit full resistance against the intended nematodes (Dutta et al. 2014; Rao and Phani 2019). Some important applications of RNAi in nematode management are described below.

7.3.1 Utilization of Neuropeptides as a Therapeutic Target

The neuropeptides responsible for coordinating vital aspects of nematode physiology and behaviour are remarkably conserved across taxonomic groups. The different types of nematode neuropeptides (FLPs, NLPs, ILPs, etc.) have been thoroughly characterized, thanks to the advances in *C. elegans* research (Li and Kim 2008). By interfering with the juvenile stage's host finding ability and invasion into roots, RNAi targeting flp-14 and flp-18 (FMRF amide like peptides) was delivered by the host reduced infection and multiplication of *M. incognita* in tobacco (Papolu et al. 2013). Two FLP genes (flp-14 and flp-18) and a 16D10 (subventral pharyngeal gland-specific gene) were used in a combinatorial in vitro RNAi experiment on *M. incognita*, resulting in a 20–30% reduction in nematode infection and multiplication (Banakar et al. 2015). Silencing neuropeptide genes nlp-3 and nlp-12 in *M. incognita* also resulted in delayed host finding and reduced infection of tomato plants, similar to what was seen with FLPs (Dash et al. 2017). Bioactive neuropeptides from the neuropeptide-like protein (NLP) family have been profiled and targeted in an effort to use them as novel targets for nematode management (Warnock et al. 2017). The feeding activities of *M. incognita* and *G. pallida* (chemosensation, host invasion, stylet thrusting, etc.) were found to be negatively impacted by a large number of separate NLPs. The nematode infection rate in tomatoes was reduced by as much as 90% when transgenic *Chlamydomonas reinhardtii* (terrestrial microalgae) and *Bacillus subtilis* were used to secrete these neuropeptides. This "non-food transgenic delivery" system may be used to deliver neuropeptides, a new type of nematicide that protects plants from pests.

7.3.2 Utilization of Parasitism Genes

The genes responsible for the synthesis and release of certain proteins from the oesophageal glands and introduced into host plants through stylet of the nematode are called parasitism genes. These genes may be crucial for nematodes that invade plants for RNAi. The SKP-1, Ring-H2, ubiquitin-like proteins (proteasome), secreted by some nematodes, control the degradation of protein in host cells. In order to invade and migrate more easily (Sindhu et al. 2009), the β-1-4 endoglucanases secreted by *H. glycines* and *G. rostochiensis* destroy plant tissues (Chen et al. 2005; Bakhetia et al. 2007). In situ hybridization analysis has suggested that the *M. incognita* and *H. glycines* cysteine proteinase genes' products are digestive enzymes, and RNAi of both of them significantly reduced the number of established nematodes on plants.

Four major *Meloidogyne* spp. contain parasitism gene 16D10, which protects secretory peptide of the nematode that promotes root growth (Huang et al. 2006). A macrophage mannose receptor, aggrecan, shares sequence homology with C-type lection (Urwin et al. 2002). The RNAi gene responsible for the synthesis of amphid protein affects the searching and invading ability of *G. rostochiensis* (Chen et al.

2005). It is possible that inhibitors for encoding these genes may be introduced into plants to control the synthesis of the amphid protein.

7.3.3 Utilization of Genes Regulating Development of the Nematode

Certain genes which regulate developmental stages of parasitic nematodes, such as embryogenesis, moulting, reproduction, etc., may be exploited in nematode management. A chitin synthase gene, regulating the production of chitin in the eggshells, was repressed by RNAi, which caused *M. artiellia* egg hatching to be delayed (Fanelli et al. 2005). The gene encoding a key sperm protein that expressed dsRNAs was found responsible for reduced reproductive potential in transgenic soybean plants. The disruption of FLP gene in PCN, *G. pallida,* resulted in motor impairment and exceptional neural sensitivity to RNAi (Kimber et al. 2007). Bioinformatics was used to identify 1508 candidate genes in *H. glycines* (Alkharouf et al. 2007). The contemporary homologous genes in *C. elegans* exhibit lethal phenotypes upon silencing in *C. elegans*. Li et al. (2010a, b) demonstrated using the same method that the RNAi of three genes encoding for a beta subunit of the coatomer (COPI) complex, a pre-mRNA splicing factor, and an unidentified protein resulted in a considerable decrease in the formation of cysts and eggs of *H. glycines*.

