Research Article

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

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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 nematicidal 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. *Escherchia 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 interefere with nematode physiology such as digestive enzymes or structural proteins of the intestine are considered.

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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. vinelandi* or *A. chroococcum*. Hence, bioengineering strategies are being developed that will provide specific and

durable resistance against plant-parasitic nematodes. These strategies come under three categories: (1) transfer of natural reistance 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 endoparsites, 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 reistance 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 harmfu l (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 foccuses on producing them on an economical scale.. The genes responsibe for producing saponins with nematicidal 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. vinelandi* 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 (Kofoid 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 nemticidal 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 filterate 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 industiral 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 has 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 x 10^{-4} haemolytic colonies (Salem, 1997).

Nematicidal activity of E. coli transformations on Meloidogyne incognita

The mortalizing actions of cell free culture filterate 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), respectivley. This may be due to the different structures of saponins produced by the different isolates. On the other hand, the cell- free culture filtlerate caused 100 % mortality on nematodes (Salem, 1997).

Utility of Mi gene resistance to mange 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 annus* L.). The fusant strain proved to be more effective than its parental strain, *P. aeruginosa* in reducing nematode parameters as well as enhacement 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 morohological characters as their parents were screened for their nematicidal efficacy against *M. javanica* J_2s 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 expressd 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 inhibotrs 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 protecolytic activity of all stages of *M. hapla*. Rice, also produces OC11 and another cystain which proved effect against *M. javanica* and *M. incognita*.

Cholestrol 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 disolved 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 laboratoty conditions and number of galls and egg masses on sunflower infected by root knot nematode, *Meloidohyne 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 eggmasses 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 *Escherichea 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 impotant in transporting carbohydrates, cell wall elogation,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.

References

Abd-Elbary, N.A., Eissa, M.F.M., Yassin, M.Y., Ismail, A.E., Badr, U.M. and Gaziea, S.M., 2010. Effect of a wild type and three different mutants of chitinolytic bacteria, *Bacillus thuringiensis* by U.V. irradiation on viability of *Meloidogyne incognita* eggs under laboratory conditions. J. Agric. Sci., Mansoura Univ., 32: 9217-9226.

Alkelany, U.S.F.M., 2016. Controlling of root-knot nematodes in eggplants using genetically improved bacteria. Ph.D. thesis, Faculty of Agriculture, Ain Shams University, Egypt, pp. 197.

Andrey, V., Chichkova, N., Lyakhov, I., Makarova, T., Evstafieva, A. and Bogdanov, A., 1992. Segments of *Escherichia coli* and genome similar to the axons of human prothymosin gene. Fed. Eur. Biochem. Soc. Lett., 213: 95-97. https://doi.org/10.1016/0014-5793(92)81420-Q

Anwar, S.A. and Mckenry, M.Y., 1998. Field performance of two genetically transformed grape rootstocks against two root-knot nematode populations. J. Nematol., 30: 486.

Barker, K.R., Lehman, P.S. and Huisingh, D., 1971. Influence of nitrogen and *Rhizopium japonicum* on the activity of *Heterodera glycines*. Nematologica, 17: 377-385. https://doi.org/10.1163/187529271X00602

Bendich, A.J. and Bolton, E.T., 1967. Relatedness among plants as measued by the DNA- agar technique. Plant Physiol., 42: 959-967. https://doi.org/10.1104/pp.42.7.959

Bonner, J.F., 1965. The molecular biology of development. Oxford Press, London and New York, pp. 155.

Borgonie, G., Claeys, M., Leyns, F., Arnaut, G., De Waele, D. and Coomans, A., 1996a. Effect of nematicidal *Bacillus thuringiensis* strains on free-living nematodes. 1. Light microscopic observations of resistant mutants of *Caenorhabditis elegans*. Fundam. Appl. Nematol.,19: 907-914.

Borgonie, G., Claeys, M., Leyns, F., Arnaut, G., De Waele, D. and Coomans, A., 1996b. Effect of nematicidal *Bacillus thurigiensis* strains on free living nematodes. 2. Ultrastruture analysis of the intoxication process in *Caenorhabiditis elegans*. Fundam. Appl. Nematol., 19: 407-414.

Borgonie, G., Claeys, M., Leyns, F., Arnaut, G., De Waele, D. and Coomans, A., 1996c. Effect of nematicidal *Bacillus thuringiensis* strains on free living nematodes. 3. Characterization of intoxication process in *Caenorhabiditis elegans*. Fundam. Appl. Nematol., 19: 323-328.

Boulter, D., Edwards, G.A., Gatehouse, A.M.R., Gatehouse, J.A. and Hildes, V.A., 1990. Additive protective effects of different plant-derived insect resistance genes in transgenic tobbaco plants. Crop Prot., 9: 351-354. https://doi.org/10.1016/0261-2194(90)90005-R

Brown, C.R., Mojtabedi, H., Santo, G.S. and Williamson, V.M., 1997. Effect of the Mi gene in tomato on reproductive factors of *Meloidogyne chitwoodi* and *M. hapla*. J. Nematol., 29: 416-418.

