

Changes in the Expression of Genes in Soybean Roots Infected by Nematodes

Benjamin F. Matthews¹, Heba M.M. Ibrahim^{1,2} and Vincent P. Klink³

¹United States Department of Agriculture, Agricultural Research Service, Soybean Genomics and Improvement Laboratory, Beltsville,

^{1,2}Genetics Department, Cairo University, Cairo,

³Department of Biological Sciences, Mississippi State University, Mississippi State, MS

^{1,3}USA

²Egypt

1. Introduction

1.1 Plant nematodes

Plant parasitic nematodes cause severe damage to plants and are responsible for billions of dollars of losses worldwide (Koenning et al. 2007). Soybean cyst nematode (SCN; *Heterodera glycines*; Fig. 1a) and root-knot nematode (RKN; *Meloidogyne* spp.; Fig. 1b) are sedentary obligate parasites of plants. SCN is the major pest of soybean and causes an estimated one billion dollars in losses annually in the US (Wrather & Koenning 2006). RKN is a major pest of vegetables and can become a serious problem on soybean, especially on edible soybean planted in areas used to grow vegetables (Adegbite & Adesiyun 2005). The genera *Meloidogyne* is widespread and is considered, economically and agriculturally, as a very important group of plant pathogens. The host range of *Meloidogyne* is very wide as it attacks almost all plant species (Sasser 1980). Both SCN and RKN are sedentary endoparasites and they cause dramatic morphological and physiological changes in plant cells while inflicting severe decreases on yield. Chemical methods of nematode control are costly and can damage the environment, especially with contamination of ground water. Therefore, the preferred method of nematode control is the use of resistant or tolerant varieties, when available. Unfortunately, a plant with resistance to one population of nematode is often susceptible to a different population due to the wide genetic variation of nematode populations.

When a plant parasitic nematode infects a plant root, the nematode and the plant enters an intricate interactive relationship with the host that is attempting to inhibit nematode development, while the nematode's goal is to develop and reproduce. The life cycle of SCN and cellular responses of soybean to SCN infection have been documented and reviewed extensively (Bird & Koltai 2000; Endo 1964; Endo, 1965; Endo, 1992; Govere et al. 2000; Lilley et al. 2005; Mitchum & Baum 2008; Niblack et al. 2006; Williamson & Gleason 2003; Abad & Williamson 2010; Klink et al. 2011a). The SCN egg can be found in soil and within the mature female. The second stage juvenile (J2) hatches from the egg, searches for a root of a plant host, penetrates the root epidermis, and migrates intracellularly, using its stylet and enzyme secretions to disrupt cells and force its way toward the vascular tissue.

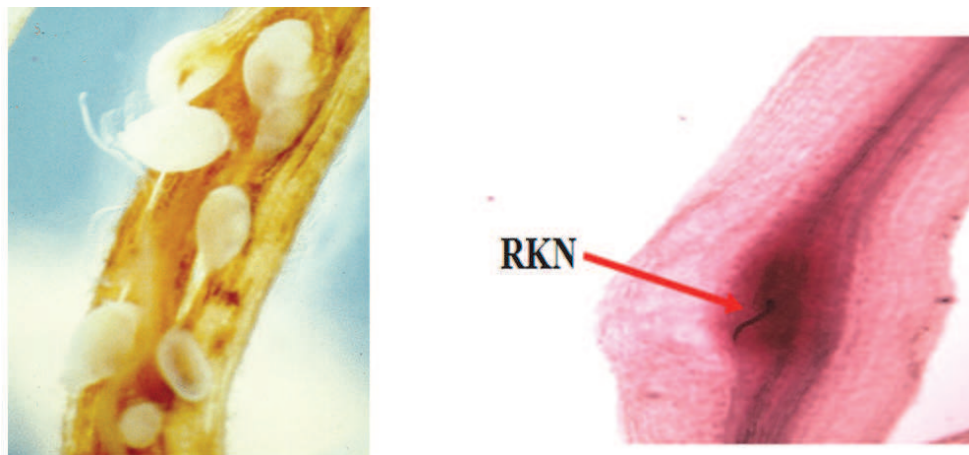


Fig. 1. (Left) Female cyst of the soybean cyst nematode at 21 days after infection(dai). (Right) Gall formed by the root-knot nematode at 14 dai. The RKN appears red after staining with acid fuchsin.

There nematode selects a feeding cell adjacent to the vascular tissue, pierces the cell wall and injects material from its esophageal gland. The proteins injected by SCN alter the physiology and metabolism of the plant cell and surrounding cells so a syncytium is formed by dissolution of the walls of surrounding cells and the fusion of those adjacent cells. The nematode becomes sedentary, feeds and molt three times to reach maturity. The anterior portion of the female SCN remains inside the root while the posterior portion breaks through the epidermis of the root at approximately 12 to 14 dai. At maturity, the outer integument of the mature female SCN hardens to protect eggs within its body, while some eggs are extruded in a gelatinous mass. SCN can complete its life cycle in three to four weeks with one female producing 200 to 600 eggs (Young, 1992). Thus, SCN can complete numerous life cycles during the soybean growing season and infest a field rapidly.

The RKN follows a similar pattern of development to that of SCN. The RKN also goes through five different developmental stages starting with the J1 which molts once inside the egg. After hatching, the motile J2 immediately searches for a plant host and infects immediately behind the root tip and migrates between the plant cells. RKN does not feed during this stage; instead it uses its lipid reserves in the gut (Eisenback & Triantaphyllou, 1991). When the RKN J2 reaches the vascular cylinder, it becomes sedentary and establishes its permanent feeding site by injection of proteins into selected parenchymal adjacent to the vascular system to form giant cells (Caillaud *et al.*, 2001). The giant cells expand and undergo multiple rounds of mitosis without cell division. After feeding for only 24 hours, the RKN molts three times to reach the adult stage (Eisenback & Triantaphyllou, 1991). The entire body of the RKN remains within the root and infection of roots by RKN can be easily recognized by the "knots" or "galls" formed where they feed and develop (Caillaud *et al.*, 2001). The mature adult female deposits its eggs in a gelatinous mass, which remain attached to the end of the female's body and can be observed on the gall surface. One adult female can lay hundreds to thousands of eggs in three months.

