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Journal of Botanical Research
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REVIEW

The Use of RNA Interference in Enhancing Plant Resistance against Nematodes

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ARTICLE INFO

Article history
Received: 17 March 2020
Accepted: 8 April 2020
Published Online: 30 April 2020

Keywords:
Plant parasitic nematodes
RNAi
dsRNA
Resistance

ABSTRACT

Plant-parasitic nematodes caused severe yield loss in major crops all over the world. The most wild-used strategies to combat the nematodes is the chamical nematicides, but the overuse of synthetic nematicides threaten sustainable agriculture development. Other strategies, like resistance cultivars and crop rotation, have limited efficiency. Thus, the utilization of molecular biotechnology like RNA interference (RNAi) would be one of the alternative ways to enhance plant resistance against nematodes. RNAi has already used as a tool for gene functional analysis in a wide range of species, especially in the non-parasitic nematode, *Caenorhabtidis elegans*. In plant-parasitic nematodes, RNAi is induced by soaking nematodes with double strand RNA(dsRNA) solution mixed with neurostimulants, which is called *in vitro* RNAi delivery method. In another way around, *in planta* RNAi method, which is Host-mediated RNAi approach also showed a great success in conferring the resistance against root-knock nematodes. Two main advantages of RNAi-based transgenics are RNAi technology do not produce any functional foreign proteins and it target organisms in a sequence-specific way. Even though the development of RNAi-based transgenics against plant-parasitic nematodes is still in the initial phase, it offers the prospect into a novel nematode control strategy in the future.

1. Introduction

Plant Parasitic Nematodes (PPN) are one of the significant constraints for crop production worldwide. Up to now, more than 4,100 species of PPNs have been recorded [24], causing about $173 billion of damage to world agriculture every year [14]. However, this damage is likely to be underestimated because many growers are not aware of these parasites in the soil [1]. The symptoms caused by PPNs are easily confused with the symptoms of other pathogens. Sedentary endoparasites such as root-knot nematodes (*Meloidogyne spp*.) and cyst nematodes (*Globodera* and *Heterodera spp*.) are the most damaging PPNs [24]. Even though the life cycles of these nematodes are different from each other, both cyst nematodes and root-knot nematodes inject their saliva into plant cells [28]. The cyst nematodes withdraw nutrients through feeding sites called syncytia, while root-knot nematodes form giant cell to take nutrients from the host. After nematode infection, plants are slowly damaged by the interruption

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DOI: https://doi.org/10.30564/jrb.v2i1.1759

of the water transportation and nutrients from the roots to the branches. In addition, the plants also become more susceptible to other diseases [15]. Also, migratory endoparasites are highly damaging as these endoparasites interact with other pathogens to enhance crop damage and yield loss [24].

Many methods can be applied to control PPNs, such as resistant cultivars, nematicides and crop rotation. However, many crops lack nematode resistance genes, and crop rotations are inadequate to manage PPNs while nematicides have adverse environmental effects [15]. Under the circumstances, defending the plant against the PPN with novel strategies such as RNA interference (RNAi) is necessary. RNAi refers to the phenomenon of efficient and specific degradation of homologous mRNA induced by double-stranded RNA (dsRNA). Fire et al. [18] first described the mechanism of RNAi using the nematode *Caenorhabditis elegans*, and a similar mechanism has been revealed in different species and kingdoms. If the sequence of the genes which are essential for PPN infection, feeding or reproduction is known, RNAi could be used as a powerful tool to enhance the resistance in plants against nematodes by silencing those pathogenic genes.

The objective of this paper is to review the research published on the use of RNAi in enhancing plant resistance against Plant-Parasitic Nematodes. The first part of this review introduces the mechanisms of RNAi. The second section presents two ways of inducing RNAi, *in vitro* RNAi and *in planta* RNAi. In *in vitro* RNAi method, the dsRNA is delivered into worms directly, while the dsRNA is transferred to the plant in *in planta* RNAi method. The third section discusses the problems of enhancing the resistance conferred by *in planta* RNAi and their solution. In this section, the off-target effect, non-specific target effect and the dilemma of RNAi phenotype detection are discussed. This review aims to identify the prospect of RNAi being utilised as a powerful tool to develop transgenic plants against PPNs. The research papers included in this review are the result of a search in the online literature database at scopus.com using the keywords ‘RNAi’, ‘nematode’, ‘resistance’, and the reference is limited to ‘not older than 2008’.