7.3.4 Utilizing Genes Regulating the mRNA Metabolism

Inhibiting development or reproduction of nematodes by genes regulating mRNA metabolism may prone to be an effective technique. According to Yadav et al. (2006), tobacco plants were protected from infection by *M. incognita* due to fragments of two dsRNA genes that encoded an integrase and a splicing factor. In another study, *H. glycines* soaked in dsRNA solution of a ribosomal gene Hg-rps-23 exhibited more than 95% mortality to the J_2 population (Alkharouf et al. 2007). Additionally, *H. glycines* cyst counts were reduced by 81 to 88% in soybean roots producing inverted repeat constructions, Hg-rps-3a, Hg-rps-4, and Hgspk-1 genes, which are implicated in the metabolism of mRNA (Klink et al. 2009). Transgenic soybean plants with Prp-17 gene, regulating mRNA splicing, inoculated with *H. glycines* showed 53 and 79% decline in the number of cysts and eggs/g root tissue, respectively. The Prp-17 gene and other similar genes operate the metabolism of mRNA, indicating that RNAi may be sensitive specifically to these genes and that they may be suitable targets for parasitic nematode control.

7.3.5 Genome-Enabled Development of Novel Chemical Nematicides

Using genomic data from *M. incognita*, a bioinformatics pipeline was used to screen candidate gene targets for novel nematicides. With the help of this approach, a shortlist of excellent target genes that might be used as a starting point for the creation of fresh chemical nematicides was produced. Functional studies took the form of in vitro feeding studies where siRNAs targeted at each potential gene were tested for their impact on phenotype or the nematode's capacity to attack and feed on plant roots. Following the identification of the necessary essential nematode target genes, targeted development or chemical testing for compounds that suppress such functions can be carried out to create new pesticides.

7.3.6 Ectopic Delivery of dsRNA: Non-transgenic RNAi

The ectopic application spraying of dsRNA on plants has good potential of introducing genes into a crop for nematode control. The BioDirect Technology, a non-transgenic alternative route of introducing RNAi into a crop for protection against herbicides, insects, and viruses, is quite effective in using this tactic. The challenge in this case is to create stable dsRNA forms and spray delivery methods for foliar part of crop and taken up systemically through the conductive tissue to the roots where they can be ingested by the nematodes. Foliage may also ingest while feeding on the host, and upon ingesting, crucial function and processes of the nematodes are inhibited.

7.4 Nematode Resistance Transgenic Crops

Some of the approaches mentioned above are being applied to cereals, vegetables, and staple crops where nematode control is critically needed. Below is a more detailed discussion of developments of nematode resistance transgenic plants in some most economically important crops.

7.4.1 Banana

Bananas and plantains (*Musa* spp.) suffer considerable production losses due to nematode infestation (Khan and Jairajpuri 2012). *Pratylenchus coffeae, P. goodeyi*, and *Radopholus similis* are commonly encountered in banana plantations, causing 20 to 40% yield losses (Haque and Khan 2021). Similarly, *Meloidogyne incognita* and *M. javanica* are other significant nematodes of banana in areas where *Pratylenchus* and *Radopholus* are less prevalent (De Waele and Davide 1998). Bananas are triploid, which makes them a particularly attractive crop for genetic modification because they of limitation in the cultivar improvement through

conventional breeding methods. The plants' sterility is advantageous in this situation because it reduces the possibility of gene flow to related plants. Recent genetic engineering efforts on bananas and other plantains have some success. Various transformation procedures based on particle bombardment, protoplast electroporation (through embryogenic cell suspension), and transformation mediated through *Agrobacterium* are available (Arvanitoyannis et al. 2008), which may prove effective.