It is important to reiterate that the SCN and RKN puncture the plant cell wall with its stylet to inject secretions from its esophageal glands. These secretions are important to altering the

plant cell morphology and metabolism to form a feeding structure, called the syncytium in the case of SCN or giant cell in the case of RKN. More than 60 genes have been identified that are expressed in the esophageal glands of SCN, many of which have no known function (Gao *et al.* 2001, 2003; Williamson & Gleason 2003; Davis *et al.*, 2004; Davis & Mitchum, 2005). Some of the genes encoding these proteins are similar to microbial genes or genes of animal-parasitic nematodes. Knowledge about these secreted proteins from the nematode and their interactions with targets within the plant cell during infection provides a better understanding of the interaction between the host cells and the parasite.

During the establishment of their feeding sites, nematodes secrete into the plant cell several different proteins and enzymes made in the esophageal gland (Davis *et al.* 2004; Gao *et al.* 2001, 2003). The SCN esophageal glands produce β -1,4-endoglucanase and pectate lyase to degrade the plant cell wall (Smant *et al.*, 1998; Hang *et al.*, 2003). Some enzymatic reactions of these nematode proteins on the cell wall may produce compounds that interact with signal transduction receptors on the plant host cells (Davis *et al.* 2004; Davis & Mitchum 2005; Mitchum & Baum, 2008). A model of a potential secretomes from plant parasitic nematode has been proposed by Davis *et al.* (2004) and shows involvement of cell wall remodeling proteins, such as endoglucanases, and expansions. Plant parasitic nematodes also produce proteins that may mimic plant proteins, such as chorismate mutase (Doyle & Lambert, 2003; Bekal *et al.* 2003; Lambert *et al.* 1999) and CLAVATA (Wang *et al.* 2005; Wang *et al.*, 2010; Replogle *et al.* 2010). Some of the secreted proteins contain a peptide sequence that targets the protein to the nucleus, while other proteins remain in the cytoplasm of the plant cell (Elling *et al.*, 2007).

2. Gene expression in soybean

Gene expression has been examined in both compatible and incompatible interactions of SCN with soybean roots using Affimetrix microarrays containing approximately 37,000 set of probes (Klink *et al.* 2007 a; 2009a, 2010, 2011b) (Ithal *et al.* 2007a,b). The identification of gene expression occurring specifically within the syncytium was first reported by Klink *et al.* (2005). The experiments provided a means for examining expression at the genomic scale. Also, changes in gene expression in the cells at the feeding site of the nematode have been examined using microarrays (Klink *et al.* 2007b, 2009a, 2010a, 2011b; Ibrahim *et al.* 2011). In all of these studies approximately two to ten per cent of the genes represented on the microarray changed in expression by over 1.5-fold. Through microarray studies, many genes were identified that are involved in metabolism, energy, defense and other areas, which provided new insights into plant-pathogen interactions. At the first phase of parasitism, which is prior to feeding or at 12 h after infection (dai), gene expression patterns in the root were found to be similar in both the susceptible and resistant reaction, when the nematode first attempts to establish itself in the host. Gene expression during the second phase depends on the defense response of the host plant (Klink *et al.*, 2007a). If the host is resistant or displays an incompatible interaction to the nematode, then gene expression patterns are different than if the host is susceptible or if the host displays a compatible reaction with the nematode, although there are some commonalities (Klink *et al.* 2007b, 2009a, 2010b). In either case a syncytium is formed. However, in the incompatible interaction, the syncytium degrades, whereas the syncytium is maintained and expands in the compatible interaction. During the formation of the nematode feeding sites, many pathways are involved in the induction of syncytia. For example, solidifying and lignifying

the cell wall of the syncytium, down-regulation of the plant defense system, such as the pathway leading to jasmonic acid, occur in the plant selected feeding cells during the nematode parasitism process (Ithal *et al.*, 2007a; Klink *et al.*, 2007b). Meanwhile other genes and pathways are utilized by the plant exhibiting an incompatible reaction (Klink *et al.*, 2007b, 2009a, 2010b), wherein the syncytium degrades.

Gene expression during only the compatible interaction has been studied between RKN and soybean using soybean Affymetrix microarrays roots (Ibrahim *et al.*, 2010). The nematode not only triggers the defense response of the root and forms a feeding site or giant cell, but also redesigns the morphology of root cells to form a gall. The giant cell is interesting in that it undergoes karyokinesis, but not cytokinesis. Furthermore, genes encoding enzymes in important biochemical pathways were found to be either highly induced or highly suppressed during the infection of the soybean roots with RKN (Ibrahim *et al.* 2010).

Analysis of microarray data can be complex and requires a great deal of time and effort. Commonly, microarray data sets are very large and take a long time to analyze, identify and understand changes in metabolic pathways. Most of the time, only genes already known to be involved in resistance are focused in on with the rest of the data never analyzed to its full potential. PAICE (Pathway Analysis and Integrated Coloring of Experiments) (PAICE (Paice_v2_90.jar) <http://sourceforge.net/projects/paice/>) (Hosseini *et al.* unpublished) software has been used to analyze microarray data and connect gene expression results between microarrays and illustrations of biochemical pathways found in the Kyoto Encyclopedia of Genes and Genomes (Ibrahim *et al.*, 2011; Klink *et al.*, 2009a, 2010b, 2011b; Tremblay *et al.*, 2010). This program provides visualization of microarray gene expression data relevant to known biochemical pathways with a color scheme coding up-regulated genes in various shades of green and down-regulated genes in various shades of red depending on gene expression level. This tool makes key changes in gene expression in biochemical pathways stand out and makes comparison of pathway changes among treatments and across time points easier. This tool will be used in this chapter to display some of the gene expression data from various relevant publications.