1.1 Mechanism of RNAi

RNAi refers to the phenomenon of efficient and specific degradation of homologous mRNA induced by double-stranded RNA (dsRNA). Fire et al. [18] firstly described this post-transcriptional silencing of endogenous genes in *Caenorhabditis elegans*. RNAi also is important in the immune response to foreign genetic material [49]. When an organism recognises dsRNA, a cascade of sequence-specific silencing processes will initiate leading to the dsRNA and its homologous mRNA degraded [47]. The RNAi pathway can be considered a two-step process. Firstly the dsRNA would be cleaved into 21–25 nucleotide long small interfering RNAs (siRNAs) in the cell cytoplasm by the RNaseIII enzyme, Dicer. The separation of double-strand siRNA in the second step allows the siRNA to attach to the RNA-induced silencing complex (RISC). These RISC then target sequence-complementary mRNA molecules and cleave them, inducing RNA silencing [41]. Similar mechanisms of action appear to function in PPNs as well [25], so RNAi can be used to develop RNAi against PPNs.

1.2 *In vitro* RNAi Delivery Method

Three different methods have been successfully applied to introduce dsRNA into the free-living nematodes. The three ways are microinjection [33], feeding the nematodes with bacteria which contain dsRNA of the target gene [52] and soaking of nematodes in dsRNA solution [50]. However, the sedentary PPNs are too small for microinjection. They are obligatory parasitic so that feeding with bacteria cannot be used to introduce dsRNA into these worms. The sedentary PPNs only can survive outside of their host plant when they are at the stage of eggs, second stage juveniles (J2s) and adult males. Eggs and J2s stages have been used for transmitting RNAi by soaking.

The most commonly used RNAi delivery way for J2s of PPNs is neurostimulant-mediated oral ingestion. Chemicals such as octopamine, serotonin and resorcinol are used in the solution to promote dsRNA ingestion. These chemicals successfully induce dsRNA ingestion in several species including *Heterodera schachtii* [53], *Meloidogyne javanica* [19] and *Heterodera glycines* [6]. Fluorescein isothiocyanate (FITC) in the solution or fluorescently-labelled dsRNAs have been used as a visual marker to show the uptake of dsRNA [12]. Although pharyngeal stimulant can induce ds RNA ingestion efficiently, some side effects have also been reported during incubation. Adam, Phillips, Jones, and Blok [2] found that the death rate of *Meloidogyne javanica* J2s would increase significantly if they were soaked in 0·5% or 1% resorcinol solution overnight.

Soaking eggs in dsRNA or siRNA solution is another way to induce RNAi in PPNs [10]. Fanelli, Di Vito, Jones, and De Giorgi [16] constructed the dsRNA targeting a chitin synthase which is responsible for chitin layer synthesis in the eggshell. After dsRNA construction, the eggs of *Meloidogyne artiellia* were soaked in dsRNA solution at 20°C for between 24 and 72 h. This result of a reduction in chitin transcript illustrated that the dsRNA could go through the eggshells with chitin. More recently, Dalzell et al. [10] performed a siRNA-mediated RNA silencing in

Meloidogyne incognita. They silenced the nuclear RNase III enzyme Drosha, a key effector of miRNA production by soaking *Meloidogyne incognita* eggs in siRNA solutions. The irregular growth of eggs and embryonic lethality suggested that eggshells of *Meloidogyne incognita* are permeable to siRNA. When it comes to other species of PPN, whether the eggs are similarly susceptible to dsRNA or siRNA still not clear, because the permeability of the PPNs eggshells would change before hatching, according to [23].

As migratory parasites are not obligate biotrophic, they can be targeted by RNAi at other stages of the life-cycle. Haegeman, Vanholme, and Gheysen [21] incubated different stages of *Radopholus similis* with dsRNA with a sequence derived from the xylanase gene. Octopamine was used in this research to stimulate dsRNA ingestion. A reduction in the transcript of the xylanase gene and reduction of infection rates were observed at the end. Cheng, Dai, Xie, and Xiao [9] found that 24h of incubation was necessary for ingestion of FITC soaked *Bursaphelenchus xylophilus*, but a pharyngeal stimulant was not required in this research. L2-L3 larvae of migratory parasites also can be induced RNAi by soaking, but intestinal microinjection of dsRNA into adult female is efficient as it leads to a 46% lethality rate in the F1 generation while soaking in dsRNA solution only induced 25% lethality [36].

