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(Review Article)

AN APPROACH TO THE PARASITISM GENES OF THE ROOT KNOT NEMATODE

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A B S T R A C T

Plant parasitic nematodes which are highly successful parasites evolved a very specialized feeding relationship with the host plant to cause the destructive root-knot disease. They initiate their parasitic relationship with the host by releasing their secretions into root cells which in turn stimulate the root cells of the host to become specialized feeding cells which are considered as the single source of nutrients essential for the nematode's survival. The parasitism genes expressed in nematode's esophageal gland cells encode secretory proteins that are released through its stylet to direct the interactions of the nematode with its host plants.

Keywords: Genes, enzymes, esophageal gland, host, root cells, stylet.

INTRODUCTION

Plant parasitic nematodes are the hidden enemy of crops and are one of the many groups of harmful organisms which depend on plants for their survival and reproduction (Khan, 2008). Plant parasitic nematodes can cause significant damage to almost all kinds of crops but due to their subterranean habit and microscopic size they remain invisible to the naked eye. The estimated annual yield losses due to plant parasitic nematodes in the world's major crops are recorded at about 12.3% and 14% in the developing countries (Sasser and Freckman, 1987). In India, recent studies shows that plant parasitic nematode is responsible for both the quantitatively and qualitatively yield losses amounting around Rs. 240 billion every year (Khan, 2008). Besides this direct damage, these nematodes also serve as predisposing agents in development of disease complexes in association with other microbial agents including fungi, bacteria and virus.

The most evolutionary advanced adaptations for plant parasitism by plant parasitic nematodes are the products called parasitism genes (Gao *et al*., 2002). The parasitism proteins are secreted by the nematode and play a direct role in plant parasitism. These parasitism proteins secretions mostly originate from the pharyngeal gland cells, but secretions from the chemosensory amphids might also be important (Davis *et al*., 2004).

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NEMATODE PARASITISM GENES

Root knot nematodes which are highly successful parasites evolved a very specialized feeding relationship with the host plant to cause the destructive root-knot disease. They initiate their parasitic relationship with the host by releasing their secretions into root cells which in turn stimulate the root cells of the host to become specialized feeding cells which are the sole source of nutrients essential for the nematode's survival (Baum *et al.,* 2007). A deeper understanding of the basic principles and mechanisms of root knot nematode parasitism is critical for discovering new targets in root knot nematodes to develop novel crop resistance using biotechnology tools.

The parasitism genes expressed in the root knot nematode's esophageal gland cells encode secretory proteins that are released through its stylet to direct the interactions of the nematode with its host plants (Huang *et al.,* 2005). The products which are collectively called parasitome, of parasitism genes secreted into susceptible host tissue modulate the complex changes in function, morphology and gene expression in host root cells to form feeding cells. Plant parasitic nematodes are well equipped with a stylet to tear the cell walls and allow exchange of solute between plant and parasite

and also have well-developed secretory gland cells associated with esophagus that produce secretions released through the stylet into host. In order to be parasitic, the nematode must be able to penetrate the roots of host and migrate through root tissues. Despite the small size of root knot and cyst nematode infective J2 stage, plant cell walls serves as an obstacles so nematodes release a mixture of cell-wall-digesting enzymes to break structural plant cell-walls (Baum *et al.,* 2007). Above this, the most interesting things appear to be the nematode directed formation of the feeding cells by both the root knot nematodes as giant cells and cyst nematodes as syncytia. Gheysen and Jones (2006) also suggested that both induction and maintenance of giant cells were controlled by stylet secretions. These secretions were originated from dorsal and subventral pharyngeal glands of feeding nematodes. They also reported that the nematode genes expressed solely in subventral gland cells were most similar to the genes those produced cell wall degradation enzymes from bacteria and were not present in symbiotic bacteria. Zenov'eva *et al*. (2004) summarized several gene products isolated from subventral glands of nematodes which included lipoprotein, cellulose-binding protein, endoglucanase, chitinase, pectinase and proteinase.

