



Research Article

The Role of Genetic Engineering in Management of Plant Parasitic Nematodes with Emphasis on Root-Knot Nematodes: A Review

Mahmoud Mohamed Ahmed Youssef* and Suzan Abd-Elazeim Hassabo

Plant Pathology Department, Nematology Laboratory, National Research Centre, Dokki, Post code 12622, Cairo, Egypt.

Abstract | Genetic engineering can be defined as the formation of new combination of heritable material (DNA) by the insertion of nucleic acid molecules produced outside the cells into any virus, bacteria, plasmid or other vector system in which they are capable of continued propagation. *Azotobacter* spp. with nematocidal activity could control root-knot nematode, *Meloidogyne incognita*. The development of such strains by genetic manipulation by transferring gene (s) required for saponins production is effective in increasing crop yield together with fixing nitrogen. *Escherichia coli* is still used as a host for the recombinant DNA technology – based industrial production of proteins and peptides to manage root-knot nematode. Mi gene resistance in tomato plants has been utilized to manage *M. incognita* and *M. javanica*. Also, protoplast fusion between *Pseudomonas fluorescens* and *P. aeruginosa* to manage *M. incognita* was utilized. Many genes expressed in nematode feeding cells or the regulatory regions that control these genes have been isolated. Transproteins toxic to different plant parasitic nematodes can be expressed into tissues and cells feed upon by nematodes. Transgenic products with a potential to interfere with nematode physiology such as digestive enzymes or structural proteins of the intestine are considered.

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***Correspondence** | Mahmoud Mohamed Ahmed Youssef, Plant Pathology Department, Nematology Laboratory, National Research Centre, Dokki, Post code 12622, Cairo, Egypt; **Email:** myoussef_2003@yahoo.com

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Introduction

Plant diseases are a threat to world agriculture. Significant yield losses due to the attack of pathogen occur in most of the agricultural and horticultural crop species. Traditional plant breeding methods have been used to develop cultivars resistant to various diseases. However, this process is time-consuming and limited availability of genetic resources for most of the crops has left little room to continued improvement by these means. Effective resistance against plant-parasitic nematode is uncommon in many crops. Yet, crop resistance is the most environmentally friendly and cost effective means of reducing yield losses in agriculture. Hence,

considerable emphasis is placed on molecular assisted breeding of natural resistance genes and engineering of synthetic resistance genes. Synthetic resistance can also be derived from the expression of toxic molecules in the plant, either to affect nematode directly or to interfere with formation and maintenance of their feeding cells (Vrain, 1999). From these toxic materials, saponins are natural plant materials having broad biological activities and have several antibiotic activities such as antiviral, antifungal and insecticidal (Small et al., 1990; Poinar, 1993). Gene(s) required for saponins production from saponins-producing plants could be transferred to *Azotobacter* sp., *A. vinelandii* or *A. chroococcum*. Hence, bioengineering strategies are being developed that will provide specific and

urable resistance against plant-parasitic nematodes. These strategies come under three categories: (1) transfer of natural resistance genes from plants that have them to plants that do not, to promote the defense in susceptible crops, (2) interference with the biochemical signals that nematodes exchange with plants, especially those inducing formation of feeding sites for the sedentary endoparasites, and (3) expression of proteins toxic to nematodes in plant cells (Vrain, 1999). These points are as follows:

Transfer natural resistance genes

To transfer natural resistance gene(s) by genetic engineering, it must first be localized (mapped) on the plant chromosomes and their sequence must be determined. This point can be discussed as follows:

Transfer of gene(s) required for saponins production in Azotobacter chroococcum

Plant saponins have many economic importance. They are antimicrobial, insecticidal and insecticidal substances. D'Addabbo *et al.* (2011) reported that dry vegetative growth and root materials of *Medicago sativa* (as source of saponins), *in vitro* and *in vivo* trials against *M. incognita* and *Heterodera carotae* with *M. sativa* pelleted meal reduced number of these nematodes in soil and roots and subsequently improved plant growth and yield. There is no evidence to suggest that consumption by humans is harmful (haemolytic saponins are highly toxic to mammals when administered intravenously, but oral toxicity is very much lower due to saponin failure to cross the gut and enter the blood stream). The justified research on saponins focuses on producing them on an economical scale. The genes responsible for producing saponins with nematocidal effect could control the root-knot nematode, *M. incognita* (Salem, 1997). The development of bacteria, *Azotobacter* spp which are free living and nitrogen fixer by genetic manipulation to transfer gene(s) required for saponins production from plants producing saponins to these bacteria is effective in controlling plant-parasitic nematodes and increasing crop yields together with benefit in fixing nitrogen. Gene(s) required for saponins production from saponins producing plants could be transferred to *Azotobacter* spp., *A. vinelandii* or *A. chroococcum*. The expression of saponins producing activity was screened using haemolytic technique i.e., transformants exhibited haemolysis zones on blood agar plate (Salem, 1997).

Nematicidal activity of transformed Azotobacter chroococcum on Meloidogyne incognita (Kofoed et White) Chitwood

Barker *et al.* (1971) determined the effect of nitrogen source and concentration on the nematode activity. They found that the application of nitrogen in soil reduced nematode hatching, penetration and cyst formation. The inhibitory effects were positively correlated with concentration of nitrogen. So, the fertilization of soil with nitrogen fixer microorganisms with nematocidal activity would prevent much of the crop damage caused by nematodes under field conditions. Korayem and Salem (2000) reported that the highest mortality percentage (50%) of the root-knot nematode, *M. incognita* after 24 hr was obtained by *A. chroococcum* transformed by saponaria DNA isolated by Bendich and Bolton's Method (1967). The same mortality value was obtained by standard saponins at concentration of 80 mg/ml. On the other hand, the filtrate of the same strain of *Azotobacter* transformed by *Poinciana regia* DNA caused 23% mortality only according to Bonner (1965). However, *Saponaria officinalis* DNA extracted by Doyle and Doyle (1987) had no effect on nematode juveniles as mentioned by the same authors.

Expression of saponins producing ability in Escherichia coli

E. coli is a promising industrial strain for many reasons, its high growth rate on simple medium and its ability to express the eukaryotic gene(s) which means that *E. coli* might have splicing enzymes (Andrey *et al.*, 1992). *E. coli* is still used as host for the recombinant DNA technology-based industrial production of proteins and peptides (Denhardi and Colastoni, 1987) and it was used as recipient. Saponaria DNA was prepared according to Doyle and Doyle (1987) method. *E. coli* cells component was transformed with different concentrations of DNA material, 30, 50 and 66 µg DNA/ml. The transformation frequency was calculated. The results indicate that the best DNA concentration was the highest one (66 µg/ml) which gave 60×10^{-4} haemolytic colonies (Salem, 1997).

Nematicidal activity of E. coli transformations on Meloidogyne incognita

The mortalizing actions of cell free culture filtrate on the root-knot nematode, *M. incognita* were assayed. The nematicidal properties of two transformant isolates and *E. coli* wild type strain were tested. It was found that the *E. coli* strain originally has a

nematicidal properties. The suspension of *E. coli* cells caused 30 % mortality. The nematicidal property was increased up, on transformation, to cause 40 % and 60 % mortalities by isolate (2) and isolate (1), respectively. This may be due to the different structures of saponins produced by the different isolates. On the other hand, the cell- free culture filtrate caused 100 % mortality on nematodes (Salem, 1997).

Utility of Mi gene resistance to manage the root-knot nematode

The nematode resistance gene has been recently used from tomato, *Lycopersicon esculentum* (Milligan *et al.*, 1998). The Mi gene, used since the 1940s, when it was introgressed from its wild relative *L. peruvianum*. This gene is important because it confers resistance to four of the major root-knot nematode species (Williamson *et al.*, 1994; Brown *et al.*, 1997) and can be used to study the biochemistry of susceptible and resistant plants. The resistance conferred by this gene has some limitations, such as the presence of naturally occurring resistance breaking biotypes and decreased resistance at high soil temperature. Rich and Olson (1999) have conducted three field trials to determine the response of *M. javanica* to tomato cultivars containing the Mi gene for resistance in sequential tests. Cultivars with Mi gene were PSR991994 and Sanibed and susceptible cultivars were Colonial and Agriset 761. The resistant cultivars greatly suppressed root galling in the three tests in spring and fall, 1997 and spring, 1998 seasons. Population densities of second stage juveniles of the root- knot nematode also were low in soil samples collected from resistant cultivars. Tomato fruit yields, were significantly increased in one test only when using resistant cultivars. No evidence of resistance breaking biotypes of *M. javanica* was observed in spring and fall, in 1997.