Generally speaking, the *in vitro* RNAi delivery method could be considered efficient to manipulate PPNs pathogenicity. However, the silencing achieved due to soaking in dsRNA solutions is often lacking stability. Rosso, Dubrana, and Cimbolini [40] accessed the duration of the silencing effect. They silenced the gene which encodes polygalacturonase (*Mi-pg-1*) in *Meloidogyne incognita* by *in vitro* RNAi technology. The suppression of *Mi-pg-1* transcription was optimal at 44 h after soaking, but this silencing effect cannot be detected for both at 68 h after soaking. Bakhetia, Urwin, and Atkinson [4] observed that the reduction in the transcript levels of β-1, 4-endoglucanase In *Heterodera glycines* after an incubation of 16 h with the corresponding dsRNA. However, after 15 days of dsRNA treatment, they reported that the transcript levels of β-1, 4-endoglucanase returned to normal. In contrast to *in vitro* RNAi technology, host delivery strategy would prevent gene suppression reversal because the transgenic plants provide continuous availability of the dsRNA to the nematode [54]. In addition, *in vitro* method only can be used in the lab for proof of RNAi principle and test constructs before transferring dsRNA into the plant. Therefore, the *in planta* RNAi method is more suitable for enhancing plants resistant to PPNs.

1.3 *In planta* RNAi Method

Host-mediated RNAi is a novel approach to confer resistance against PPNs in plants. In this technology, plants are genetically modified to express dsRNA molecules with sequence derived from the target gene. RNAi constructs are made by cloning the sense and antisense cDNA sequences of the target gene. In order to initiate the expression of the dsRNA, a tissue-specific or constitutive promoter is used during vector construction. After transcription, a loop or hairpin structure is formed by the self-complementary sense and antisense strand. Then dsRNA with a loop or hairpin structure is cleaved by the plant enzyme called Dicer into siRNA, and the siRNA is ingested by the nematodes subsequently. This dsRNA can also directly be ingested by nematodes [13].

Using the conventional methods to develop dsRNA constructs for host delivered RNAi is time- consuming, so the Gateway cloning system, which is much easier and effective, has been employed for the development of RNAi constructs [35]. The Gateway cloning system uses two different enzyme mixtures, each of which performs a different type of recombination reaction [39]. The BP clonase enzyme mix recombines the *attB* sites and the *attP* sites, generating *attL* and *attR* sites. On the contrary, the LR clonase enzyme mixes catalysis the reverse reaction (Figure 1).

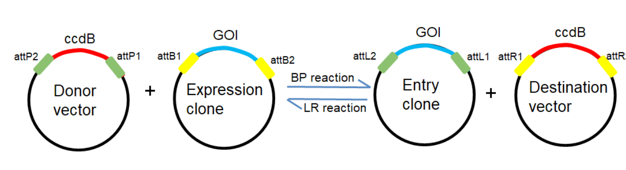

Figure 1. The mechanism of BP reaction and LR reaction

Initially, the gene of interest is amplified by PCR using long-tailed primers. The 3' part of the primers is specific for the DNA of interest, and the 5' tail of the primers containing appropriate *attB* sites. This PCR product is transferred into a Donor vector. The gene of interest in a donor vector is available for transfer into destination vectors by a Gateway LR cloning reaction. The vectors containing the gene of interest are introduced into plants through *Agrobacterium tumefaciens* mediated transformation. With the developing of experimental techniques, the Gateway cloning system becomes compatible to include RNA interference. Rual et al. [42] reported that completed *Caenorhabditis elegans* genome sequence allows application of high-throughput (HT) approaches for phenotypic analyses using RNAi, but HT-RNAi resources are limited by lack of flexibility. In order to overcome this disadvantage, they created the complete set of ORFs in genome

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DOI: https://doi.org/10.30564/jrb.v2i1.1759

(ORFeome) resources of the nematode *Caenorhabditis elegans* through the Gateway cloning system. Then they showed these ORFeome resources could be used to create alternative HT-RNAi resources with enhanced flexibility. This flexibility suggests that additional HT-RNAi libraries can be generated to perform gene knockdowns under various conditions, which increase the possibilities for phenome mapping of *Caenorhabditis elegans*.

The genes which are targeted in the *in planta* RNAi method could be classified into three categories: effector genes, housekeeping genes and genes implicated in nematode development or reproduction. The first demonstration of plant-mediated RNAi was accomplished by Yadav, Veluthambi, and Subramaniam [55]. The dsRNA they developed targets two housekeeping genes: an integrase and a splicing factor gene of *Meloidogyne incognita*. The results show a decline of more than 90% of PPN infection in transgenic tobacco as the expression of the targeted gene was repressed. Klink et al. [26] reported that four embryonic lethal genes encoding ribosomal protein 3a and 4, spliceosomal SR protein and synaptobrevin could be used for PPNs control. They silenced these four genes by using *in planta* RNAi method resulting in a restriction of the female development of *Heterodera glycines* in their host. Another example of silencing housekeeping genes is given by J. Li, Todd, and Trick [30]. The *Heterodera glycines Y25* gene encoding a β-subunit of the coatomer (COPI) complex, was used as a template to synthesise dsRNA. The transgenic soybean expressing RNAi constructs effectively suppressed *Heterodera glycines* infection and development. When splicing factor and integrase genes in *Meloidogyne incognita* were used for RNA interference in *Arabidopsis thaliana*, a dramatic decrease in the number of galls, females and egg masses was observed by Kumar et al. [27]. Based on the examples mentioned above, housekeeping genes could be considered as a good choice for RNAi targeting. Before the development of dsRNA targeting housekeeping genes, a risk assessment is always needed because most of these genes are highly conserved across different species and the dsRNA constructs could have non-specific effects on host plants or other beneficial species [46].

