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Review Paper

ROLE OF ESOPHAGEAL GLAND SECRETIONS IN PLANT-NEMATODE RELATIONSHIP

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Abstract

Plant parasitic nematodes are bio-trophic parasites and can only obtain nutrients for development and reproduction from the cytoplasm of living plant cells. Evolutionary adaptations of nematodes for plant parasitism led to the development of the protusible stylet which marked the morphological and physiological modifications of the esophagus. The esophagus has a muscular metacarpus containing a triradiated pump chamber and three large and complex specialized secretory gland cells *viz.*, the dorsal and sub-ventral glands in the basal bulb. This secretory gland cells are the principle source of secretions involved in plant parasitism. Earlier, Endo (1987), reported that the dorsal gland are more important than the sub-ventral gland as the dorsal gland becomes very active in terms of production of secretory granules which are involved in parasitism in *Heterodera glyssines* while the sub-ventral glands appear to be inactive. But recent studies in molecular biology revealed that cellulase enzymes are expressed in sub-ventral gland cells of *Heterodera glyssines* and *Globodera rostochiensis*. During parasitism of plant cell, nematode stylet protruded through the plant cell wall but does not pierce the plasma membrane which becomes invaginated around the stylet tip. Gland secretions from the esophageal gland cells may be injected or deposited outside the plasma membrane of recipient cell. In either case, specific compounds in the secretion bind plant cell receptors giving a signal transduction cascade to modulate gene expression in the cell. The relative importance of secretions from dorsal gland cell versus sub-ventral gland cells in the host nematode interaction is still an issue of debating. Studies on the role of parasitism of the two gland cells secretion will provide us the knowledge in developing transgenic resistance plants against nematode leading to increase in crop yield.

INTRODUCTION

Nematodes are vermiform or thread like, bilaterally symmetrical, unsegmented, psuedocoelomic and triploblastic lacking respiratory and circulatory system. The body is flexible, usually elongate, filiform, cylindrical to fusiform and tapering towards the end. Adult of plant parasitic nematodes may ranges from 0.3 – 0.5 mm to 11 mm long and moves in the soil through pore space between the soil particles needing some moisture for their survival. Food of nematodes is invariably some source of protoplasm, such as plants, fungal hyphae, algae, bacteria, protozoa and even nematodes. On the basis of food and habit, the nematodes are

divided into four categories viz., microbial feeders or microbivorous nematodes, predatory nematodes, plant feeders or phytonematodes and miscellaneous nematodes (including those nematodes whose feeding habits are non-specific, and are referred to as saprophytic nematodes or free-living nematodes, food requirement of these nematodes is limited and any organic source of plant or animal origin may sustain them).

PLANT FEEDER or PHYTONEMATODES

Plant-parasitic nematodes are biotrophic parasites and can only obtain nutrients for development and reproduction from the cytoplasm of living plant cells. These nematodes have evolved diverse parasitic strategies and feeding relationships with their host plant to obtain the nutrients that are necessary for their survival. Depending upon species, they feed from the cytoplasm of unmodified living plant cells or have evolved to modify root cells into elaborate discrete feeding cells. Plant-parasitic nematodes use a hollow protusible stylet to penetrate the wall of a plant cell, inject gland secretions into the cell, and withdraw nutrients from the cytoplasm. Based on the feeding strategy, the plant parasitic nematodes are broadly divided into two types: Migratory nematodes and Sedentary nematodes.

Migratory feeding nematodes remove the cell cytoplasm from the parasitized cell, frequently causing cell death, and then move to another cell to repeat the feeding process.

The other plant parasites become sedentary and feed from a single cell or a group of cells for prolonged periods of time, to sustain the feeding for long period of time. The sedentary parasites dramatically modify the root cells of susceptible hosts to elaborate feeding cells, including modulating complex changes in cell morphology, function, physiology and gene expression (Bird, 1996; Gheysen and Fenoll, 2002). The drastic phenotypic changes of root cells is the result of nematode-mediated changes, directly or indirectly, in the developmental program of the parasitized cells (Williams and Hussey, 1996).