PARASITISM GENE IDENTIFICATION

A number of approaches to identify the nematode parasitism genes and proteins have been devised and tried. Many of these approaches mainly focus on esophageal-glands due to their active involvement in parasitism. The peptide sequence from an antigen purified with an esophageal-gland-specific monoclonal antibody was previously used to isolate the first parasitism gene from a plant parasitic nematode (Smant *et al*., 1998), encoding a β-1,4-endoglucanase enzyme (cellulase). Immunoaffinity purification was used to enrich secreted proteins, which results in the finding of a secreted protein (endo-1, 4- β-glucanase) from the subventral glands of the cyst nematode *Globodera rostochiensis* (Smant *et al*., 1998). Another method to be mentioned is the analysis of collected nematode secretions by 2D gel electrophoresis and microsequencing. This has also proved successful for the beet cyst nematode *Heterodera schachtii* (De Meutter *et al.,* 2001) and the root knot nematode, *Meloidogyne incognita* (Jaubert *et al*., 2002b). Recently, mass spectometry is also used for direct identification of proteins secreted by *M. incognita*, revealing proteins with host cell reprogramming potential (Bellafiore *et al*., 2008). Further studies also reveal the expression of cyst nematode endoglucanase genes and their associated products specifically from subventral gland cells of the nematode, cellulolytic activity of the enzymes, and secretion of cellulases from the stylet in plant during migration of infective J2 stage inside the roots (Wang *et al.,* 1999). These studies also confirmed and refined the previous reports of secreted cell-wall-modifying enzymes from plant parasitic nematode (Deubert and Rohde, 1971). An important discovery of the nematode endoglucanases enzymes was their strong resemblance to prokaryote (glycosyl hydrolase family 5) endoglucanases and little similarity to endoglucanases of eukaryotes and no similarity to any gene of *Caenorhabditis elegans* (Smant *et al*., 1998).

ORIGINS OF NEMATODE PARASITISM GENES

Plant-parasitism is believed to have evolved at least three times independently (Blaxter *et al*., 1998). The genes that were evolved from nematode ancestors of contemporary species are one likely possible mechanism for the origin of nematode parasitism genes and the other mechanism may be horizontal gene transfer (HGT). It was reported that those genes expressed in the esophageal gland cells of plant parasitic nematodes show strongest similarities to the bacterial genes which strengthened the existing hypothesis that parasitism genes in plant-nematodes may have been acquired, at least in part, by horizontal gene transfer from bacteria and other microorganisms that inhabit the same parasitic environment. The genes *Mj-cm-1*and *Mi-cbp-1* shows strongest similarities to the genes of bacteria (Ding *et al*., 1998 and Lambert *et al*., 1999). The complementation of a bacterial mutant with *Mj-cm-1* was also used to provide functional analysis of the gene (Lambert *et al*., 1999). Most of the parasitism genes are found to be highly similar to bacterial sequences thereby suggesting that these parasitism genes could have been acquired from bacteria through horizontal gene transfer. For example, the nematode endo-1,4-β-glucanases from the Tylenchomorpha, which belong to Glycosyl Hydrolase Family (GHF5), show less similarity to plant endoglucanases but show resemblance to the bacteria. The genes encoding the cellulases enzymes of both nematode and bacteria may have evolved from an ancient cellulase of a common ancestor of both the bacteria and nematodes. The endoglucanases from nematode shows the highest similarity with the bacterial one, which also points to a HGT from bacteria to an ancestor of the cyst nematode.

However, it is not possible and advisable to provide the conclusive evidence for a horizontal gene transfer (HGT) from one organism to another organism germ line. There are examples of putative cases of horizontal gene transfer from eukaryote to prokaryote, prokaryote to prokaryote and from prokaryote to eukaryote (Smith *et al*., 1992 and Syvanen, 1994). On the other hand, the presence of bacterial symbionts in nematode ancestors, such as the bacterium *Wolbachia* symbiont found in filarial nematodes (Blaxter *et al*., 1998), may also represent a source for transfer of genetic material from bacteria to nematodes.

NEMATODE PLANT CELL WALL DEGRADING ENZYMES

CELLULASE

The most widely studied cell wall degrading enzyme in nematode till date is cellulase or endo-1, 4-β-glucanase. These enzymes degrade the cellulose which is the structural component of the plant cell wall. This enzymes Cellulases were identified in the sedentary nematode genera *Heterodera*, *Globodera* and *Meloidogyne* (Smant *et al*., 1998). The plant cell wall digesting enzymes cellulase and pectinase genes are already described for root knot nematodes (Huang *et al*., 2004; Huang *et al*., 2003; Huang *et al*., 2005 and Rosso *et al*., 1999) and cyst nematode species (Smant *et al*., 1998: Gao *et al.,* 2003; Wang *et al.,* 2001 and Yan *et al*., 2001). The first major achievement in parasitism gene discovery was the discovery of cellulase genes from the soybean and potato cyst nematodes. The discovery of cellulase genes was of very important since no cellulase genes had been reported from animals at that time (Smant *et al.,* 1998). The enzymes beta-1, 4-endoglucanase genes from *Pratylenchus penetrans* (Uehara *et al*., 2001), a migratory parasite that also requires enzymes to enter the plant cell-walls was also reported. An enzyme cellulase of the beta-1, 3-endoglucanase type was also recently reported from *Bursaphelenchus xenophilus*, the pinewood nematode where it is hypothesized of being involved in nematode feeding from the fungal mycelium (Kikuchi *et al.,* 2005).