Some literature indicates that nematode effector genes can also confer resistance against PPNs when targeted by host-mediated RNAi. Dinh, Brown, and Elling [11] constructed dsRNA molecules which are complementary to the effector gene, *Mc16D10L*, and these dsRNA molecules were transferred into three potato cultivars. The number of *Meloidogyne chitwoodi* eggs and egg masses in transgenic potato was reduced by up to 68% compared to empty vector control plants. Recently, Niu et al. [34] found that the effector gene *MiMSP40* of *Meloidogyne incognita* could downregulate plant immunity to help parasitism. Overexpression of *MiMSP40* makes Arabidopsis more susceptible, but *in planta* RNAi targeting against this gene caused a reduction in parasitism in Arabidopsis. Similarly, Zhuo et al. [56] reported that overexpression of effector gene *MeTCTP* in *Meloidogyne enterolobii* promotes infection, but host generated RNAi aiming at *MeTCTP* resulted in less parasitism.

The genes involved in nematode development and reproduction are also used to inhibiting the growth and reproductive nematodes. In the case of cyst nematodes, silencing the major sperm protein (MSP) by RNAi leads to a 68% reduction in the number of eggs [48]. The impaired fecundity of *Heterodera glycines* carried over to the next generation of nematodes regarding the ability to reproduce declined in their progenies. Two genes from *Meloidogyne incognita*, a dual oxidase gene and signal peptidase gene, were revealed to be of relevance to the development and reproduction of root-knot nematode [8]. Silencing of these two genes led to more than 50% reduction in nematode numbers in the roots and diapause of the egg-producing female. Antonino et al. [3] found that serine protease in *Meloidogyne incognita* is relevant to root-knot nematode reproduction. In their study, they knocked-down serine protease gene *Mi-ser-1* by RNAi. Nematodes that infected modified tobacco produced fewer eggs, and the progeny of nematodes matured in these plants has a declined success in egg hatching. This result indicated that serine protease is involved in different processes during nematode development, like reproduction and embryogenesis.

2. The Problems of Enhancing the Resistance by RNAi and Their Solution

2.1 Off-target Effects and Their Solution

RNAi has emerged as a potent and successful technology for crop protection in recent years, but there remain certain limitations that need to be addressed before adopting this technology in the field. One major concern regarding the employment of RNAi-based nematode management strategy is the potential for off-target effects. Non-target genes with similar sequence to the target gene are likely to be silenced by mRNA degradation or translational repression as the basis of RNAi is sequence identity [32]. J. F. Rual, Klitgord, and Achaz [43] reported that when an mRNA shares more than 95% identity over 40 nucleotides with the dsRNA in nematode *Caenorhabditis elegans*, the off-target effects will happen.

Due to the issues of off-target effects, more emphasis should be put on avoiding the off-target effects when

RNAi is employed as a novel method in PPNs management. Some strategies could be used to prevent off-target effects. Recently Banerjee, Gill, Jain, and Sirohi [7] reported that using a database for *in silico* homology searches to identify off-target sequences is efficient in avoiding off-target effects. Thorat [51] used nematode-induced promoter and root-specific promoter of *Arabidopsis thaliana* to transform tomato with GUS gene. Then they observed a strong expression of the GUS gene in the nematode infection sites. Therefore the root-specific promoters and nematode inducible promoters could be used to prevent RNAi in the non-target parts of the plant. In addition, sequences of the 5'-untranslated region (5'-UTR) and the 3'-untranslated region (3'-UTR) also could be used as RNAi targets to reduce off-target effects because they are less conserved than coding regions [20].