Further the sedentary nematodes are classified into two categories: sedentary endoparasites (they remain in the feeding cell which is a sole source of nutrients throughout the life. e.g. *Meloidogyne*, *Globodera* and *Heterodera*) and sedentary ectoparasites (Use a single feeding cell as nutrient source for several days, then moves on to establish another feeding cell. e.g. *Criconebella xenoplax* (Hussey and Grundler, 1998)

Evolutionary adaptations of nematodes of plant parasitism led to the development of the protusible stylet as well as marked the morphological and physiological modification of the esophagus (Bird, 1971; Maggenti, 1987 and Hussey, 1989a).

Secretory gland cells in the nematode esophagus are the principle sources of secretions involved in plant parasitism, and these gland enlarged considerably as nematodes evolved from microbial feeding nematodes to become parasites of higher plants. Likewise the function of the secretions produced by the esophageal gland cells also evolved to enable nematodes to feed on the plant cells and modify them into complex feeding cells (Hussey, 1989 and Davis *et al.*, 2000). Recent discoveries also suggest that some genes encoding esophageal gland secretions of plant parasitic nematodes may have been acquired *via* horizontal gene transfer from prokaryotic microbes (Smant *et al.*, 1998 and Davis *et al.*, 2000).

Discoveries have been made in identifying parasitism genes in cyst and root-knot nematodes because these nematodes induce the most dramatic and evolutionary advanced changes observed in host cell phenotype (Hussey and Grundler, 1998). Cyst and root-knot nematodes have evolved to alter gene expression in specific root cells to modify them into very specialized and metabolically active feeding cells, called syncytia or giant cells, respectively. Cell fusion following cell wall degradation gives rise to the syncytia whereas abnormal cell growth following repeated mitosis without cytokinesis produces giant cells. A number of genes with known or putative functions have been found to be up-regulated or silenced in these feeding cells suggesting that root-knot and cyst nematodes induce transcriptional changes in the parasitized cells (Bird, 1996; Gheysen and Fenoll, 2002).

THE ESOPHAGUS

The nematode body is broadly divided into three parts viz., Stomodeum/ Foregut (stoma, esophagus and cardia), Mesenteron/ Midgut (contains intestine) and Proctodeum/ Hindgut (contains rectum in female, cloaca in male). The esophagus is the second and largest part of stomodeum which lies between stoma and cardia and function as food transporter that transport the food material from low pressure stoma to high pressure intestine. The esophageal lumen is generally tri-radiated, one arm pointing ventrally and the other two subdorsally. The radii divide the esophagus into three sectors a dorsal and two subventrals. The esophagus is intimately connected with the feeding and thereby shows diversity of structure. It provides very useful information on the phylogenetic relationships of nematodes and is thus, considered as a reliable and important taxonomic character at all levels of classification but more so at higher levels.

MODIFICATION OF ESOPHAGUS

To survive in the environment, certain nematodes have evolved for their existence which leads to the modification of their esophagus in some nematodes, this modification are as one part esophagus/ cylindrical, two part esophagus/ bipartite and three part esophagus/ tripartite. In the phylum Nematoda, the gland cells present in the esophagus region appears to be 5 in number, an anterior and two posterior pair but in many groups there appear to be a loss of an anterior pair of sub-ventral glands. The one part and two part esophagi have 5 gland cells, one dorsal and four sub-ventrals but differ in their esophageal gland openings. The one part esophagus, specially found in order Mononchida opens just below the nerve ring, and the two part esophagi (Dorylaimid nematodes) open in the post corpus. The three part esophagus specially found in Tylenchid nematodes, have three gland cells, one dorsal side and two sub-ventrals. The dorsal gland opens at the base of the stylet knobs whereas the sub-ventral glands open at the base of pump chamber / valval apparatus in metacarpus.

ESOPHAGEAL GLAND CELL MORPHOLOGY

In this literature, more emphasis will be given to the largest group of plant parasitic nematodes, the Tylenchids, which are well adapted for plant parasitism. In addition to the stylet, tylenchid nematodes have a well developed esophagus designed for feeding on plants (Hussey, 1989a). The esophagus has a muscular metacarpus with a triradiate pump chamber and three large transcriptionally active secretory gland cells, one dorsal and two sub ventral (Endo, 1984; Hussey and Mims, 1990) in the basal bulb.