Burrows, P.R., Barker, A.D., Newell, C.A. and Hamilton, W.D., 1998. Plant- derived enzymes inhibitors and lectins for resistance against plant-parasitic nematodes in transgenic crops. Pestic. Sci., 52: 176-183. https://doi.org/10.1002/(SICI)1096-9063(199802)52:2<176::AID-PS680>3.0.CO;2-T

Chrispeel, M.J. and Raikhel, N.V., 1991. Lectins, lectin genes and their role in plant defense. Plant Cell, 3: 1-9. https://doi.org/10.1105/tpc.3.1.1

D'Addabbo, T., Carbonara, T., Leonetti, P., Radicci, V., Tava, A. and Avato, P., 2011. Control of plant parasitic nematodes with active saponins and biomass from *Medicago sativa*. Phytochem. Rev., 4: 503-509. https://doi.org/10.1007/s11101-010-9180-2

Denhardi, D.T. and Colastoni, J., 1987. A survey of vectors for regulating expression of closed DNA in *Escherichia coli*. In: Rodriguez, R.L. and Denhardt, D.T. (Eds.) Vectors: A survey of molecular cloning vectors and their uses. Butterworth Publishers. Stoneham, Massachusetts, pp. 179-204.

Doyle, J.J. and Doyle, J.L., 1987. Isolation of plant DNA from fresh tissue. Phytochem. Bull., 19: 11-15.

Eissa, M.F.M., Ismail, A.E., Abd-Elbary, N.A., Yassin, M.Y., Badr, U.M. and Gaziea, S.M., 2010. Control of *Meloidogyne incognita* using a wild type and three mutants of the chitinolytic bacteria, *Bacillus thuringiensis* under greenhouse conditions. Bull. Natl. Res. Center, 35: 139-149.

El-Hamshary, O.I.M., El-Nagdi, W.M.A. and Youssef, M.M.A., 2006. Genetical studies and antagonistic effects of a newly bacterial fusant against *Meloidogyne incognita*, root-knot nematode, and a plant pathogen *Fusarium oxysporum* infecting sunflower. Pak. J. Biotechnol., 3: 61-70.

Feitelson, J.S., Payne, J. and Kim, L., 1992. Bacilus thuringiensis. Insects Beyond Biotechnol., 10: 271-273. https://doi.org/10.1038/nbt0392-271

Freeman, B.C. and Beattie, G.A., 2008. An overview of plant defenses against pathogens and herbivores. The plant health instructor. https://doi.org/10.1094/PHI-I-2008-0226-01

Goverse, A., de Almeida, E.J., Vehees, J., van der Krol, S., Helder, J. and Gheysen, G., 2000. Cell cycle activation by plant parasitic nematode. Plant Mol. Biol., 43: 747-761. https://doi.org/10.1023/A:1006367126077

Hussey, R.S., 1989. Disease inducing secretions of plant-parasitic nematodes. Ann. Rev. Phytopathol., 27: 123-141. https://doi.org/10.1146/annurev.py.27.090189.001011

Ismail, A.E., Eissa, M.F.M., Abd-Elbary, N.A., Yassin, M.Y., Badr, U.M. and Gaziea, S.M., 2009. Influence of a wild type and three mutants of the chitinolytic bacteria, *Bacillus thuringiensis* by U.V. irradiation on *Meloidogyne incognita* juveniles under laboratory and greenhouse conditions. Pak. J. Nematol., 27: 383-392.

Ismail, A.E., Eissa, M.F.M., Abd-Elbary, N.A., Yassin, M.Y., Badr, U.M. and Gaziea, S.M., 2010. Impact of a wild type and its three mutants of the chitinolytic bacteria, *Bacillus thuringiensis* on egg-masses and infective juveniles of *Meloidogyne incognita* under laboratory and greenhouse conditions. Egypt. J. Agric. Res., 3: 1-10.

Jones, M.G.K., 1981. Host cell response to endoparasitic nematode attack: structure and function of giant cells and syncytia. Ann. Appl. Biol., 97: 353-372. https://doi.org/10.1111/j.1744-7348.1981.tb05122.x

Korayem, A.M. and Salem, H.H., 2000. Effect of saponin produced by genetically constructed microbial strains on root-knot nematodes. Egypt. J. Agronematol., 4: 105-110.

Larry, A.G. and Manoil, C., 2001. *Pseudomonas aeruginosa* PAOI kills *Caenorhabditis elegans* by cyanide poising. J. Bacteriol., 183: 6207-6214. https://doi.org/10.1128/JB.183.21.6207-6214.2001

Mena, J., Vazquez, R., Fernandez, M., Perez, L., Garcia, M., Pimentel, E., Lopez, A., Mencho, J.D., Zaldua, Z., Garcia, R., Somontes, D. and Moran, R., 1996. Use of *Bacillus thuringiensis* var. kurstaki to control *Meloidogyne incognita* and *Radopholus similis*. Centro Agricola, 23: 31-38.