2.2 The confirmation of RNAi effectiveness

Even though several studies reported the success of RNAi phenotype detection, it is still difficult to determine if RNAi results in the phenotype or not [45]. Most commonly, detection of the RNAi phenotype is based on the number of nematodes which establish an infection successfully, the fecundity of females or the ratio of males to females in cyst nematodes. However, under some unfavourable conditions such as insufficient nutrition, the process of establishing a feeding site is slower (Lilley, Atkinson, & Urwin, 2005). RNAi targeting the genes which express in pharyngeal gland cells of *Heterodera glycines* would lead to more males [5]. Because the number of phenotypes which can be observed is easily affected by other factors, some phenotypes changes would not be appeared or overserved [31].

RT-PCR has been used to overcome the difficulties of RNAi phenotyping. This method would reveal a decline in transcript level of the target gene. Patel et al. [37] reported that the presence of dsRNA could be detected by RT-PCR of the intron or spacer region of the hairpin construct. Other studies have used RT-PCR to illustrate the decline of control gene transcripts in dsRNA expressing plants [22]. However, this method may not be the suitable for confirmation of RNAi effectiveness, because RT-PCR has not always detected the down-regulation of the target gene despite observing an RNAi phenotype in the nematodes [36].

2.3 Conclusion and Future Prospects

RNAi was used as a successful technology for enhancing crops resistance to different pathogens in recent years, but there remain some limitations that impede the commercialisation of RNAi-modified plants. Usually, RNAi-modified crops are only resistant to one nematode species because of the high specificity level of dsRNA or siRNA. However, plants are infected with multiple species of PPN in soils [17]. In the meantime, the public suspicions on biosafety aspects of RNAi-based GM plants have widespread even though the risk assessment of GM crops is obligating before releasing them to market. Many people think the risk assessment of commercialised GM plants is not suitable for RNAi-based GM plants, because the current risk assessment does not include the identification of potential off-target effects. These problems remind us that we still have a way to go in the future due to the acceptance of that technology by the general public

Hopefully, the rapid growth of RNAi research will help to increase public trust in this technology. Development genome databases of related species and the stacking of dsRNA sequences to target multiple genes would contribute to effective nematode control [28]. Use of nematode-induced promoters and plant tissue-specific promoters would limit dsRNA gene expression to specific plant tissue in response to the particular nematode [44]. More efficient algorithmic programs facilitate more reliable risk assessment of RNAi-based GM plants [38].

In conclusion, some problems and limitation have to be taken into consideration in order to ensure RNAi successful and safe application. Nevertheless, RNAi surely will become a powerful strategy to control multiple pest and pathogens as we are moving toward the goal of sustainable development.

Reference

[1] Abd-Elgawad, M. M., & Askary, T. H.. 1 Impact of Phytonematodes on Agriculture Economy. In Biocontrol Agents of Phytonematodes, CABI Wallingford, UK, 2015: 3-49.

[2] Adam, M. A. M., Phillips, M. S., Jones, J. T., & Blok, V. C.. Characterisation of the cellulose-binding protein Mj-cbp-1 of the root knot nematode, Meloidogyne javanica. Physiological and Molecular Plant Pathology, 2008, 72(1-3): 21-28.
DOI: 10.1016/j.pmpp.2008.05.002

[3] Antonino, Coelho, R. R., Lourenço, I. T., da Rocha Fragoso, R., Viana, A. A. B., de Macedo, L. L. P., de Almeida-Engler, J.. Knocking-down Meloidogyne incognita proteases by plant-delivered dsRNA has negative pleiotropic effect on nematode vigor. PLoS One, 2013, 8(12): e85364.

[4] Bakhetia, Urwin, & Atkinson. qPCR analysis and RNAi define pharyngeal gland cell-expressed genes of Heterodera glycines required for initial interactions with the host. Molecular Plant-Microbe Interac-

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tions, 2007, 20(3): 306-312.

[5] Bakhetia, M., Urwin, P., & Atkinson, H. J.. qPCR analysis and RNAi define pharyngeal gland cell-expressed genes of Heterodera glycines required for initial interactions with the host. Molecular Plant-Microbe Interactions, 2007, 20(3): 306-312.

[6] Bakhetia, M., Urwin, P. E., & Atkinson, H. J.. Characterisation by RNAi of pioneer genes expressed in the dorsal pharyngeal gland cell of Heterodera glycines and the effects of combinatorial RNAi. Int J Parasitol, 2008, 38(13): 1589-1597.
DOI: 10.1016/j.ijpara.2008.05.003

[7] Banerjee, S., Gill, S. S., Jain, P. K., & Sirohi, A.. Isolation, cloning, and characterization of a cuticle collagen gene, Mi-col-5, in Meloidogyne incognita. 3 Biotech, 2017, 7(1): 64.
DOI: 10.1007/s13205-017-0665-1

