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

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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 focusses 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 focusses 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-tocophoral, 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 NEAMTODES: 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-pinen, amonoterpene 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.

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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
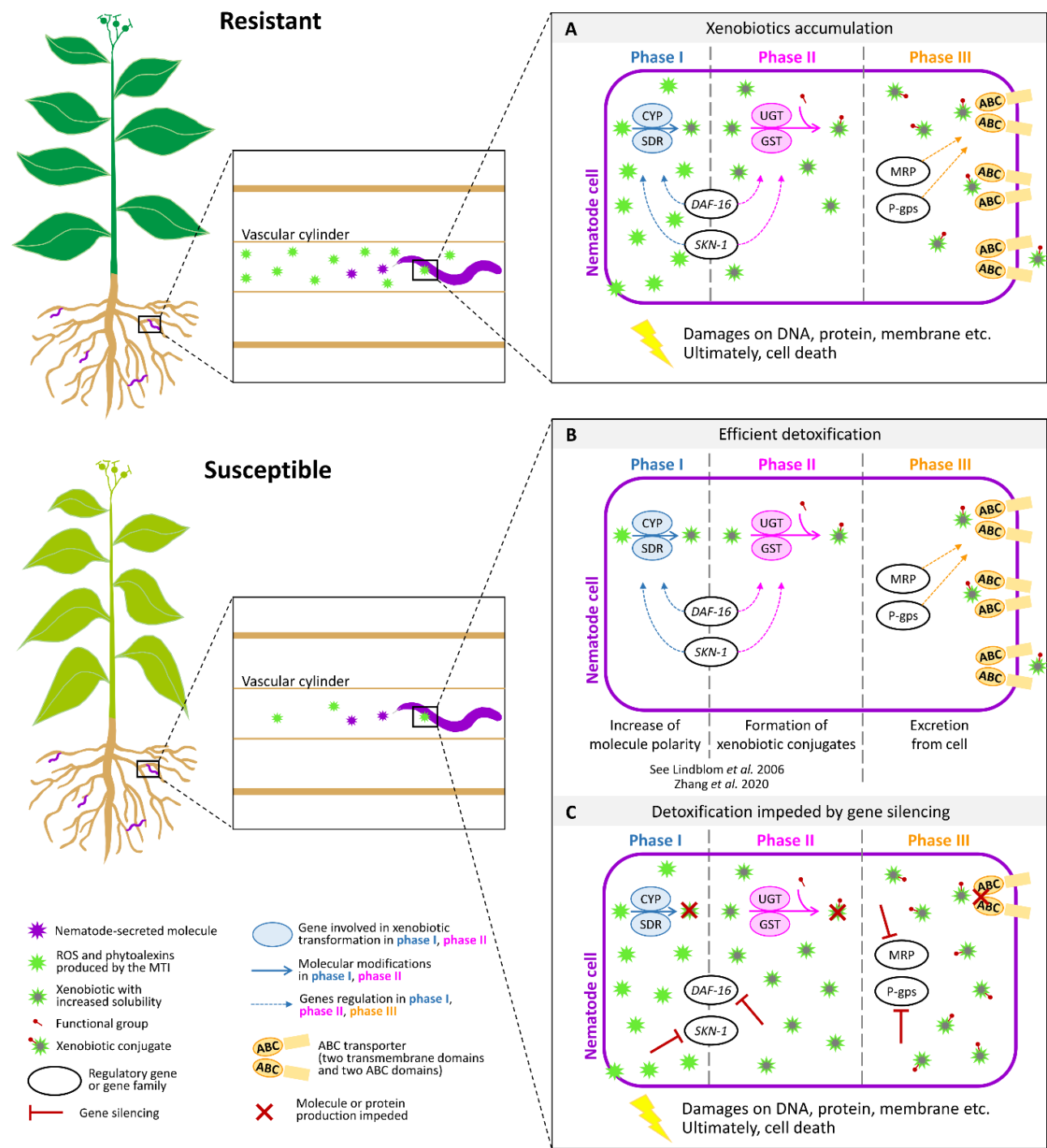
Figure 1. Response of plant parasitic nematodes in resistant and susceptible plants. A resistant plant

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, gluthatione 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.