ATP-Binding Cassette (ABC) Transporter Genes in Plant Parasitic Nematodes: An Opinion for Development of Novel Control Strategy

Rinu Kooliyottil¹,²*, Koushik Rao Gadhachanda³, Nejra Solo², Louise-Marie Dandurand²*

¹Citrus Budwood Registration Program, Division of Plant Industry, Florida Department of Agriculture and Consumer Services, Alachua, FL 32615

²Department of Entomology, Plant Pathology and Nematology, University of Idaho, Moscow, ID 83844

³739 Cook Hill Rd, Cheshire, CT, 06410

*Corresponding Authors:

Rinu Kooliyottil, rinu.kooliyottil@fdacs.gov, rinukmicro@gmail.com
Louise-Marie Dandurand, lmd@uidaho.edu

Abstract:

The molecular interaction between the nematode and the host plant cells is complex and sophisticated. Initial contact with the plant parasitic nematodes (PPNs) triggers immune response in the host plant system which includes the release of toxic molecules. To put a bridle on this immune response, PPNs trigger pivotal cytoprotective mechanisms, such as antioxidant and detoxification pathways. Mechanisms of these pathways have been studied in PPNs and the specific genes involved have been targeted for gene silencing research in view of developing novel control measures. However, one of the important group of proteins involved in detoxification pathways known as ABC-transporters are not being studied until recently in PPNs.
This opinion article focuses on the current knowledge and future prospects of ABC transporters in PPNs.

**Keywords:** plant parasitic nematodes; xenobiotic metabolism; plant resistance; gene silencing

**INTRODUCTION**

Nematodes and plants have interacted for millions of years. These interactions have led to the evolution of plant parasitic nematodes (PPNs) to be able to overcome plant defenses. With years of evolution, PPNs have developed effective immune-suppression and growth strategies. Today, nematodes have complex feeding structures along with other highly adaptive features, which suit their environment (Ali et al., 2017). Along with nematode evolution, plants have also adapted and changed to recognize changes in pathogens for continued effective defense response. Initial contact with the plant parasitic nematodes (PPNs) triggers immune response in the host plant system which includes the release of toxic molecules. To put a bridle on this immune response, PPNs trigger pivotal cytoprotective mechanisms, such as antioxidant and detoxification pathways (Gillet et al. 2017). Mechanisms of these pathways have been studied in PPNs and the specific genes involved have been targeted for gene silencing research in view of developing novel control measures (Gillet et al., 2017; Qiu et al., 2019). However, one of the important group of proteins involved in detoxification pathways known as ABC-transporters are not being studied until recently in PPNs. This opinion article focuses on the current knowledge and future prospects of ABC transporters in PPNs.
PLANT NEMATODE INTERACTIONS

Plants use a set of induced and constitutive strategies to protect themselves against pathogens. The protective measures are activated when pathogen-derived compounds called pathogen-associated molecular patterns (PAMPs) are recognized. In resistant plants, PAMP perception activates pattern-triggered immunity (PTI), which initiates signals that facilitate resistance to the growth of pathogens (Jones and Dangl, 2006). In resistant plants, the NB-LRR (Nucleotide-binding Site Leucine-rich Repeat) proteins recognize the pathogen effectors which leads to effector-triggered immunity (ETI). Effector triggered immunity directs one of the most effective plant defense mechanism known as the hypersensitive response (HR) (Bigeard et al., 2015); whereby a few cells surrounding the ingressing pathogen or pest die to ward off the nematode. Some early signs of HR are rapid influxes of free calcium (Ca^{2+}), production of the reactive oxygen species (ROS), nitric oxide, and changes in the phytohormone expression (Garcia-Brugger et al., 2006; Lozano and Smant, 2011). Among several important roles during the plant defense response, rapid influxes of Ca^{2+} are considered to be crucial for the activation of the NADPH oxidase found on the membrane of the plant cell (Kadota et al., 2015). Oxidases produce extracellular ROS which initiates a cascade of events leading to an oxidative burst (Lozano and Smant, 2011). An oxidative burst along with the production of ROS is also an important part of the plant defense, since ROS create a cytotoxic environment for the pathogen or pest, as well as act as signaling molecules for local and systemic defense responses (Rosso, 2009; Gillet et al., 2017).