2.1 Carbohydrate and energy

The female nematode requires large amounts of energy from its host so it can develop and produce large quantities of eggs. In syncytia formed during both a compatible interaction at 5 and 10 dai and the incompatible interaction at 6 dai of soybean roots with SCN (Ithal *et al.* 2007a,b; Klink *et al.* 2007b, 2009a, 2010a); Fig 2) and in galls formed by RKN at 12 dai in a compatible interaction (Ibrahim *et al.* 2010), genes involved in glycolysis are up-regulated. Genes that are in common and up-regulated between the compatible and incompatible interactions of SCN with roots include genes encoding enzymes encompassing the entire pathway between α -D-glucose-6-phosphate and pyruvate. Also, transcripts of genes encoding enzymes between β -D-Fructose-6-phosphate and α -D-glucose and β -D-glucose are elevated in both cases. There are two differences in gene expression levels in the glycolysis/gluconeogenesis pathway that are striking. First the amount transcript of the gene encoding aldose 1-epimerase (EC 5.1.3.3), catalyzing the first step in galactose metabolism that converts β -D-glucose into α -D-glucose, is moderately lower at 10 dai in syncytia formed by SCN, but is elevated in the SCN incompatible reaction at 6 dai and in root galls formed by RKN at 12. An increase in this enzyme is associated with a decrease in

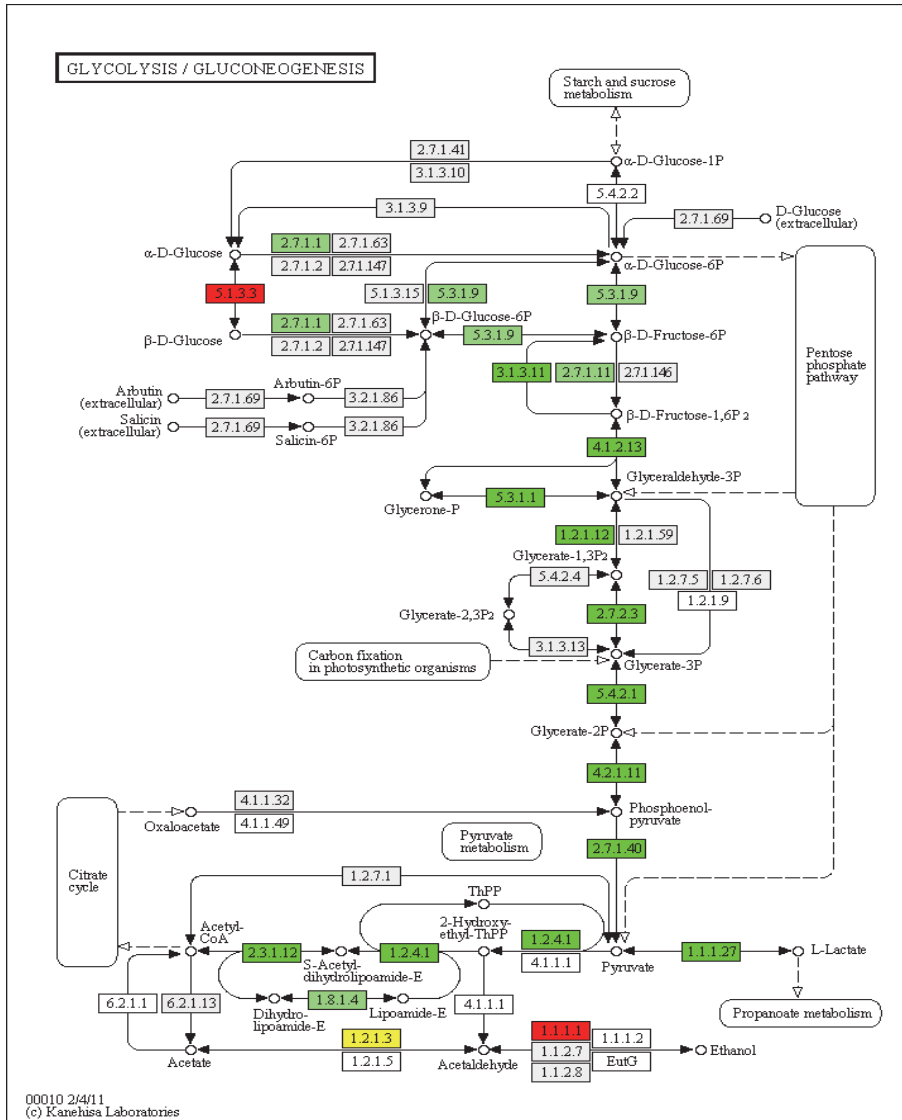


Fig. 2. Expression profiles at 10 dai in a susceptible reaction of Williams 82 with SCN are displayed for the genes encoding enzymes in glycolysis/ gluconeogenesis on the KEGG pathway diagram. Enzyme commission numbers in the rectangles are provided by KEGG. Rectangles are colored light green for genes up-regulated in the first 50%, medium green for genes up-regulated in the 50 to 75 quartile and dark green for genes up-regulated in the top 25 %. Enzymes colored in red are encoded by down-regulated genes using a similar scheme. Enzymes colored in yellow are encoded by more than one gene and different copies of that gene are up- and down-regulated, respectively. Rectangles colored light gray indicate that the genes encoding those enzymes are not annotated in our soybean microarray database.

the production of cellulose (Fekete *et al.* 2008). The second pronounced difference is that the gene encoding fructose-bisphosphatase (EC 3.1.3.11) is not elevated in galls, whereas it is one of the genes with the most highly elevated abundance of transcripts in syncytia during the compatible interaction at 5 and 10 dai in syncytia. It is not elevated at 9 dai in the incompatible interaction of SCN with soybean. The reaction of fructose-bisphosphatase is in the direction of starch formation. This supports metabolite studies of the interaction of *Arabidopsis* with the sugar beet nematode, *Heterodera schachtii*, indicate that syncytia accumulate starch during this interaction (Hofmann & Grundle 2008a,b, 2010).