PECTATE LYASE

The enzyme, Pectate lyases can cleaves the internal (1, 4)-α-linkages of pectate and were identified in *Meloidogyne* spp., but also known to be reported from other genera such as *Heterodera, Globodera* and *Bursaphelenchus* (Popeijus *et al*., 2000; Doyle & Lambert, 2002; Huang *et al.,* 2005; Kikuchi *et al*., 2005 and Vanholme *et al*., 2007). The pectinase proteins obtained from nematode was of the type pectate lyase which is found in fungi and bacteria, cyst and root knot nematodes; (Popeijus *et al*., 2000; Huang *et al*., 2003; Huang *et al*., 2005; De Boer *et al*., 2002 and Doyle & Lambert, 2002) or to the polygalacturonase type of bacteria (Jaubert *et al*., 2002 a). The involvement of these enzymes in penetration and migration is well supported by the evidence that these enzymes are produced and released during the nematode penetration and migration and to a smaller extent, or not at all during the sedentary stages of the nematodes (Huang *et al*., 2005; Rosso *et al*., 1999; De Boer *et al.,* 1999 and Goellner *et al.,* 2000).

POLYGALACTURONASE

The *Mi-pg-1* gene encoding a functional polygalacturonase (PG) from *M. incognita* was the first known animal polygalacturonases (PGs) cloned from plant parasitic nematode (Jaubert *et al.,* 2002a). These enzymes help in catalyzing the hydrolysis of pectic polygalacturonic acid and in turn release oligogalacturonides. PGs are classified into two classes namely exo-PGs and endo-PGs depending on their mode of action. The gene *Mi-pg-1* encodes for 633 amino acid protein. Phylogenetic analysis reveals that *Mi-pg-1* is closer to PGs from prokaryotes than to eukaryotic enzymes. The close similarity between bacterial PGs and *Mi-pg-1* provides strong evidence supporting the hypothesis that the parasitism genes in nematodes may have been acquired through gene transfer from microorganisms. More interestingly, *M. incognita* PG could play an important role in weakening the plant cell walls of root tissue during nematode penetration and intercellular migration by the parasite like other nematode parasitism genes.

XYLANASE

Most abundant polysaccharide in nature next to cellulose is xylan and is composed of (1, 4)-β-linked xylopyranose units (Collins *et al*., 2005). The enzymes endo-1, 4-β-xylanases depolymerise the nonhydrolysed xylan polymer by cleaving the xylan backbone (Subramaniyan and Prema, 2002). The characterization of the first functional animal endo-1, 4-β-xylanase gene was reported from *Meloidogyne incognita*, the southern root-knot nematode. The nematode endoxylanase, *Mi-XYL1*, has similarity to bacterial endoxylanases (Mitreva- Dautova *et al*., 2006). Most of these enzymes have been designated to the family GHF5, although there is some confusion about this classification since the proteins have similarity to

GHF30 enzymes as well. Expressed sequence tag (EST) study on *Radopholus similis,* the migratory nematode revealed some interesting genes, including an EST with homology to an endo-1, 4-β-xylanase was also reported recently by Jacob *et al*. (2008).

EXPANSINS

There is evidence that the potato cyst nematode also secretes a protein that has the ability to break the non-covalent bonds in plant cell walls in addition to the ability of breaking down the covalent bonds found in plant cell-walls through the enzyme cellulases and pectinase (Baum *et al*, 2007). This type of activity is accomplished by expansin-like protein found in the potato cyst nematode (Qin *et al*., 2004), which is also the first confirmed report of such protein from outside the plant kingdom. The expansins is involved in softening the plant cell-walls by breaking the non-covalent bonds between cell-wall-fibrils.

The resultant cell-wall softening could also be demonstrated for the potato cyst nematode expansin parasitism protein (Qin *et al*., 2004). Till now, no such genes have been reported from root knot nematodes or other cyst nematodes.