Both the glands contain large lobed nucleus with a prominent nucleolus, abundant golgi complexes, rough endoplasmic reticulum, secretory granules and other organelles typical of secretory cells (Endo, 1984). The secretory proteins are synthesized in nuclear region of gland cells and sequestered in spherical membrane- bounded granules, which are transported along microtubules in the cytoplasmic extension to accumulate near the valves in the ampulla prior to the content being secreted. Association of nerve processes and neurosecretory cells with cytoplasmic extension and ampulla of esophageal glands indicates the secretion of glandular substances is controlled by nervous system (Aderson and Byers, 1975; Endo, 1984). During secretion, gland cells are triggered rapidly to release secretory proteins in the granules by exocytosis into membranous end-sac of the valve. From this sac, the proteins pass through a duct to enter the lumen of esophagus to be injected through the stylet into host tissue. Spherical secretory granules vary in size, composition and morphology among nematode genera and species and between the dorsal and sub-ventral gland cells within a specific life stage.

DORSAL AND SUB-VENTRAL GLANDS OF *Meloidogyne incognita* (Hussey and Mims, 1990)

- ✓ Dorsal and sub-ventral glands secretory granules in *Meloidogyne incognita* changed during parasitism of plants.
- ✓ Sub-ventral glands shrank and dorsal gland enlarged with the onset of parasitism.
- ✓ Secretory granules formed by both types of glands are spherical, membrane-bound.

- ✓ Sub-ventral gland extensions in pre-parasitic second stage juveniles are packed with secretory granules having diameter of about 700-1000 nm with finely granular matrix.
- ✓ Within the matrix of each sub-ventral granule, electron- transparent core is present containing minute spherical vesicles.
- ✓ Dorsal gland extension in pre-parasitic juveniles contains fewer granules as compared to sub-ventral gland.
- ✓ Matrix of dorsal gland secretory granules formed during parasitism are homogenous and more electron dense than the matrix of sub-ventral gland granules.
- ✓ The sub-ventral gland secretory granules of parasitic juveniles and adult female appeared to be degenerated.

NATURE OF SECRETIONS

The bioactive molecules synthesized in the esophageal gland cells and secreted through nematode's protusible stylet regulate plant-nematode interactions. These stylet secretions may function in:

- a) hatching
- b) penetration and migration through root tissue
- c) modification and maintenance of feeding sites (plant cell).
- d) formation of feeding tubes.
- e) digestion of host cell content to facilitate nutrient acquisition

a. Hatching: Egg hatching may be strictly a mechanical process in which the second-stage juvenile pierces the egg shell or enzymes may be secreted to aid the hatching process. In several Tylenchid nematodes, secretory granules accumulate in the sub-ventral glands while the second-stage juvenile is still in eggs. Bird (1968a) speculates that the contents of the granules in second-stage juvenile of *Meloidogyne javanica* were secreted to hydrolyze the lipid layer of the egg and assist in penetration. But it is not important in *Globodera rostochiensis* since they use stylet to cut a slit in eggshell.

b. Penetration and migration: Invasion of plant tissue by endoparasitic nematodes or penetration through cells by stylet of ectoparasites were presume to be mechanical force but recently it has been reported that enzymes are also involved in degradation of cells by releasing cellulase and pectinase.

c. Modification and maintenance of feeding sites (plant cell): Endoparasitic nematodes use to modify the original plant cell for their survival; these modification differs from genus to genus such as in *Cryphodera* species, they form single uninucleate giant cell; Cyst nematode form syncytia, Root-knot nematode forms giant cell while Citrus nematode form nurse cell. These modifications lead to increase in metabolic activity and reduce when nutrient shrinks.

d. Formation of feeding tubes: During feeding, sedentary nematodes also injects dorsal gland secretions that form unique tube-like structure called feeding tubes within the cytoplasm of the feeding cell (Rebios, 1980; Endo, 1991; Hussey and Mims, 1991). The function of feeding tube is that it acts as a seal during feeding. Microinjection studies with fluorescent of different molecular weights showed that the walls of feeding tubes serve as a molecular sieve during nutrient uptake by the parasite (Bockenhoff and Grundler, 1994).

e. Digestion of host cell content to facilitate nutrient acquisition or Extracorporeal digestion: Example- *Ditylenchus destructor*, digest the cytoplasm of fungal hyphae cell prior to ingestion. This secretion apparently originated from granules that are formed in the dorsal gland only after nematode begins feeding.