2.2 Cell wall modification and remodeling in soybean

Syncytial cells formed by SCN may encompass 200 to 400 cells, while giant cells formed by RKN sometimes reach more than 400-times the size of a normal cell and may contain more than one hundred nuclei (Caillaud *et al.*, 2008). The expansion of the syncytium and the giant cell are accompanied by extensive cell wall modification. Microarray data indicate that the expression of many genes involved in cell wall extension and remodeling is altered (Klink *et al.* 2007b, 2009a,b; Ithal *et al.* 2007; Ibrahim *et al.* 2011). For example more pectinases are expressed in the syncytium during a compatible interaction at 10 dai than in an incompatible reaction at 9 dai (Fig 3). One gene represented by

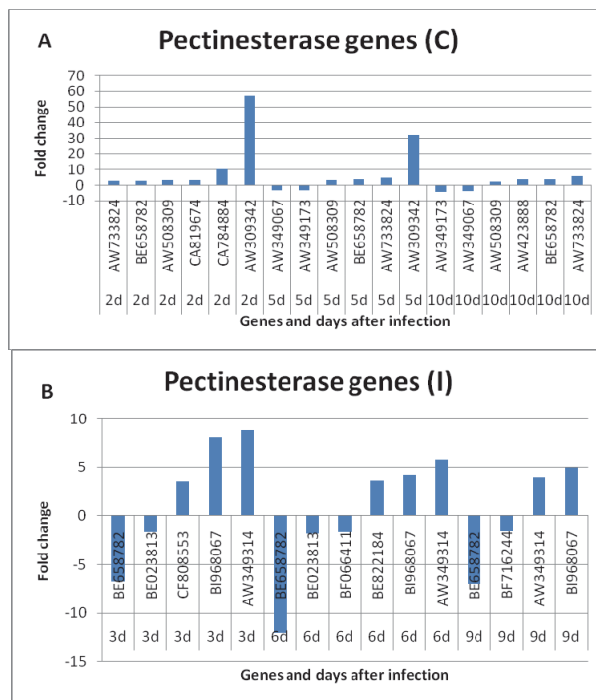


Fig. 3. (A) Fold change in expression of pectinesterases in syncytia in a compatible interaction (C) at 2, 5 and 10 dai. Data from Ithal. *et al.* (2007b) and (B) an incompatible interaction (I) at 3, 6 and 9 dai. Data from Klink *et al.* (2009a). Genes are represented by GenBank numbers.

GenBank number AW309342 experiences more than a 50-fold increase in expression at 2 dai and over 30-fold increase in expression at 5 dai in syncytia in the susceptible reaction. Only three genes encoding pectinesterase are overexpressed in syncytia of the incompatible reaction at 3 and 6 dai and one gene represented by BE658782 is over 5-fold decreased in expression.

Nine genes encoding xyloglucanases are up-regulated in syncytia at 2 and 5 dai during the susceptible reaction. At 5 dai three genes, represented by GenBank numbers BU764179, AW707175, and BQ298739 are more than 15-fold increased in transcript abundance (Fig. 4a). Only four genes encoding xyloglucanases are up-regulated in syncytia during the incompatible reaction at 3 and 6 dai, while one gene represented by AW310549 is down-regulated approximately 30-fold (Fig. 4b). The lack of sustained upregulation and in some cases the actual downregulation of cell remodeling genes in the incompatible reaction is indicative of the fact that the syncytium is not sustained in the incompatible reaction for more than two or three days before it degrades.

Numerous cellulases, endo-1,4- β -glucanases, are altered in regulation in soybean roots upon SCN infection. Two genes encoding cellulases are increased in expression over 60-fold at 3 dai in the incompatible reaction, BI969418 and BI785739. The first, BI969418, decreases to 10-fold over expression at 6 and 9 dai, while the second, BI785739, returns to control levels, while CF806812 increases over 50-fold in expression at 6 and 9 dai in the incompatible interaction (Klink *et al.*, 2009a). In contrast in the compatible reaction, two genes, represented by CD394414 and BI971040, encoding cellulases are increased at 2 dai 5- and 10-fold, respectively, while genes represented by BM091956 and BI968056 are increased approximately 28- and 46-fold at 5 dai. At 10dai two genes are increased over 36- and 78--fold, MI968056 and BN091956, respectively (Ithal. *et al.* 2007b).

Expansion of giant cells formed by RKN also requires extensive cell wall remodeling and modification. After infection with RKN (12 dai and 10 wai (weeks after infection)) soybean genes encoding cell-wall modifying xyloglucan endotransglycosylase/hydrolase and endoxyloglucan transferase A2 are differentially expressed (Ibrahim *et al.* 2011). These enzymes are known to have an important role in cell wall softening and degradation (Nishitani, 1998). In addition, some β -endo-1,4-glucanases family members, involved in cell wall remodeling and expansion, were shown to be up-regulated at both 12 dai and 10 wai. Many genes encoding endo-1,4- β -glucanases family members were up-regulated at both time points, 12 dai and 10 wai (Ibrahim *et al.* 2011). This enzyme is also involved in cell wall remodeling and expansion. Some, members of the endo-1,4- β -glucanase gene family are expressed in feeding cells formed by RKN and cyst nematode in tobacco plants (Goellner *et al.*, 2001). The promoter of one of these genes is strongly activated in feeding cells formed by *Meloidogyne incognita* as indicated by strong GUS expression (Mitchum *et al.*; 2004). Also, there is an increase in expression of the gene encoding expansin A, which is consistent with other investigations, wherein the expansin (LeEXPA5) genes in *A. thaliana* and tomato were shown to be up-regulated in developing giant cells after infection of roots with *Meloidogyne* (Jammes *et al.*, 2005; Gal *et al.*, 2006). Moreover, down-regulation of cellulose synthase and over-expression of pectinesterase that degrades pectin to pectate coincide with a breakdown of the cell wall during the early time points of infection with RKN. These results are consistent with those of Jammes *et al.* (2005), wherein genes encoding pectin esterases and pectate lyases were activated in *Arabidopsis thaliana* (roots after infection with *Meloidogyne incognita* and the cell walls loosening process occurred during the development of the giant cell as well.