OTHER PARASITISM GENES

CHORISMATE MUTASE

Chorismate is the precursor for a number of compounds like cellular aromatic amino acids and the plant hormone indole-3-acetic acid, related to salicyclic acid and other secondary metabolites (Dewick, 1998). This chorismate-derived compound plays an important role in plant growth and development, in plant defense, and also in interactions with other organisms (Schmid and Amrhein, 1995 and Weaver and Hermann, 1997). The enzyme chorismate mutase catalysed the pericyclic claisen-like rearrangement of chorismate to prephenate in the shikimate pathway, which is a primary metabolic pathway found in plants and other micro-organisms (Romero *et al,* 1995). This enzyme is well characterized from microbes and plants, and not described from any other animals outside plant-parasitic nematodes (Roberts *et al*., 1998; Romero *et al*., 1995; Schmid and Amrhein, 1995). The first animal chorismate mutase gene (*Mj-cm-1*) was cloned from *Meloidogyne javanica* and found to be expressed in the oesophageal gland cells of the nematode (Lambert *et al.,* 1999). The enzyme chorismate mutase is known to be involved in early development of the feeding sites induced by plant parasitic nematodes, but how this enzyme alters the development of plant cells is not properly known (Doyle and Lambert, 2002). Chorismate mutase was also identified from soybean and potato cyst nematodes recently. (Bekal *et al*., 2003; Gao *et al*., 2003 and Jones *et al*., 2005).

CHITINASE

Chitinase is a putative parasitism protein, identified from the subventral glands of the soybean cyst nematode (Gao *et al*., 2002). This parasitism protein has a clearly defined function but no clear role for this function during the production of protein. The occurrence of chitin in nematode has been found only in the egg shell (Bird *et al*., 1991) and the presence of this parasitism protein chitinases has been discussed as having a role in the hatching of nematode (Baum *et al.,* 2007). *In situ* expression (Gao *et al.,* 2002) and microarray expression studies demonstrate that chitinase is not found to be expressed in the eggs of nematodes but has a strong expression peak in the early phases of parasitism after penetration inside the plants (Baum *et al*., 2007).

ANNEXIN

Annexin genes represents a family that codes for calcium dependent phospholipid binding proteins and has a broad range of reported functions. The mRNA for a secretory isoform of an annexin-like protein was also reported to be expressed from the dorsal gland of the soybean cyst nematode (Gao *et al.,* 2002). However, no clear confirmation about its role in parasitism can be drawn at this time. This gene is also reported from *Globodera pallida* , the potato cyst nematode (Baum *et al.,* 2007).

CALRETICULIN

Calreticulin-like proteins are also reported to be secreted from other plant parasitic nematodes and are regarded as good candidates for a role in parasite-host interactions (Nakhasi *et al*., 1998 and Pritchard *et al*., 1999). A calreticulin-like protein preceded by a signal peptide was also reported to be secreted from the subventral glands of a root knot nematode (Jaubert *et al.,* 2002 b). The confusing array of putative or demonstrated calreticulin functions reported (Nakhasi *et al*., 1998) make it difficult to confirm its role in host parasitism by root-knot nematodes.

SMALL BIOACTIVE PEPTIDES

The most commonly expressed parasitism gene in *Heterodera glycines* was first identified as clone *HG-SYV46* (Wang *et al*., 2001) through the secretion of signal peptide of an esophageal gland cell cDNA library.

The computational analyses found out that the C-terminal domain of *HG-SYV46* is related to the members of the *CLAVATA3-ESR*-like (*CLE*) family of signaling proteins in *Arabidopsis* (Olsen and Skriver, 2003). The CLAVATA3 in *Arabidopsis* has been identified as a key factor determining the shoot meristem differentiation (Fletcher *et al*., 1999). The expression of the cDNA of *Heterodera glycines* CLAVATA3-like peptide in the *CLAVATA3* (*CLV3*) *Arabidopsis* mutant was found to restore the wild-type phenotype confirming the first report of ligand mimicry in plant nematode interactions (Wang *et al.,* 2005). It will be interesting to find out if the small C-terminal extension of the cyst nematode ubiquitin extension proteins when considering the importance of this small peptides in signaling roles of plant development and plant nematode interactions (Tytgat *et al., 2004 and* Gao *et al*., 2003) will have the regulatory functions in plant cell. It is also supported by the fact that the role of small peptides in nematode-plant interactions is also presented by an unknown peptide fraction smaller than 3 kilo Dalton isolated from potato cyst nematode secretions (Baum *et al.,* 2007).

CONCLUSION

Root knot nematode release certain plant cell wall modifying proteins to disrupt the plant cell wall in addition to the mechanical shearing of plant cell wall by the stylet of the nematode. The parasitism proteins secreted by root-knot nematode might include plant cell wall modifying enzymes likely to be of prokaryotic origin besides proteins that are capable of localizing in the host plant cell nucleus, suppressors of host defense, proteins that can mimic plant proteins as reported in other cyst and root knot nematodes.

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