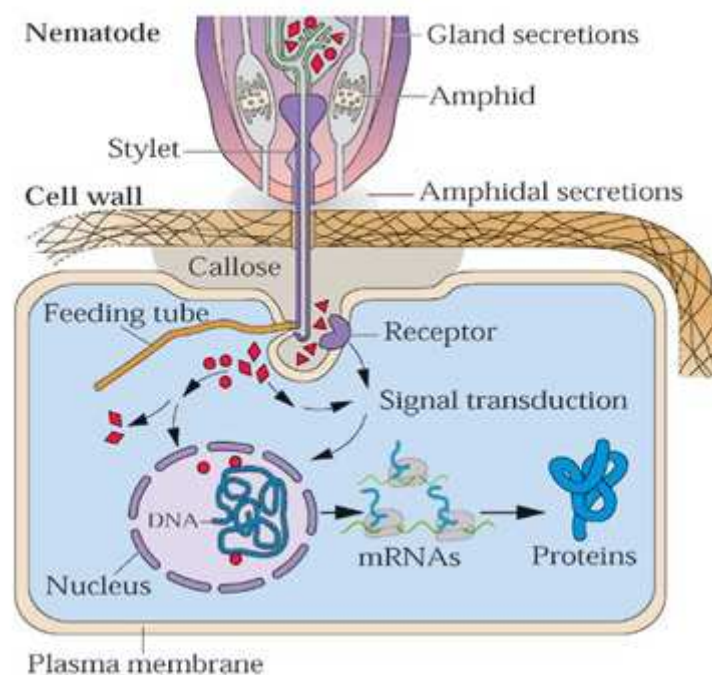


Figure 1. Schematic model of interaction of a sedentary parasitic nematode with its feeding cell (Williamson and Hussey, 1996)

Secretory molecules from sedentary endoparasites are particularly intriguing due to complex changes in plant cell phenotype, function and gene expression that they modulate. During parasitism of plant cell, nematode's stylet penetrates the cell wall but does not pierce the plasma membrane and invaginates around the stylet tip. The gland cell secretions injected through the stylet transform root cells in susceptible plants into metabolically active feeding cells. These unidentified gland secretions modify, directly or indirectly, gene expression to induce morphological, physiological and molecular changes in the recipient cells to enable them to function as a continuous source of nutrient for nematode parasitic stages. Although the mechanism(s) by which these nematodes alter plant gene expression is unknown, studies suggest that factor(s) in the nematode stylet secretions induce novel gene regulatory cascades causing the parasitized root cells to differentiate into a unique feeding cells (Bird, 1996). The gland secretions may be deposited outside the plasma membrane or inject directly into the cytoplasm of the recipient cell through a perforation in the plasma membrane at the stylet orifice. In either case, specific compounds in the secretions could bind to plant cell receptors to elicit a signal transduction cascade to modulate gene expression in the cell. Alternatively, the secretions may function as transcription factors and enter the nucleus and directly modify gene expression in recipient plant cell.

Linford (1937) gave the first evidence that root-knot nematodes inject secretions directly into host cells through stylet leading to development of giant cells. In a detailed early study of the nature of the contents of esophageal gland cells, secretion proteins but no nucleic acids were detected in sub-ventral gland cell extension in pre-parasitic *Meloidogyne javanica* second stage juveniles (Bird and Saurer, 1967). Ultrastructural cytochemical analyses performed on granules of sub-ventral gland cells of pre-parasitic second stage juveniles of *Meloidogyne incognita* were positive for acid phosphatase activity and negative for peroxidase, cellulase, DNase, RNase and nucleic acids (Sundermann and Hussey, 1988).

Adult females of *Meloidogyne* species dissected from galls and placed in an aqueous solution produce stylet secretions that precipitate at lip region. The fine structure of stylet secretions from adult females consists of electron-dense strands that usually surround a finely granular core (Bird, 1969 and Davis *et al.*, 1994). These secretions stain positive for basic protein but stain negative for several enzymes and nucleic acids (Bird and Saurer, 1967). Dorsal gland cell

secretory granules stained positive for nucleic acid, and peroxidase but not for acid phosphatase, glucuronidase, DNase, catalase, polyphenoloxidase or cellulase activity (Sundermann and Hussey, 1988).