2.3 Plant defense system

When a nematode invades a plant root, it must repress or control the plant defense response, so it can successfully establish its permanent feeding site (Caillaud *et al.*, 2001). These defense responses may include the production of jasmonic acid and salicylic acid, the hypersensitive response, cell wall strengthening, the production of pathogenesis related (PR) proteins, and other cellular defense responses. There are changes in the expression of genes involved in many of these defense responses in both compatible and incompatible interactions of SCN with soybean and with RKN and soybean in the compatible interaction. Many of the same genes are altered in expression in both the compatible and incompatible interaction. However, the amount of change in transcript abundance may be very important and in some cases a gene is up regulated in one interaction and down regulated in another interaction

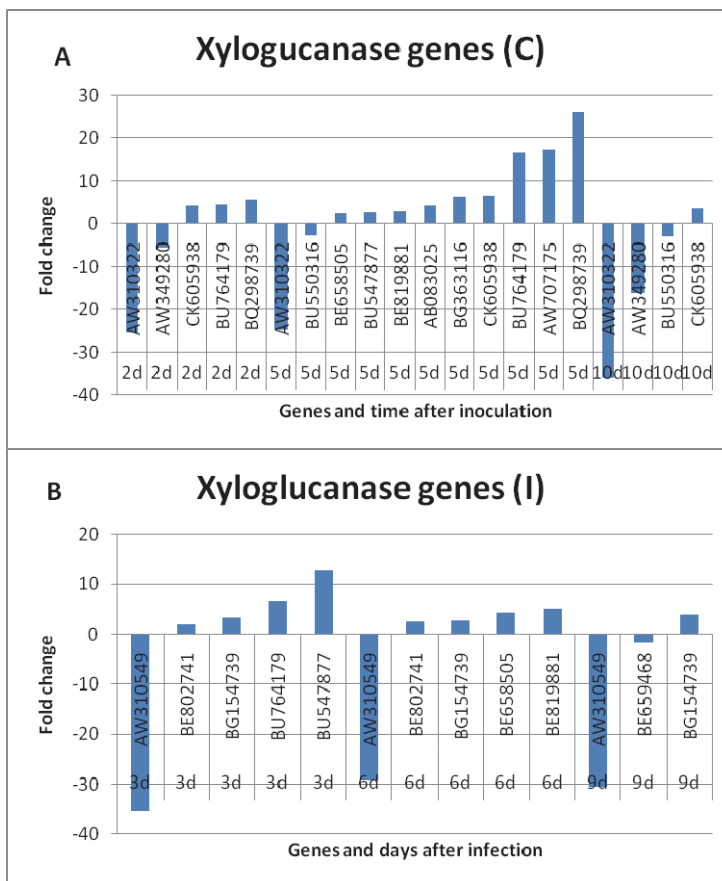


Fig. 4. (A) Fold change in expression of genes encoding xyloglucanases in syncytia in a compatible interaction (S) at 2, 5 and 10 dai. Data from Ithal. *et al.* (2007b) and (B) an incompatible interaction at 3, 6 and 9 dai Data from Klink *et al.* (2009a). Genes are represented by GenBank numbers.

2.3.1 Alpha-linolenic acid and jasmonic acid biosynthesis

The pathway leading to jasmonic acid biosynthesis is one of the pathways associated with pathogen resistance that was significantly affected by both SCN and RKN infection. In soybean there are several lipoxygenase gene family members. Several members of this gene family are expressed higher in the compatible reaction of SCN with soybean at 2, 5 and 10 dai, specifically CF808603, CD409280 and BM092012, which are elevated 2.4- to 6.3-fold (Data from Ithal *et al.* 2007b). In contrast, in the incompatible reaction of SCN with soybean, several members of the gene family are down-regulated, while others are up-regulated, ranging between approximately -22- to 22-fold (Klink *et al.* 2007a). Genes encoding allene oxide synthase (AOS) and allene oxide cyclase (AOC) are not greatly changed in the compatible interaction at 2, 5 and 10 dai fold (data from Ithal *et al.* 2007b). However, three members of the AOS gene family are down regulated in the incompatible interaction at 3 dai, while syncytia are forming. Then expression of one gene family member is increased at 6 and 9 dai as the syncytia collapse and become non-functional (Fig. 5 A; Klink *et al.* 2007b). Expression of genes encoding AOC is increased in syncytia during the incompatible reaction, especially at 3 dai, then decreases in expression at 6 and 9 dai (Fig. 5B; data from Klink *et al.* 2007a). A genes encoding 12-oxyphytyldienoate reductase 1 (OPR1), represented by BI968944, is strongly down-regulated in the compatible interaction of SCN with soybean roots (Ithal *et al.* 2007b), while a gene encoding OPR3, represented by BU765938, is up regulated 14-fold at 6 dai in the incompatible reaction (Fig 5C; Klink *et al.* 2007b). Thus, there is an increase in transcripts for specific gene members encoding enzymes through the pathway leading to JA biosynthesis in the incompatible reaction of SCN with soybean roots, while there is either no effect on genes encoding AOS and AOC or a decrease in transcript levels in the case of the gene encoding OPR1 in the compatible reaction. JA biosynthesis is one of the pathways affected in soybean roots by infection with RKN at 12 dai and 10 wai (Ibrahim *et al.*, 2011). At 12 dai, most of the genes encoding enzymes encoding lipoxygenase family members were up-regulated. Lipoxygenase is important in the biosynthesis of oxylipins and it is important in the response of plants during wounding and attack by pathogens (Gobel *et al.*, 2001). Reduction of the expression of the gene encoding this lipoxygenase resulted in an increase in susceptibility of transgenic potato plants to insect attack (Gobel *et al.*, 2001). Over-expression of the gene encoding lipoxygenase could mean a high accumulation of 9-HPOTrE, as it is one of the major products of lipoxygenase (Fig. 6). Interestingly, 9-HPOTrE is involved in the activation of the plant defense response directly or through its metabolites. In potato plants, 9-HPOTrE is produced in response to injury or infection. The role of 9-HPOTrE in the plant defense response suggests that there may be a new pathway leading to LOX-mediated defense responses (Reddy *et al.*, 2000). The same results have been observed in pigeon pea seedlings after infection with *Fusarium udum* (Reddy *et al.*, 2000).