XENOBIOTIC METABOLISM TO DEFEND PLANT RESISTANCE
Plant parasitic nematodes have evolved xenobiotic metabolic pathways to counteract the cytotoxic defense response of plants. There are three phases of xenobiotic metabolism (Figure 1). Phase I metabolism, which mainly involves cytochrome P450, makes xenobiotics and endobiotics more soluble, while phase II metabolism is a detoxification step. In this phase enzymes, such as uridine dinucleotide phosphate glucuronosyl transferases (UGT) and glutathione S-transferases (GST), catalyze conjugate formation of xenobiotics and endobiotics with glutathione, amino acids, acetate, sulfate, propionate, or phosphate marking them for excretion (Kurutas, 2015; Laing et al., 2015). Most commonly this involves conjugation to glutathione (GSH), which is a tripeptide (γ-Glu-Cys-Gly) which has a major role in detoxification and redox buffering processes. In its reduced form it acts as a nucleophile that attacks electrophilic carbon, nitrogen, or sulfur atom on the toxic nonpolar compound (Edwards et al., 2000; Islam et al., 2017). Together with other antioxidants, such as ascorbate, α-tocopheral, and cysteine, it is an important aspect of non-enzymatic protection against oxidative stress (Kurutas, 2015). In animals, including nematodes, phase III involves excretion of these conjugates by ATP-binding cassette (ABC) transporters (Lindblom and Dodd, 2009). ABC transporters play a major role in the pumping of xenobiotic and endogenous metabolites through extra- and intracellular membranes, which helps to reduce the cellular concentrations of toxic compounds.

**ABC TRANSPORTERS IN PLANT PARASITIC NEMATODES: MUCH TO BE KNOWN**

Presence of a strongly conserved ATP binding motif is a signature key for ABC transporters, and fundamental functional arrangement of an ABC transporter in membranes is conserved from bacteria to humans (Higgins, 1992; Childs and Ling, 1994; Linton and Higgins, 1998). With a
total 60 genes, ABC transporters constitute the largest family of transporters in the genome of *Caenorhabditis elegans*, where it has been shown to be associated with the drug resistance (Sheps et al., 2004; Pohl et al., 2011; Ardelli, 2013). Furthermore, elevated expression of ABC transporter genes has been reported in animal parasitic nematodes (APNs) in association with drug resistance (Xu et al., 1998; Prichard and Roulet, 2007; Stitt et al., 2011). However, very few reports on the role of ABC transporters in PPNs exist. The genome of the PPN *Bursaphelenchus xylophilus* has 106 ABC transporters which is almost double the number found in the genome of *C. elegans* and about three times more than what is found in the genome of *Meloidogyne incognita* (Kikuchi et al., 2011). Upregulation of ABC transporter genes in response to a α-pinene, a monoterpene produced by plants in response to pathogen attack, has been found recently (Li et al., 2019). Diao et al. (2020) investigated the multi drug resistant protein coding (MDR) genes in *B. xylophilus* with a focus on screening nematicides for the control of these devastating nematodes and found that MDR genes encode the ABC transporter and the ABC transporter transmembrane region. Recent investigation by Cox et al. (2019) on root-expressed ABC transporter genes in tomato shed light on this topic for the first time in plant-microbe interactions in a natural environment. In this study they found that silencing root-expressed ABC transporter genes triggers the repulsion of both *Meloidogyne* and *Globodera* spp. (Cox et al., 2019). Fu et al. (2020) recently investigated the patterns of gene expression between hydrated and 24-hr desiccated nematodes in foliar nematode *Aphelenchoides fragariae*, this study shows differential expression of detoxification genes. Interestingly, this cited study showed the regulation of *pgp-14*-like multi-drug resistance protein (MRP/PGP), which are part of the ABC transporter system (Figure 1).
FUTURE PROSPECTIVE: NEMATODE ABC TRANSPORTERS AS POTENTIAL TARGET TO CONTROL PLANT PARASITIC NEMATODES

Interactions of *Globodera pallida* a sedentary endoparasitic cyst nematode with its natural host *Solanum tuberosum* and a resistant plant *Solanum sisymbriifolium* was investigated previously (Kooliyottil et al., 2016; Kooliyottil et al., 2019). A hyper sensitive response was evident as early as 24 hours post infestation in the root cells of *S. sisymbriifolium* (Kooliyottil et al., 2016).