Transfer gene(s) by protoplast fusion between Pseudomonas fluorescens and P. aeruginosa to manage Meloidogyne incognita

P. fluorescens has been found to have a potential of controlling root-knot nematode (Siddiqui and Shaukat, 2004). Also, *P. aeruginosa* has potential for controlling root-knot nematode (Larry and Manoil, 2001). The fusant strain between these two microorganisms (Psa: Psf) was used as soil drench or as seed soaking for controlling *M. incognita* infecting sunflower (*Helianthus annuus* L.). The fusant strain proved to be more effective than its parental strain, *P. aeruginosa* in reducing nematode parameters as well

as enhancement plant growth (El-Hamshary *et al.*, 2006). Zaid *et al.* (2009) found that the protoplast fusion between *Serratia* and *Pseudomonas* strains induced high mortality levels against nematodes when compared with parents which may be due to producing antibiotics, chitinolytic enzymes, chitinases and bacteriocin more than their parents. Alkelany (2016) reported that ten protoplast fusants with the same morphological characters as their parents were screened for their nematicidal efficacy against *M. javanica* J₂s and egg hatching. He found that the tested fusants were more effective than their parents in suppressing nematode reproduction and improving plant growth parameters. Also, one dose from the fusants was better than two doses from their parents.

Utility of nematode cell-specific genes to manage nematodes

The first sign of giant-cell induction by root-knot nematode is the formation of a binucleate cell (Jones, 1981; Melillo *et al.*, 2006) which are found associated with juveniles, 24 h after root infection, and become multinucleate within 48 h. Nuclei in the giant cells are enlarged and may contain 14-16 times more DNA than do normal root tip nuclei. Many genes expressed in nematode feeding cells, or the regulatory regions that control these genes have been isolated. Nematodes secrete signaling molecules that are thought to induce giant cells and syncytium development (Hussey, 1989; Williamson and Hussey, 1996). When the nematode-induced shortened cell cycles are regularized into normal ones, they would prevent the infected cells from forming into giant cells. If the feeding site becomes non-functional or absent, the nematode will die (Goverse *et al.*, 2000).

Utility of toxic proteins expressed in plant cells to manage nematodes

Transproteins toxic to nematodes, but not to plant can be expressed into tissues and cells fed upon by nematodes. Transgenic products with a potential to interfere with nematode physiology such as digestive enzymes or structural proteins of the intestine are considered here:

Proteinase inhibitors: Many defensive proteins are only made in significant quantities after a pathogen or pest has infected the plant. However, when defensive proteins and enzymes are activated, they effectively inhibit fungi, bacteria, nematodes, and insect herbivores (Freeman and Beattie, 2008). All living cells have a variety of proteinase inhibitors to

regulate their endogenous proteolytic activity. But, plant organs that accumulate these inhibitors are often protected from pests and parasites because these inhibitors bind strongly, sometimes irreversibly, to the active site of digestive proteinase enzymes (Ryan, 1990; Vrain, 1999). For example, proteinase inactivators in the diet of plant-parasitic nematodes probably bind to digestive proteinases in the gut to prevent protein hydrolysis and absorption of amino acids. The cyst nematodes entered and developed in the roots, but caused a shift in sex ratio with five times more males than females in the transgenic roots. The proteinase inhibitor changed the nutritional value of the plants, and their altered diet influenced the sexual fate of the cyst nematode juveniles. Root-knot nematodes developed normally, but females produced fewer eggs in the transgenic roots (Vrain, 1999). Michaud *et al.* (1996) found major cysteine proteinase activity in three species of root-knot nematodes. A cysteine proteinase inhibitor from rice, oryzacystatin 1 (OC1) completely inhibited the proteolytic activity of all stages of *M. hapla*. Rice, also produces OC11 and another cysteine which proved effective against *M. javanica* and *M. incognita*.