Transcript abundance of genes encoding lipoxygenase was much lower at 10 wai (weeks after infection) than at 12 dai in roots infected by RKN (Ibrahim *et al.* 2011). Three of seven gene family members encoding lipoxygenase were down-regulated. Also, all of the allene oxide synthase gene family members were greatly down-regulated at 10 wai This suggests that at 12 dai the plant defense system is still struggling to fight the infection, but after prolonged infection (10 wai) most of the genes that encode enzymes responsible for the production of jasmonic acid were turned off in the compatible interaction. Genes in this pathway could be a target for testing to determine if resistance to nematode infection can be increased in transformed plants by over-expression of these genes.

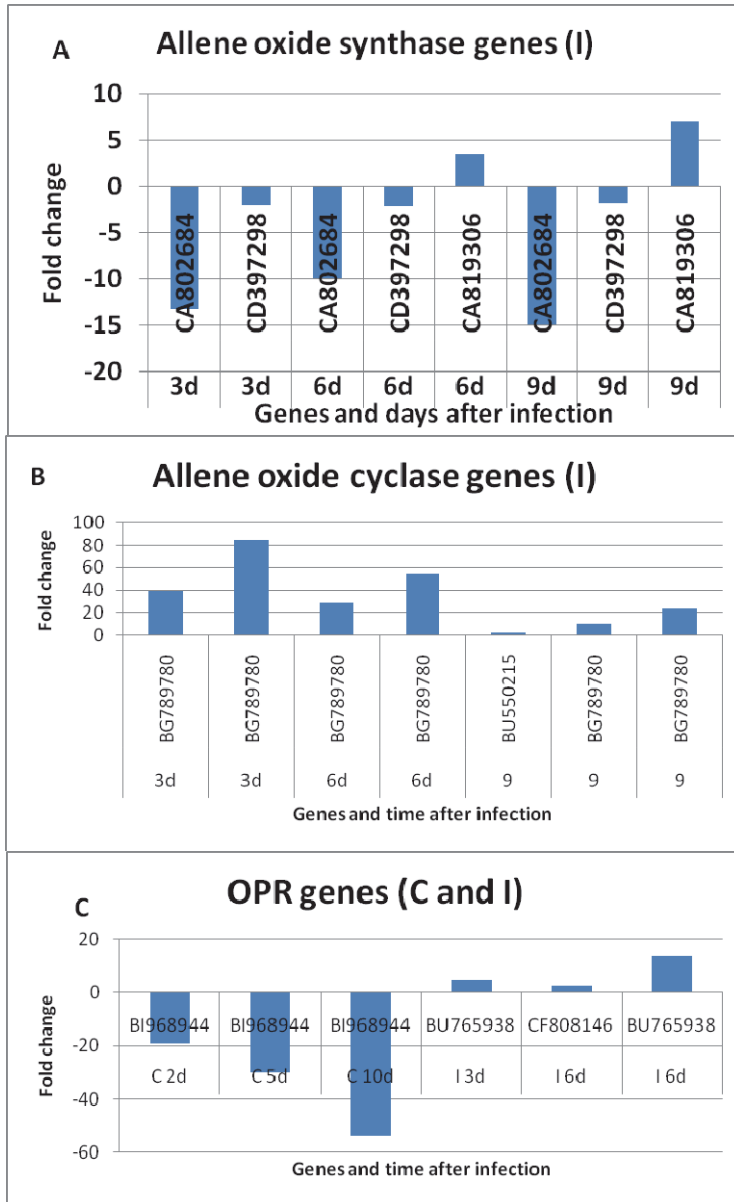


Fig. 5. A) Fold change in expression of genes encoding allene oxide synthase (AOS); (B) fold change in expression of genes encoding allene oxide cyclase (AOC) in sysncytia of an incompatible reaction of SCN with soybean; and C) fold change in expression of genes encoding 12-Oxyphytodiienoate reductase in sysncytia in a compatible interaction (C) at 2, 5 and 10 dai (data from Ithal. *et al.* 2007b) and incompatible interaction (I) at 3, 6 and 9 dai (data from Klink *et al.* 2009b). Genes are represented by GenBank numbers.

2.3.2 Pathogen related protein (PR) and transcription factors:

Pathogen related (PR) proteins are induced systemically by the interaction of a pathogen with its host (Van Loon & Van Strien, 1999). PR-1 and PR-2 are induced by SA (Ohshima *et al.*, 1990, Hennig *et al.*, 1993), while basic PR genes are induced by JA (Niki *et al.* 1998). Genes encoding enzymes involved in JA synthesis were discussed above. Unfortunately, genes important to salicylic acid biosynthesis were either not represented on the microarray chip or were not annotated. However, genes encoding proteins of the PR-1, PR-2 and PR-5 families were up-regulated at 3, 6 and 9 dai in the incompatible interaction of soybean with SCN, suggesting that salicylic acid or its derivatives may be synthesized at these time points.

The PR1 gene, represented by CF806816, was increased 900, 2100 and 1600-fold at 3, 6, and 9 dai, respectively in the incompatible interaction of SCN with soybean, while the PR1 gene, represented by BQ628525, was over expressed 70, 240, 160-fold at 3, 6, and 9 dai (Klink *et al.* 2009b). During the compatible interaction, few PR1 genes were increased in expression and only one gene, represented by BU548404, was increased over 10-fold (Ithal. *et al.*, 2007b) and this was at 2 dai, when the nematode first initiates feeding. At 5 dai only two genes were increased in expression and this was at 5.6-fold and 2.8-fold, respectively. Only one PR-1 gene was increased in expression at 10 dai in the compatible interaction and that was only 5-fold increased in expression. Transcript levels of genes encoding PAL are also more strongly up-regulated in tomato roots displaying an incompatible interaction with the potato cyst nematode (*Globodera rostochiensis*), than in the compatible interaction (Uehara *et al.*, 2010). Arabidopsis roots infected with beet-cyst nematode (*Heterodera schachtii*), transcript levels of PR-1, PR-2, and PR-5 were increased, while PR-3 and PR-4 remained at similar levels to control plants (Hamamouch *et al.* 2010). Transcript levels of genes encoding PR-1 and PR5 were also increased in the incompatible interaction of Arabidopsis with the RKN, *M. incognita*, while transcript levels of PR-3 were elevated to a lesser extent. PR-3 and PR-4 are different types of chitinase. Seven chitinase genes are increased in expression at 3 dai in the incompatible reaction of soybean with SCN; three are approximately 20-fold over-expressed. At six dai, three genes encoding chitinase are expressed; one is 74-fold; A second gene is 33-fold increased in expression. No genes encoding chitinase are over-expressed in the incompatible reaction at 2 dai, and only one gene is over expressed at 5 and 10 dai, 6- and 15-fold, respectively (Fig 6). PR10 genes, represented by X60043, CF921432 and CF805736, are increased in expression 200-fold or more at all time points in both the compatible and incompatible interactions of SCN with soybean roots.