Banana and plantain are being used as test crops for the above nematode resistance techniques. The resistance diploid banana hybrid against *R. similis* (Uganda population) is regulated by two dominant genes. Dochez et al. (2009) found that 37 out of 81 hybrids were resistant to the nematode. In a glasshouse test, Cavendish dessert bananas with a 70 ± 10% resistance to *R. similis* expressed the OcIΔD86 transgenic version of rice cystatin (Atkinson et al. 2004). It was found that giant cells in plants which expressed cystatin production exhibited 83.4% resistance to *M. incognita* (Green et al. 2002; Lilley et al. 2004). This technique is being exploited in developing different *Musa* types (Lilley et al. 2011b).

The banana plants in East African Highland expressing the maize cystatin showed suppressed population, while the plantain cv. Gonja has been modified to express cystatin as well as a repellent peptide (Lilley et al. 2011b). Different East African Highland banana types have been introduced with similar additive cystatin plus repellant constructions (NARO, Uganda). Cystatin prevents banana weevils from feeding and growing, it is possible that cystatin-mediated nematode resistance in bananas has additionally benefits in host resistance (Kiggundu et al. 2010). According to Lilley et al. (2011a), *R. similis* quickly absorbs molecules, and uptake of dsRNA results in effective suppression of transcript; however, the degree of silencing can vary depending on the nematode target gene and the environmental conditions (Haegeman et al. 2009). The *R. similis* infestation in *Medicago truncatula* later was reduced by up to 60% when it was soaked in dsRNA identical to xylanase gene (gland cell) (Haegeman et al. 2009).

7.4.2 Potato

Globodera, Meloidigyne, Pratylenchus, and *Ditylenchus* constitute important nematode pests of potato in temperate countries as well as in cooler areas of subtropical and tropical regions (Haque and Khan 2021). The *H*1 resistance gene is found quite effective against the infestation with *G. rostochiensis*, but not effective in preventing reproduction of *G. pallida* on potato. In potato, proteinas inhibitor (PI) based engineered resistance has been thoroughly tested, primarily against *G. pallida*. The serine PI cowpea trypsin inhibitor (CpTI), a plant-based PIs as anti-nematode effectors that has been examined first for effectiveness. Hepler and Atkinson reported that the sexual fate of freshly hatched *G. pallida* was affected by CpTI expressed in transgenic potatoes. This led to development of much greater number of less harmful male individuals in the *G. pallida* population. Successive field tests of transgenic potatoes were conducted for further study on cystatins. Urwin et al.

(2001) reported that best line among healthy susceptible transgenic potato cv Desiree demonstrated 70% field resistance to PCN when it expressed chicken egg white cystatin via the constitutive CaMV35S promoter. Similarly, from potato cv. Sante and cv Maria Huanca, the best lines which exhibited natural partial resistance to PCN were improved to complete resistance when the identical design was applied to them. The field tests later showed that the sunflower cystatin produced in cv. Desiree and modified rice cystatin (OcI-D86) both provided comparable degrees of resistance to chicken egg white cystatin (Urwin et al. 2003). Lilley et al. (2004) observed that potato cultivars with OcI-D86 cystatin expression restricted primarily to the roots, particularly to the syncytia (*G. pallida*), and giant cell (*M. incognita*) exhibiting comparable levels of resistance to both nematodes.

The peptide repellent technique and its potential and application in developing transgenic potato plants have also been evaluated. A containment trial recorded a 52% decrease in the *G. pallida* females in the roots of best line expressing the acetylcholinesterase-inhibiting peptide over control (Liu et al. 2005). Lilley et al. (2011b) further increased the resistance to 95% in best line by employing localized production of the same peptide with a root tip-specific promoter.