Transcriptome analysis of *G. pallida* juveniles isolated from resistant *S. sisymbriifolium* or the susceptible host, potato, 24 hours post infestation showed expression of several genes related to the xenobiotic metabolism (Kooliyottil et al., 2019). While performing a comparative analysis of *G. pallida* isolated from *S. tuberosum* and *S. sisymbriifolium* author’s preliminary observations showed the expression of 18 *G. pallida* ABC transporters (Table 1). Although the expression was not significantly different whether from a resistant or susceptible plant species, expression of genes coding for ABC transporter proteins upon infection may provide insight into the role of these genes in parasitism by the nematode. Transcriptome information of PPNs isolated from resistant plant species is scanty. Most of the available information is focused on nematodes that are infecting susceptible plant species, but those studies that are available do not provide evidence on expression of ABC transporters (Shukla et al., 2018; Cotton et al., 2014). An investigation about ABC transposers in PPNs, especially when interacting with resistant plant species may provide useful information about how nematodes are able to overcome plant defenses such as ROS (Figure 1). Considering the existing knowledge of the importance of ABC transporter in APNs, characterization of ABC transporter genes may contribute to the identification of gene targets for silencing and provide novel PPN control. Understanding the
role and mechanisms of ABC transporters in PPNs will be helpful to identify the strategies for achieving sustainable pest control and may even facilitate development of PPN resistant plants.

ACKNOWLEDGMENTS

The support received from Dr. Morgane Gillard, Department of Plant Sciences, University of California, Davis, CA for the illustration on xenobiotic metabolism in plant parasitic nematodes in resistant and susceptible plants is acknowledged.

REFERENCES

1. Ali, M. A., Azeem, F., Li, H., and Bohlmann, H. (2017). Smart parasitic nematodes use multifaceted strategies to parasitize plants. *Front. Plant Sci.* 8, 1699. doi:10.3389/fpls.2017.01699

2. Ardelli, B. F. (2013). Transport proteins of the ABC systems superfamily and their role in drug action and resistance in nematodes. *Parasitol. Int.* 62(6), 639-646. doi:10.1016/j.parint.2013.02.008

3. Bigeard, J., Colcombet, J., and Hirt, H. (2015). Signaling mechanisms in pattern-triggered immunity (PTI). *Mol. plant* 8(4), 521-539. doi:10.1016/j.molp.2014.12.022

4. Childs, S., and Ling, V. (1994). “The MDR superfamily of genes and its biological implications,” in *Important Advances in Oncology*, Eds. V.T. DeVita, S. Hellman, S. A. J.B. Rosenberg (Lippincott: Philadelphia), 21–36.
5. Cox, D. E., Dyer, S., and Weir, R. (2019). ABC transporter genes ABC-C6 and ABC-G33 alter plant-microbe-parasite interactions in the rhizosphere. *Sci. Rep.* 9, 19899. https://doi.org/10.1038/s41598-019-56493-w

6. Cotton, J. A., Lilley, C. J., Jones, L. M., Kikuchi, T., Reid A. J., Thorpe, P., et al. (2014). The genome and life-stage specific transcriptomes of *Globodera pallida* elucidate key aspects of plant parasitism by a cyst nematode. *Genome Biol.* 15, R43. https://doi.org/10.1186/gb-2014-15-3-r43

7. Diao, J., Hao, X., and Ma, W. (2020). Bioinformatics analysis of structure and function in the *MRP* gene family and its expression in response to various drugs in *Bursaphelenchus xylophilus*. *J. For. Res.* https://doi.org/10.1007/s11676-019-01086-6

8. Edwards, R., Dixon, D. P., and Walbot, V. (2000). Plant glutathione S-transferases: enzymes with multiple functions in sickness and in health. *Trends Plant Sci.* 5(5), 193-198. doi:10.1016/s1360-1385(00)01601-0

9. Fu, Z., Agudelo, P., and Wells, C. E. (2020). Detoxification-related gene expression accompanies anhydrobiosis in the foliar nematode (*Aphelenchoides fragariae*). *J. Nematol.* 52, 1–12. https://doi.org/10.21307/jofnem-2020-047

10. Garcia-Brugger, A., Lamotte, O., Vandelle, E., Bourque, S., Lecourieux, D., Poinssot, B., et al. (2006). Early signaling events induced by elicitors of plant defenses. *Mol. Plant Microbe Interact.* 19(7), 711-724. doi:10.1094/MPMI-19-0711