During the interaction of soybean roots with RKN, many genes encoding several PR proteins were altered in expression (Ibrahim *et al.*, 2011). Transcripts of the gene encoding PR-1 were increased 78-fold at 12 dai in the compatible interaction of soybean roots with RKN. After prolonged infection by RKN at 10 wai, transcript levels of two genes encoding PR-1 were 17- and 350-fold increased. Genes encoding chitinase (PR-3 and PR-4) were down-regulated 4.6-fold at 12 dai in the compatible interaction of soybean roots with RKN, however, by 10 wai transcripts of two chitinase genes were up-regulated 15- to 26-fold, respectively. Transcripts of genes encoding PR-10 (SAM22) were increased 5- to 10-fold at 12 dai and remained at a similar level at 10 wai.

The increase in PR-1 protein suggests that there may be an increase in the level of salicylic acid. Interestingly, there are two different possible routes to salicylic acid production (Chen *et al.* 2009). Salicylic acid is known as a signal molecule for defense against nematodes (Branch *et al.*, 2004).

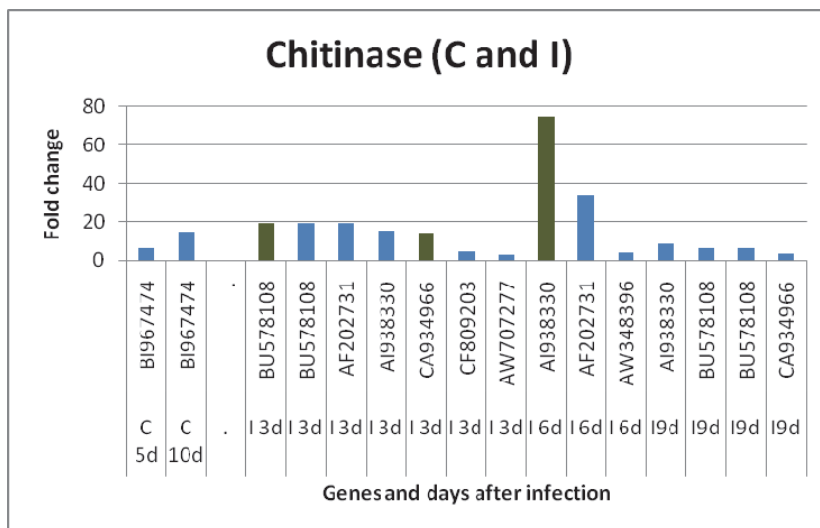


Fig. 6. Fold increase in expression of chitinase genes in the compatible (C) interaction (data from Ithal. *et al.* 2007b) and incompatible interaction (I) at 3, 6 and 9 dai (data from Klink *et al.* 2009b). Genes are represented by GenBank numbers.

The pathway that has the most scientific support involves isochorismate synthase (Wildermuth *et al.* 2001) and is not represented or is not annotated on the microarray. The other pathway involves phenylalanine. In the latter pathway, we found a high increase in the tyrosine aminotransferase enzyme (EC:2.6.1.5) which would lead to high level of phenylalanine. Genes encoding phenylalanine ammonia-lyase (EC:4.3.1.24) and salicylate 1-monooxygenase (1.14.13.-) were over-expressed at 6.9 and 2.9 F.C, respectively. Loon *et al.* (2006) reported that the PR-1- type proteins and also proteinase inhibitors were induced in abscission zones, which suggest the involvement of these proteins in cell wall loosening and degradation of the scarified cells as a defense response against fungal and bacterial pathogens. Also, transgenic tobacco over-expressing PR-1 was more resistant to blue mold and black shank caused by *Peronospora tabacina* and *Phytophthora parasitica f. sp. nicotianae*, respectively (Loon *et al.*, 2006). In addition, PR-3 and PR-4 showed chitinase activity that is required for embryogenesis during the globular stage in carrot (Loon *et al.*, 2006). Genes encoding PR-3 and PR-4 family proteins are reported to be up-regulated by jasmonic acid and ethylene (Niki *et al.*, 1998). Also, PR-4 showed ribonuclease activity against fungal protein in wheat (Loon *et al.*, 2006).

2.3.3 Phenylpropanoid biosynthesis

The phenylpropanoid pathways leads to the synthesis of coumarins, flavonoids, phytoalexins, lignins, and lignans, all which can play roles in plant defense. Several genes encoding enzymes in this complex pathway are up regulated in the incompatible interaction at 6 dai (Fig . 7; data from Klink *et al.* 2007b). And there are notable differences in the expression of genes encoding enzymes in this pathway between the compatible interaction at 2, 5 and 10 dai and the incompatible reaction at 6 and 9 dai. One major difference is in the expression of the genes encoding enzymes involved in the production of phenylpropanoids.