Monoclonal antibody technology has been used to identify secretory components produced by plant parasitic nematodes. These monoclonal antibodies are also important to monitor the developmental expression of different esophageal antigens at various stages of nematode development (Atkinson and Harris, 1989; Davis *et al.*, 1994; Goverse *et al.*, 1994; Smant *et al.*, 1997).

DORSAL GLAND vs. SUB-VENTRAL GLAND (in term of its role)

The relative importance of secretion from the dorsal gland cell versus the sub-ventral gland cells in host- nematode interactions has been previously debated. The location of the dorsal gland valve near the stylet knobs readily allows dorsal gland cell secretions to release through the stylet. Labeling of stylet secretion produced by the adult females of *M. incognita* with the monoclonal antibody specific for dorsal gland granules (Hussey 1989b) and video-enhanced observations of nematode secretory activity *in vivo* (Wyss and Zunke, 1986) provide evidence that dorsal gland cell secretions can be released through stylets of plant parasitic nematodes. In contrast, the release of secretory proteins from the sub-ventral gland cells into the lumen of esophagus directly behind the tri-radiate pump chamber in the metacarpus and the rigid and circular lumen of esophagus anterior to the pump chamber was considered to restrict anterior flow of sub-ventral gland secretions (Doncaster, 1971). Therefore, secretions of the sub-ventral gland cells were originally thought to pass only posteriorly in the esophageal lumen to the intestine to function in intracorporeal digestion (Hussey, 1989a and Wyss *et al.*, 1992). But studies using monoclonal antibodies specific for sub-ventral gland secretions demonstrated that antigens in the nematode stylet secretions originate in the sub-ventral gland cells (Davis *et al.*, 1994; Goverse *et al.*, 1994 and Smant *et al.*, 1997). In addition to this, the recent discovery that Beta-1, 4- endoglucanase (cellulase) are synthesized in the sub-ventral gland cells of cyst nematodes and secreted through the nematode's stylet *in planta* unequivocally establishes a role for sub-ventral gland secretions in plant parasitism (Smant *et al.*, 1998 and Wang *et al.*, 1999). The predicted morphological resistance to anterior flow of sub-ventral gland secretions in the esophageal lumen during maximal pumping of the metacarpus must be minimal during secretion phase of a feeding cycle. Furthermore, production of stylet secretions *in vitro* by pre-parasitic juveniles and adult females involves very little movement of the metacorporeal pump chamber, also when the tri-radiate pump chamber is closed; there is a slight gap between the sclerotized walls of the pump chamber that might permit anterior flow of the sub-ventral gland cell secretions during phase of the feeding cycle.

NEMATODE PARASITISM GENES

Analysis of differential nematode gene expression has been used as an alternative method to isolate genes expressed specifically within the esophageal gland cells of plant-parasitic nematodes.

Nematode parasitism genes targets those genes that code for proteins released from the nematode where it directly interact with the host molecules to promote the parasitic interaction. Parasitism proteins produced in esophageal glands are the most studied examples, to know the host- nematode relationship. It is synthesized as pre-proteins with N-terminal signal peptides and matured in Endoplasmic reticulum of gland cells where signal peptide is cleave off and matured protein passes along the secretory pathway.

The products of parasitism genes expressed in esophageal glands which are secreted through stylet into host tissue are the most evolutionary advanced adaptations for plant parasitic nematodes (Davis *et al.*, 2000). A parasitome and a secreted products of parasitism genes known as *Herein*, expressed in nematode esophageal gland helps to mediate parasitism (Greenbaum *et al.*, 2001) in the host. During parasitism, nematode's stylet penetrates the host cell wall releasing cellulase enzyme expressed in the two sub-ventral glands but does not pierce

plasma membrane due to which it provide an opening exclusively at the stylet orifice (Rebios, 1990).