[8] Charlton, W. L., Harel, H. Y., Bakhetia, M., Hibbard, J. K., Atkinson, H. J., & McPherson, M. J.. Additive effects of plant expressed double-stranded RNAs on root-knot nematode development. Int J Parasitol, 2010, 40(7): 855-864.
DOI: 10.1016/j.ijpara.2010.01.003

[9] Cheng, X.-Y., Dai, S.-M., Xie, B.-Y., & Xiao, L.. Influence of cellulase gene knockdown by dsRNA interference on the development and reproduction of the pine wood nematode, Bursaphelenchus xylophilus. Nematology, 2010, 12(2): 225-233.
DOI: 10.1163/138855409x12469541205044

[10] Dalzell, J. J., McVeigh, P., Warnock, N. D., Mitreva, M., Bird, D. M., Abad, P., . . . Maule, A. G.. RNAi effector diversity in nematodes. PLoS Negl Trop Dis, 2011, 5(6): e1176.
DOI: 10.1371/journal.pntd.0001176

[11] Dinh, P. T., Brown, C. R., & Elling, A. A.. RNA interference of effector gene Mc16D10L confers resistance against Meloidogyne chitwoodi in Arabidopsis and potato. Phytopathology, 2014, 104(10): 1098-1106.

[12] Dutta, T. K., Papolu, P. K., Banakar, P., Choudhary, D., Sirohi, A., & Rao, U.. Tomato transgenic plants expressing hairpin construct of a nematode protease gene conferred enhanced resistance to root-knot nematodes. Front Microbiol, 2015, 6(260). DOI: 10.3389/fmicb.2015.00260

[13] Dutta, T. K., Papolu, P. K., Banakar, P., Choudhary, D., Sirohi, A., & Rao, U.. Tomato transgenic plants expressing hairpin construct of a nematode protease gene conferred enhanced resistance to root-knot nematodes. Frontiers in Microbiology, 2015, 6(260).
DOI: 10.3389/fmicb.2015.00260

[14] Elling, A. A.. Major emerging problems with minor Meloidogyne species. Phytopathology, 2013, 103(11): 1092-1102.

[15] Fairbairn, D. J., Cavallaro, A. S., Bernard, M., Mahalinga-Iyer, J., Graham, M. W., & Botella, J. R.. Host-delivered RNAi: an effective strategy to silence genes in plant parasitic nematodes. Planta, 2007, 226(6): 1525-1533.

[16] Fanelli, E., Di Vito, M., Jones, J. T., & De Giorgi, C.. Analysis of chitin synthase function in a plant parasitic nematode, Meloidogyne artiellia, using RNAi. Gene, 2005, 349: 87-95.
DOI: 10.1016/j.gene.2004.11.045

[17] Ferguson, C. M., Barratt, B. I., Bell, N., Goldson, S. L., Hardwick, S., Jackson, M., Rennie, G.. Quantifying the economic cost of invertebrate pests to New Zealand's pastoral industry. New Zealand Journal of Agricultural Research, 2018: 1-61.

[18] Fire, A., Xu, S., Montgomery, M. K., Kostas, S. A., Driver, S. E., & Mello, C. C.. Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. nature, 1998, 391(6669): 806.

[19] Gleason, C. A., Liu, Q. L., & Williamson, V. M.. Silencing a candidate nematode effector gene corresponding to the tomato resistance gene Mi-1 leads to acquisition of virulence. Molecular Plant-Microbe Interactions, 2008, 21(5): 576-585.

[20] Gu, S., Jin, L., Zhang, F., Sarnow, P., & Kay, M. A.. Biological basis for restriction of microRNA targets to the 3′ untranslated region in mammalian mRNAs. Nature structural & molecular biology, 2009, 16(2), 144.

[21] Haegeman, A., Vanholme, B., & Gheysen, G.. Characterization of a putative endoxylanase in the migratory plant-parasitic nematode Radopholus similis. Mol Plant Pathol, 2009, 10(3): 389-401.
DOI: 10.1111/j.1364-3703.2009.00539.x

[22] Ibrahim, H. M., Alkharouf, N. W., Meyer, S. L., Aly, M. A., Gamal El-Din Ael, K., Hussein, E. H., & Matthews, B. F.. Post-transcriptional gene silencing of root-knot nematode in transformed soybean roots. Exp Parasitol, 2011, 127(1): 90-99.
DOI: 10.1016/j.exppara.2010.06.037

[23] Jones, Tylka, G., Perry, R., & Wright, D.. The Physiology and biochemistry of free-living and plant-parasitic nematodes. In: CABI Publishing London,1998.