Mena, J., Pimental, E., Vazquez, R., Fernandez, M., Garcia, M., Zaldua, Z., Somontes, D., Lopez, A., Gomez, M. and Mencho, J.D., 1997. Results of the use of *Bacillus thuringiensis* var. kurstaki in the control of *Radopholus similis* in banana and plantain plantations. Centro Agricola, 24: 41-49.

Michaud, D., Cantin, I., Bonade, B., Jouarim, L. and Vrain, T.C., 1996. Identification of stable plant cystain/nematode proteinas complexes using middly dematuring gelatin/polyacylamide gel electropheresis. Electrophoresis, 77: 1378-1379. https://doi.org/10.1002/elps.1150170816

Milligan, S.B., Bodeau, J., Yagboobi, J., Kaloshian, I., Zabel, P. and Williamson, Y.M., 1998. The root-knot resistance gene Mi from tomato is a member of the leucine zipper, nucleotide binding, Lecino-rich repeat family of plant genes. Plant Cell, 10: 1307-1321. https://doi.org/10.2307/3870642

Melillo, M.T., Leonetti, P., Bongiovanni, M., Castagnone-Sereno, P. and Bleve-Zacheo, T., 2006. Modulation of ROS activities and H_2O_2 accumulation during compatible and incompatible tomato/root-knot nematode interactions. New Phytol., 170: 501-512. https://doi.org/10.1111/j.1469-8137.2006.01724.x

Peumans, W.J. and van Damme, E.J.M., 1995. Lectins as plant defence proteins. Plant Physiol., 109: 347-352. https://doi.org/10.1104/pp.109.2.347

Poinar, Jr G.O., 1993. Control of nematodes. In: Library of congress cataloging in publication data. The natural history of nematodes, Eng-Wood Cliffr, New Jersy. pp. 289-298.

Rich, J.R. and Olson, S.M., 1999. Utility of Mi gene resistance in tomato to mange *Melodigoyne javanica* in North Florida. J. Nematol., 31: 715-718.

Ryan, C.A., 1990. Proteinase inhibitors in plants. Genes for improving defenses against insects and pathogens. Annu. Rev. Phytopathol., 28: 425-449. https://doi.org/10.1146/annurev.py.28.090190.002233

Salem, H.H., 1997. Study of the biological control of billarzian snails and soil nematodes using a genetically constructred microbial strain. M. Sc. thesis, Fac. Sci., Zagazig Univ., Egypt. pp. 123.

Sampson, M. and Gooday, G.W., 1998. Involvement of chitinase of *Bacillus thuringiensis* during pathogenesis of insects. Microbiology, 144: 2189-2194. https://doi.org/10.1099/00221287-144-8-2189

Siddiqui, I.A. and Shaukat, S.S., 2004. Systemic resistance in tomato induced by biocontrol bacteria against roo-knot nematode *Meloidogyne javanica* is independent of salicylic acid production. J. Phytopathol., 152: 48-54. https://doi.org/10.1046/j.1439-0434.2003.00800.x

Small, E., Jurzysta, M. and Nozzo, C., 1990. The evaluation of hemolytic saponins content in wild and cultivated alfalfa. Econ. Bot., 44: 226-235. https://doi.org/10.1007/BF02860488

Vadlamudi, R.K., Weber, E., Ji, I., Ji, T.H. and Bulla, L.A., 1995. Cloning and expression of a receptor for an insecticidal toxin of *Bacillus thuringiensis*. J. Biol. Chem., 270: 5940-5994. https://doi.org/10.1074/jbc.270.10.5490

Vrain, T.C., 1999. Engineering natural and synthetic resistance for nematode management. J. Nematol., 31: 424-436.

Warnock, N.D., Wilson, L., Patten, C., Fleming, C.C., Maule, A.G., Dalzell, J.J., 2017. Nematode neuropeptides as transgenic nematicides. PLoS Pathog., 13: e1006237. https://doi.org/10.1371/journal.ppat.1006237

Williamson, V.M. and Hussey, R.S., 1996. Nematode pathogenesis and resistance in plants. Plant Cell, 8: 1735-1745. https://doi.org/10.1105/tpc.8.10.1735

Williamson, V.M., Lamberti, K.N. and Kaloshian, I., 1994. Molcular biology of nematode resistance in tomato. In: Lamerti, F., De Giorgi, C. and Bird, D.N. (Eds). Adv. Mol. Plant Nematol., New York: Plenum Press, pp. 211- 219. https://doi.org/10.1007/978-1-4757-9080-1_18

Zaid, K.A., Kawther, S., Kash, S., Ibrahim, A. and Tawfik, T.M., 2009. Improving nematicidal activity of bacteria via protoplast. Aust. J. Basic Appl. Sci., 3: 1412-1427.