POTENTIAL ROLE OF PARASITISM PROTEINS

- Digestion of cell wall barrier
- Formation of feeding cells
- Formation of feeding tubes (allow 28-40kd molecules only to be ingested)
- Maintenance of feeding cells

ROOT-KNOT AND CYST NEMATODE PARASITISM GENES

Only expressed in the esophageal gland and has a signal peptide.

- i. **RanBPM:** Expressed in dorsal gland, has complex functions including the regulation of cell cycle (reported from potato cyst nematode).
- ii. **Annexin:** Expressed in dorsal gland of soybean cyst nematode.
- iii. **Venom-allergen proteins:** Reported from root-knot and cyst nematode. Involved in early parasitism.
- iv. **Calcirectulin:** Produced in sub-ventral glands of root-knot nematode. Involved in early parasitism.
- v. **Chitinase:** Expressed in sub-ventral glands of soybean cyst nematode. It has no role in egg hatching but are expressed during early phases of parasitism after penetration.

MORE NEMATODE GENES INVOLVED IN PARASITISM

Expressed elsewhere and has a no signal peptide.

- i. **Peroxidase:** Found in potato cyst nematode hypodermis and nematode body surface. Presumably detoxify ROS (antioxidant).
- ii. **FAR:** Retinol and fatty acid binding protein, found in *Globodera pallida*.
- iii. **SXP/RAL-2:** Found in *G. rostochiensis*, function not known.
- iv. **14-3-3:** Dorsal gland of *Meloidogyne incognita*, has no signal peptide, function not known.

CONCLUSION AND FUTURE PROSPECTS

Nematode secretions are undoubtedly found to be involved in the plant cellular responses to nematode feeding. The feeding behavior of migratory endoparasites and parasitic nematodes indicates a simple digestive function for secretion they inject into the plant cells. However, highly evolved sedentary endoparasites does not evoke a destructive process, the secretion they injected are compatible with the cytoplasm of the host cell.

Significant progress are now being made in identifying parasitism genes expressed in nematode esophageal gland cells whose products are secreted into the plant tissue to control the complex process of parasitism. These stylet secretions facilitate nematode migration in roots and mediate the modification of root cells into the elaborate feeding cells, which are the sole source of nutrients for sedentary endoparasitic nematodes. Identifying the complete profile of parasitism genes expressed throughout the parasitic cycle of a nematode is the key to understanding the molecular basis of nematode parasitism of plants and defining what makes a nematode a plant parasite previously. Interspecific and intraspecific comparison of the structure of parasitism genes encoding stylet secretions that induce feeding cell formation will also provide the knowledge that should lead to establishing a genetic basis for host range specificity among nematode species or races. Understanding the genetic variability will have an important positive effect on the development and deployment of sustainable nematode management strategies.

The role of the sub-ventral gland cells in the infection process differs from that of the dorsal gland cell in plant parasitic nematodes during the parasitic cycle. Parasitism genes expressed in the sub-ventral gland cells primarily encode cell-wall digesting enzymes used by the nematode during migration in roots. The sub-ventral gland cells primarily function during the early stages of the infection process is also supported by the morphological changes in these gland cells, which become smaller and contain fewer secretory granules in later nematode stages during the parasitic cycle (Hussey and Mims 1990).

Characterization of parasitism genes encoding stylet secretions is essential for understanding the molecular genetics of the nematode-host interactions. The discovery of a comprehensive profile of a nematode parasitome represents a significant first step towards dissecting the molecular interactions of a nematode with its host.

Antibodies generated against over expressed parasitism gene products can be used to immune localize nematode proteins secreted into root tissue during parasitism (Wang *et al.*, 1999).

Use of RNAi method as a molecular genetic tool will allow us to study parasitism gene function in plant-parasitic nematodes using certain specific nematode genes to be silenced by soaking pre-parasitic nematodes in dsRNA complementary to the coding region of the gene-of-interest. Once parasitism genes essential for parthenogenesis in sedentary endoparasitic nematodes are characterized, then the knowledge will allow several approaches to be implemented for developing transgenic resistance plants including the expression of peptide, plantibodies or dsRNA that specifically inhibit target nematode parasitism genes directly or indirectly. The use of biotechnology to manage pests and pathogens in agriculture is coming to fruition, and the recent discoveries of nematode parasitism genes provide an unprecedented opportunity for limiting nematode damage to multiple crop plant.

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