11. Gillet, F. X., Bournaud, C., Antonino de Souza Júnior, J. D., and Grossi-de-Sa, M. F. (2017). Plant-parasitic nematodes: towards understanding molecular players in stress responses. *Ann. Bot.* 119(5), 775-789. doi:10.1093/aob/mcw260
12. Higgins, C. F. (1992). ABC transporters: from microorganisms to man. *Annu. Rev. Cell Biol.* 8, 67–113. https://doi.org/10.1146/annurev.cb.08.110192.000435

13. Islam, S., Rahman, I. A., Islam, T., and Ghosh, A. (2017). Genome-wide identification and expression analysis of glutathione S-transferase gene family in tomato: Gaining an insight to their physiological and stress-specific roles. *PloS one* 12(11), e0187504. doi:10.1371/journal.pone.0187504

14. Jones, J. D., and Dangl, J. L. (2006) The plant immune system. *Nature* 444, 323–329. doi:10.1038/nature05286

15. Kadota, Y., Shirasu, K., and Zipfel, C. (2015). Regulation of the NADPH oxidase RBOHD during plant immunity. *Plant Cell Physiol.* 56(8), 1472-1480. doi:10.1093/pcp/pcv063

16. Kikuchi, T., Cotton, J. A., Dalzell, J. J., Hasegawa, K., Kanzaki, N., McVeigh, P., Takanashi T., Tsai, I. J., Assefa, S. A., Cock, P. J., et al. (2011). Genomic insights into the origin of parasitism in the emerging plant pathogen *Bursaphelenchus xylophilus*. *PLoS Pathog.* 7, e1002219. doi: 10.1371/journal.ppat.1002219

17. Kooliyottil, R., Dandurand, L. M., Govindan, B. N., Knudsen, G. R. (2016). Microscopy method to compare cyst nematode infection of different plant species. *Adv. Biosci. Biotechnol.* 7 (06), 311-318. doi: 10.4236/abb.2016.76029

18. Kooliyottil, R., Dandurand, L. M., Kuhl, J. C., Caplan, A., Xiao, F., Mimee, B., et al. (2019). Transcriptome analysis of *Globodera pallida* from the susceptible host *Solanum tuberosum* or the resistant plant *Solanum sisymbriifolium*. *Sci. Rep.* 9, 13256. https://doi.org/10.1038/s41598-019-49725-6
19. Kurutas, E. B. (2015). The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state. Nutr. J. 15 (71), https://doi.org/10.1186/s12937-016-0186-5

20. Laing, R., Bartley, D. J., Morrison, A. A., Rezansoff, A., Martinelli, A., Laing, S. T., et al. (2015). The cytochrome P450 family in the parasitic nematode Haemonchus contortus. Int. J. Parasitol. 45(4), 243-251. doi:10.1016/j.ijpara.2014.12.001

21. Li, Y., and Meng, F., and Deng, X. (2019) Comparative transcriptome analysis of the pinewood nematode Bursaphelenchus xylophilus reveals the molecular mechanism underlying its defense response to host-derived α-pinene. Int. J. Mol. Sci. 20(4), 911. doi:10.3390/ijms20040911

22. Lindblom, T. H., and Dodd, A. K. (2006). Xenobiotic detoxification in the nematode Caenorhabditis elegans. J. Exp. Zool. A Comp. Exp. Biol. 305(9), 720-730. doi: 10.1002/jez.a.324

23. Linton, K. J., and Higgins, C. F. (1998). The Escherichia coli ATP-binding cassette (ABC) proteins. Mol. Microbiol. 28, 5–13. doi: 10.1046/j.1365-2958.1998.00764.x

24. Lozano, J., and Smant, G., (2011). “Survival of plant-parasitic nematodes inside the host,” in Molecular and Physiological Basis of Nematode Survival, Eds. R. N. Perry, D. A. Wharton (CABI: Oxfordshire), 28-62.