[24] Jones, J. T., Haegeman, A., Danchin, E. G., Gaur, H. S., Helder, J., Jones, M. G., Wesemael, W. M.. Top 10 plant-parasitic nematodes in molecular plant pathology. Molecular Plant Pathology, 2013, 14(9): 946-961.

[25] Jones, L. M., Giorgi, C. D., & Urwin, P. E.. C.

elegans as a Resource for Studies on Plant Parasitic Nematodes. In J. Jones, G. Gheysen, & C. Fenoll (Eds.), Genomics and Molecular Genetics of Plant-Nematode Interactions. Dordrecht: Springer Netherlands, 2011: 175-220.

[26] Klink, V. P., Kim, K. H., Martins, V., Macdonald, M. H., Beard, H. S., Alkharouf, N. W., Matthews, B. F.. A correlation between host-mediated expression of parasite genes as tandem inverted repeats and abrogation of development of female Heterodera glycines cyst formation during infection of Glycine max. Planta, 2009, 230(1): 53-71.
DOI: 10.1007/s00425-009-0926-2

[27] Kumar, A., Kakrana, A., Sirohi, A., Subramaniam, K., Srinivasan, R., Abdin, M. Z., & Jain, P. K.. Host-delivered RNAi-mediated root-knot nematode resistance in Arabidopsis by targeting splicing factor and integrase genes. Journal of General Plant Pathology, 2017, 83(2): 91-97.
DOI: 10.1007/s10327-017-0701-3

[28] Li, J., Todd, T. C., Lee, J., & Trick, H. N.. Biotechnological application of functional genomics towards plant-parasitic nematode control. Plant biotechnology journal, 2011, 9(9): 936-944.

[29] Li, J., Todd, T. C., Oakley, T. R., Lee, J., & Trick, H. N.. Host-derived suppression of nematode reproductive and fitness genes decreases fecundity of Heterodera glycines Ichinohe. Planta, 2010, 232(3): 775-785.
DOI: 10.1007/s00425-010-1209-7

[30] Li, J., Todd, T. C., & Trick, H. N.. Rapid in planta evaluation of root expressed transgenes in chimeric soybean plants. Plant Cell Rep, 2010, 29(2): 113-123.
DOI: 10.1007/s00299-009-0803-2

[31] Lilley, C. J., Bakhetia, M., Charlton, W. L., & Urwin, P. E.. Recent progress in the development of RNA interference for plant parasitic nematodes. Mol Plant Pathol, 2007, 8(5): 701-711.
DOI: 10.1111/j.1364-3703.2007.00422.x

[32] Meister, G., & Tuschl, T.. Mechanisms of gene silencing by double-stranded RNA. nature, 2004, 431(7006): 343.

[33] Mello, C. C., & Conte Jr, D.. Revealing the world of RNA interference. nature, 2004, 431(7006): 338.

[34] Niu, J., Liu, P., Liu, Q., Chen, C., Guo, Q., Yin, J., Jian, H.. Msp40 effector of root-knot nematode manipulates plant immunity to facilitate parasitism. Sci Rep, 2016, 6: 19443.
DOI: 10.1038/srep19443

[35] Papolu, P. K., Gantasala, N. P., Kamaraju, D., Banakar, P., Sreevathsa, R., & Rao, U.. Utility of host delivered RNAi of two FMRF amide like peptides, flp-14 and flp-18, for the management of root knot nematode, Meloidogyne incognita. PLoS One, 2013, 8(11): e80603.

[36] Park, J.-E., Lee, K. Y., Lee, S.-J., Oh, W.-S., Jeong, P.-Y., Woo, T., . . . Koo, H.-S.. The efficiency of RNA interference in Bursaphelenchus xylophilus. Molecules & Cells (Springer Science & Business Media BV), 2008, 26(1).

[37] Patel, N., Hamamouch, N., Li, C., Hewezi, T., Hussey, R. S., Baum, T. J., . . . Davis, E. L.. A nematode effector protein similar to annexins in host plants. J Exp Bot, 2010, 61(1): 235-248.
DOI: 10.1093/jxb/erp293

[38] Ramon, M., Devos, Y., Lanzoni, A., Liu, Y., Gomes, A., Gennaro, A., & Waigmann, E.. RNAi-based GM plants: food for thought for risk assessors. Plant biotechnology journal, 2014, 12(9): 1271-1273.

[39] Reboul, J., Vaglio, P., Tzellas, N., Thierry-Mieg, N., Moore, T., Jackson, C., Thierry-Mieg, J.. Open-reading-frame sequence tags (OSTs) support the existence of at least 17,300 genes in C. elegans. Nature genetics, 2001, 27(3): 332.