Cholesterol oxidase: *Bacillus thuringiensis* Bt is a common soil bacterium that accumulates large protein crystals when it sporulates. Bt proteins are ingested by insects on plant parts and they were dissolved in the insect midgut and are processed by digestive proteases into smaller polypeptides. These polypeptides bind to receptors and disrupt the insect midgut membranes (Vadlamudi *et al.*, 1995). As a result, osmotic balance is lost and the cells of the midgut membranes lose their function and the insects stop feeding and die. Several Bt strains have been found to produce polypeptides that kill bacterial-feeding nematodes (Borgonie *et al.*, 1996a, b, c; Feitelson *et al.*, 1992; Mena *et al.*, 1996, 1997). Toxicity of Bt strains against nematodes is similar to that with insects. On the other hand, Sampson and Gooday (1998) found that Bt subsp. *israelensis* IP576 and subsp. *aizawai* HD133 both secreted exochitinase enzyme when grown on a chitin medium. Also, Ismail *et al.* (2009) stated that the chitinolytic bacterium Bt mutant no. 24, which were produced by UV irradiation for different periods, exhibited the highest percentage mortality of nematode juveniles under laboratory conditions and number of galls and egg masses on sunflower infected by root knot nematode, *Meloidogyne incognita* under greenhouse conditions followed by mutants nos.

10 and 32. Ismail *et al.* (2010) obtained the same results on egg masses and juveniles under laboratory conditions and number of galls and egg masses under greenhouse. Eissa *et al.* (2010) and Abd El-Bary (2010) obtained the same results regarding the number of root knot nematode eggs under greenhouse and laboratory, respectively. Alkelany (2016) showed that chitinase gene from *B. subtilis* was cloned and expressed in *Escherichia coli*. Results showed that the supernatants of the transformed *E. coli* T.CHI-NRC-4 and T.CHI-NRC-6 resulted in 95 and 96% inhibition in egg hatching, respectively.

Lectins

Lectins are proteins that bind to carbohydrates with high specificity. These proteins accumulate in large quantities in many seeds and other storage organs of plants which may be important in transporting carbohydrates, cell wall elongation, cell-cell interactions or growth regulation. Lectins may recognize receptors in membranes, have enzymatic properties or may act as storage proteins and may also act as defense proteins because most lectins are toxic to animals, including insects and humans (Chrispeel and Raikhel, 1991; Peumans and van Damme, 1995). A mannose-binding lectin engineered in various crops is toxic to aphids, plant hoppers and several nematodes (Anwar and Mckenry, 1998; Boulter *et al.*, 1990; Burrows *et al.*, 1998).

Nematode neuropeptides

Warnock *et al.*, (2017) reported that exogenous peptides can be assimilated by nematodes by retrograde transport along the chemosensory amphid neurons. Peptides can accumulate within cells of the central nerve ring, and can elicit physiological effects when released to interact with receptors on adjoining cells. The same authors identified bioactive neuropeptides from the neuropeptide-like protein (NPL) from family of plant parasitic nematodes as novel nematicides, and have identified numerous discrete NLPs that negatively impact on chemosensation, behaviours that influenced host-finding and invasion of the root knot nematode, *Meloidogyne incognita* and the potato cyst nematode, *Globodera pallida*. Some soil-dwelling microbes as *Bacillus subtilis* which generate and secrete these neuropeptides into the soil were developed in the presence of nematode infective juveniles. As a result, these transgenic microbes could protect host plants from infection.

Novelty Statement

This review discussed and elucidated the most important trends and achievements in the field of genetic engineering in the management of plant parasitic nematodes, especially root-knot nematode.

Author's Contribution

MMAY suggested this concept supporting by the most important citations that can serve in that trend. SA-EH helped in collecting the most important citations and the two authors wrote and approved the review.

Conflict of interest

The authors have declared no conflict of interest.

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