7.4.3 Rice

Rice is a major cereal, and is commonly consumed throughout the world, particularly in South-East Asia (Haque and Khan, 2021). About 90% of world paddy is cultivated and consumed in tropical and subtropical regions. Rice is recorded to host around 300 species of nematodes belonging to 35 genera (Khan et al. 2022). About 10 genera are economically significant in rice cultivation, which are accounted for nearly 10% of annual yield decline equivalent to US$ 16 billion in rice world over. Rice is grown in a wide range of ecological climates. Important nematodes infecting rice in irrigated ecosystems include *Meloidogyne graminicola, Aphelenchoides besseyi*, and *Hirschmanniella* spp. (Khan and Ahamad 2020). Deepwater rice is infected with the ufra nematode *Ditylenchus angustus*, while upland rice is attacked by *Pratylenchus* spp. and *M. graminicola* (Khan et al. 2022). Transgenic nematode-resistant varieties offer enormous scope for the production of rice throughout the world. Rice, for having a modest genome size (389 Mb), may serve as a model for monocot. A number of resistance genes against *M. graminicola* have been identified in *O. longistaminata* and *O. glaberrima* which have been introduced into *O. sativa* (Soriano et al. 1999). However, the cystatin-based defence is the only nematode-resistant technology that has yet to be introduced into rice. Vain et al. (1998) observed that modified rice cystatin OcIΔD86 was constitutively expressed in transgenic plants of some important African rice types, and these plants demonstrated 55% resistance to root-knot nematode. Only a minimal amount of cystatin expression was seen, which may be related to a poor CaMV35S promoter in conjunction with the native OcI gene. In order to increase expression levels, a maize

ubiquitin gene intron region was additionally incorporated leading 91–97% resistance to *M. incognita* in the best transgenic lines (Green et al. 2002).

7.4.4 Other Crops

Application of biotechnological methods for resistance against nematode has been tried in some other agricultural crops also. To prevent the *Heterodera avenae* invasion in wheat, a potato serine proteinase inhibitor (PIN2) was encoded in transgenic wheat which performed tolerance to the nematode and gave a good yield (Vishnudasan et al. 2005). It has been found that the proteinase inhibitor had a preventive impact against the nematode infection. Chen et al. reported that a tomato cultivar that was sensitive to the root-knot nematode when constitutively expressed a cystatin from the taro root, prevented the nematode attack to a considerable level. Comparing transgenic plants to wild-type plants, *M. incognita* developed 50% fewer galls on the transgenic plants, and these plants also produced lesser egg masses.

7.5 Biosafety Issue of Nematode-Resistant Transgenics

The benefits of transgenic crops for food security can only be realized if every biosecurity concern is scientifically as well as convincingly sorted out, and the crops are grown commercially with access of farmers to seeds at the reasonable cost in third world countries. Numerous individual studies have found that transgenic nematode-resistant crops do not affect non-target organisms (Atkinson et al. 2009). There have also been multiple investigations into whether or not the introduction of transgenic plants modifies the micro-environments of soil, thereby altering the web of life there (Ferris et al. 2001), it has been concluded that transgenic nematodes-resistant crops pose no threat to the natural world. To further alleviate the impact on plants, tissue-specific promoters can be used to lessen the risk to non-target organisms. Depending on the degree of similarity between the target gene sequence of nematode and that of other organisms, it is possible that the host-delivered RNAi technology will have unintended consequences for those organisms. Therefore, in order to reduce the amount of unintended silencing of off-targets, speedy and accurate bioinformatics analysis should be performed to select unique and novel targets (Atkinson et al. 2012). Finally, substantial political support is needed for the widespread adoption of transgenic crops at the field level. In India, Bt cotton has become widely planted as a proof that a transgenic crop can help poor farmers. Similarly, in the U.S.A., over 90% of cultivated maize is transgenic crop and accounted for the annual 33 Mha area (Pellegrino et al. 2018). Moreover, China has established an autonomous capability in the development of transgenics, serving as a model for developing countries (Atkinson et al. 2012).

7.6 Conclusion and Future Perspectives

Plant nematodes are one of the major pests of crops in today's highly mechanized agricultural system. It is not uncommon for nematode populations in the soil to balloon over time due to the pest's microscopic appearance and the farmer's failure to recognize it as a threat, which leads to serious quantitative and qualitative losses to their valuable crops. Research on nematode management has shown that; no single tactic has proven to be effective enough to eradicate the entire problem. Nematode population density and damage decrease considerably with the implementation of different management practices, but to a variable extent. Several new targets and novel technological strategies for nematode control have emerged especially due to advances in biotechnology. All of these measures are aimed to suppress nematode parasitism and to enhance crop yields. However, most targets have been evaluated in the laboratory or a greenhouse on selected model plant species, e.g., *Arabidopsis*. Therefore, in order to use these findings singly or in an integrative manner to achieve maximum nematode management, extensive field-level research is of utmost need to validate technology for commercial application.

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