[40] Rosso, Dubrana, & Cimbolini.. Application of RNA interference to root-knot nematode genes encoding esophageal gland proteins. Molecular Plant-Microbe Interactions, 2005, 18(7): 615-620.

[41] Rosso, M. N., Jones, J. T., & Abad, P.. RNAi and functional genomics in plant parasitic nematodes. Annu Rev Phytopathol, 2009, 47: 207-232.
DOI: 10.1146/annurev.phyto.112408.132605

[42] Rual, Ceron, J., Koreth, J., Hao, T., Nicot, A.-S., Hirozane-Kishikawa, T., . . . van den Heuvel, S.. Toward improving Caenorhabditis elegans phenome mapping with an ORFeome-based RNAi library. Genome research, 2004, 14(10b): 2162-2168.

[43] Rual, J. F., Klitgord, N., & Achaz, G.. Novel insights into RNAi off-target effects using C. elegans paralogs. BMC Genomics, 2007, 8(106).
DOI: 10.1186/1471-2164-8-106

[44] Siddique, S., Wieczorek, K., Szakasits, D., Kreil, D. P., & Bohlmann, H.. The promoter of a plant defensin gene directs specific expression in nematode-induced syncytia in Arabidopsis roots. Plant Physiology and Biochemistry, 2011, 49(10): 1100-1107.

[45] Sindhu, A. S., Maier, T. R., Mitchum, M. G., Hussey, R. S., Davis, E. L., & Baum, T. J.. Effective and specific in planta RNAi in cyst nematodes: expression interference of four parasitism genes reduces parasitic success. J Exp Bot, 2009, 60(1): 315-324.
DOI: 10.1093/jxb/ern289

[46] Singer, G. A., Lloyd, A. T., Huminiecki, L. B., &

 Distributed under creative commons license 4.0 DOI: https://doi.org/10.30564/jrb.v2i1.1759

Wolfe, K. H.. Clusters of co-expressed genes in mammalian genomes are conserved by natural selection. Molecular biology and evolution, 2004, 22(3): 767-775.

[47] Sontheimer, E. J.. Assembly and function of RNA silencing complexes. Nat Rev Mol Cell Biol, 2005, 6(2): 127-138.
DOI: 10.1038/nrm1568

[48] Steeves, R. M., Todd, T. C., Essig, J. S., & Trick, H. N.. Transgenic soybeans expressing siRNAs specific to a major sperm protein gene suppress Heterodera glycines reproduction. Functional Plant Biology, 2006, 33(11).
DOI: 10.1071/fp06130

[49] Stram, Y., & Kuzntzova, L.. Inhibition of viruses by RNA interference. Virus Genes, 2006, 32(3): 299-306.
DOI: 10.1007/s11262-005-6914-0

[50] Tabara, H., Grishok, A., & Mello, C. C.. RNAi in C. elegans: soaking in the genome sequence. Science, 1998, 282(5388): 430-431.

[51] Thorat, Y. E.. Develop Root-Knot Nematode, Meloidogyne incognita Specific Gene Expression System in Tomato, Solanum lycopersicum L. Division of Nematology Icar-Indian Agricultural Research Institute New Delhi, 2017.

[52] Timmons, L., Court, D. L., & Fire, A.. Ingestion of bacterially expressed dsRNAs can produce specific and potent genetic interference in Caenorhabditis elegans. Gene, 2001, 263(1-2): 103-112.

[53] Vanholme, B., W, V. A. N. T., Vanhouteghem, K., J, D. E. M., Cannoot, B., & Gheysen, G.. Molecular characterization and functional importance of pectate lyase secreted by the cyst nematode Heterodera schachtii. Mol Plant Pathol, 2007, 8(3): 267-278.
DOI: 10.1111/j.1364-3703.2007.00392.x

[54] Wani, S. H., Sanghera, G. S., & Singh, N. B.. Biotechnology and plant disease control-role of RNA interference. American Journal of Plant Sciences, 2010, 1(02): 55.

[55] Yadav, B. C., Veluthambi, K., & Subramaniam, K.. Host-generated double stranded RNA induces RNAi in plant-parasitic nematodes and protects the host from infection. Mol Biochem Parasitol, 2006, 148(2): 219-222.
DOI: 10.1016/j.molbiopara.2006.03.013

[56] Zhuo, K., Chen, J., Lin, B., Wang, J., Sun, F., Hu, L., & Liao, J.. A novel Meloidogyne enterolobii effector MeTCTP promotes parasitism by suppressing programmed cell death in host plants. Mol Plant Pathol, 2017, 18(1): 45-54.
DOI: 10.1111/mpp.12374