25. Pohl, P. C., Klafke, G. M., and Carvalho, D. D. (2011). ABC transporter efflux pumps: a defense mechanism against ivermectin in Rhipicephalus (Boophilus) microplus. Int. J. Parasitol. 41(13-14), 1323-1333. doi:10.1016/j.ijpara.2011.08.004
26. Prichard, R. K. and Roulet, A. (2007). ABC transporters and beta-tubulin in macrocyclic lactone resistance: prospects for marker development. Parasitology 134(8), 1123-32. doi:10.1017/S0031182007000091

27. Qiu, X., Yang, L., Ye, J., Wang, W., Zhao, T., Hu, H., et al. (2019). Silencing of cyp-33C9 gene affects the reproduction and pathogenicity of the pine wood nematode, Bursaphelenchus xylophilus. Int. J. Mol. Sci. 20 (18), 4520. https://doi.org/10.3390/ijms20184520

28. Rosso, L. C. (2009). Cloning, sequence, and expression analysis of a new MnSOD-encoding gene from the root-knot nematode Meloidogyne incognita. J. Nematol. 41(1), 52-59.

29. Sheps, J. A., Ralph, S., Zhao, Z., Baillie, D. L., and Ling, V. (2004) The ABC transporter gene family of Caenorhabditis elegans has implications for the evolutionary dynamics of multidrug resistance in eukaryotes. Genome Biol. 5(3), R15. https://doi.org/10.1186/gb-2004-5-3-r15

30. Shukla, N., Yadav, R., Kaur, P., Rasmussen, S., Goel, S., Agarwal, M., et al. (2018). Transcriptome analysis of root-knot nematode (Meloidogyne incognita)-infected tomato (Solanum lycopersicum) roots reveals complex gene expression profiles and metabolic networks of both host and nematode during susceptible and resistance responses. Mol. Plant Pathol. 19(3), 615–633. https://doi.org/10.1111/mpp.12547

31. Stitt, L. E., Tompkins, J. B., Dooley, L. A., Ardelli, B. F. (2011). ABC transporters influence sensitivity of Brugia malayi to moxidectin and have potential roles in drug resistance. Exp. Parasitol. 129(2), 137-144. doi: 10.1016/j.exppara.2011.06.018
32. Xu, M., Molento, M., Blackhall, W., Ribeiro, P., Beech, R., and Prichard, R., (1998). Ivermectin resistance in nematodes may be caused by alteration of P-glycoprotein homolog. *Mol. Biochem. Parasitol.* 91(2), 327-335. doi: 10.1016/s0166-6851(97)00215-6

Table 1

ABC Transporter genes identified from the transcriptome of *Globodera pallida* (Kooliyottil et al. 2019)

| GPLIN_000375400, GPLIN_001624000, GPLIN_000079800, GPLIN_000593000, GPLIN_000607700, GPLIN_000662100, GPLIN_000762600, GPLIN_000764700, GPLIN_000165600, GPLIN_001513300, GPLIN_000934100, GPLIN_001038000, GPLIN_001558100, GPLIN_001072100, GPLIN_000043700, GPLIN_001213800, GPLIN_001600700, GPLIN_000055000 |
Figure 1. Response of plant parasitic nematodes in resistant and susceptible plants. A resistant plant produces reactive oxygen species (ROS) and phytoalexins through the microbe- or pathogen-associated
molecular patterns (MAMPs or PAMPs), in response to the detection of nematode-secreted molecules. A susceptible plant produces less compounds to defend the nematode presence. Plant parasitic nematodes have evolved xenobiotic metabolic pathways to counteract the cytotoxic defense response of plants (A). During phase I of xenobiotic metabolism the polarity, and solubility of xenobiotics is increased, often by oxidation, reduction or hydrolysis reactions. In phase II, a functional group is added to form xenobiotic conjugates. The production of proteins involved in phase I and II is orchestrated by two transcription factors, DAF-16 and SKN-1. In phase III, ABC transporters excrete xenobiotic conjugates from the cell. The expression of genes coding for ABC transporters is regulated by gene families such as MRP and P-gps (B). Silencing genes involved in xenobiotic detoxification compromises nematode cell survival. The model propose that silencing genes involved in Phase III would lead to the impediment the production of ABC transporters, and thus to a lethal accumulation of xenobiotic conjugates that could not be excreted from the cell (C).

ABC, ATP-binding cassette; CYP, cytochrome P450; GST, glutathione S-transferase; SDR, short-chain dehydrogenase; UGT, UDP-glucuronosyl or UDP-glycosyl transferase; DAF-16, dauer formation 16; SKN-1, skinhead transcription factor-1; MRP, multi-drug resistance protein; MTI, MAMP-triggered immunity; P-gps, P-glycoproteins.