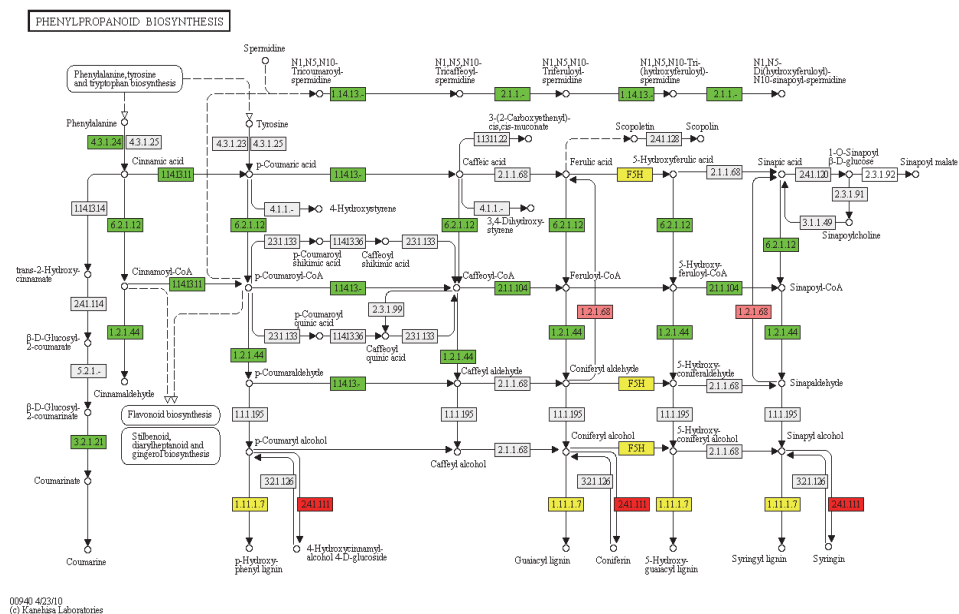


Fig. 7. Expression of genes encoding enzymes involved in phenylpropanoid biosynthesis (A) at 10 dai in a compatible reaction between Williams 82 with SCN and (B) at 6 dai in an incompatible reaction between cv. Peking and SCN are displayed on the KEGG pathway diagram. Enzyme commission numbers in the rectangles are provided by KEGG. Rectangles are colored light green for genes up-regulated in the first 50%, medium green for genes up-regulated in the 50 to 75 quartile and dark green for genes up-regulated in the top 25 %. Enzymes colored in red are encoded by down-regulated genes using a similar scheme. Enzymes colored in yellow are encoded by more than one gene and different copies of that gene are up- and down-regulated, respectively. Rectangles colored light gray indicate that the genes encoding those enzymes are not annotated in our soybean microarray database.

Phenylalanine ammonia-lyase (EC 4.3.1.34; PAL) can be considered a control point for entry into the phenylpropanoid pathway. There is no major change in expression of genes encoding PAL in the compatible interaction, however at 3, 6 and 9 dai in the incompatible interaction genes encoding PAL are increased in expression, thus suggesting an increased metabolic flow into the pathway. Genes encoding PAL and represented by BI701520, CK606172, and AW351172 are 20- to more than 40-fold increase in expression over that time course (Klink *et al.* 2007a). Increased PAL enzyme activity has been noted in resistant tomato roots infected with RKN, while PAL activity was depressed in susceptible tomato roots (Brueske, 1980). Similarly, in potato PAL activity is higher in resistant plants (Giebel, 1973). Certainly certain genes involved in isoflavonoid production are increased in expression in the incompatible reaction. For example, the gene encoding chalcone synthase (EC 2.3.1.74), represented by BQ081473, is more than 40-fold increased in expression at 3 and 9 dai in the incompatible interaction, but there is no change in the compatible interaction, while one gene encoding chalcone isomerase is elevated 4-, 6- and 17-fold in the incompatible interaction (Klink *et al.* 2007b).

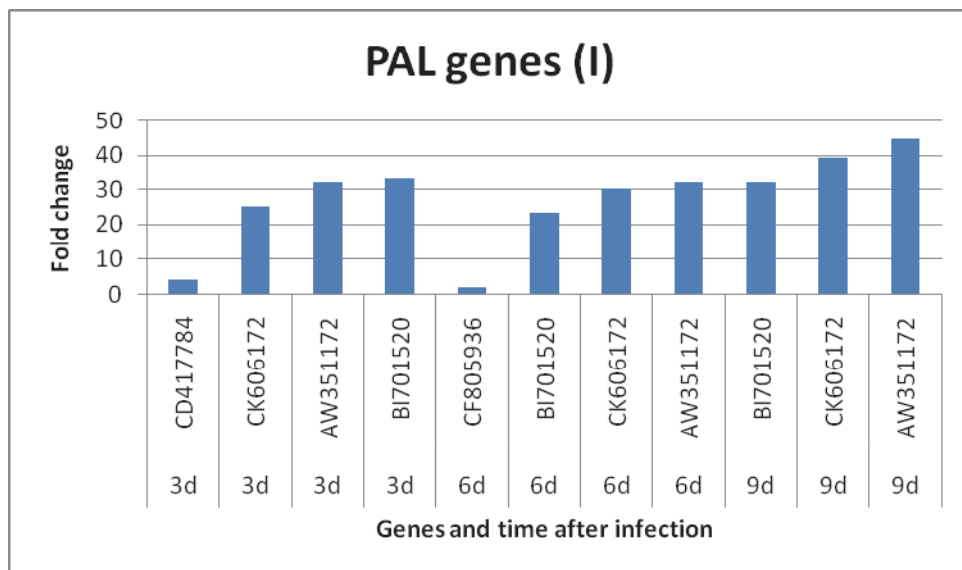


Fig. 8. Expression of genes encoding phenylalanine ammonia-lyase (EC 4.3.1.24) at 3, 6 and 9 dai after infection with SCN in an incompatible interaction with soybean roots (Data from Klink *et al.* 2007b) phenylalanine ammonia-lyase (PAL; EC 4.3.1.24; Fig. 7; Data from Klink *et al.* 2007b).

While microarray studies of genes expressed in the incompatible reaction of soybean plants against SCN revealed an increase in transcript levels of certain genes encoding enzymes involved in glycolysis/gluconeogenesis, jasmonic acid biosynthesis, phenylpropanoid biosynthesis, pathogenesis related proteins, flavonoid biosynthesis, and the methionine salvage pathway (Klink *et al.*, 2010; Alkharouf *et al.*, 2006), the expression of many genes encoding proteins having regulatory and signaling functions, such as cyclins, phosphokinases and transcription factors, were also affected. Genes encoding enzymes belonging to pathways depicted in KEGG and that were highly preferentially expressed were related to those KEGG pathways using PAICE software (Hosseini *et al.*, in preparation) to make interpretation of the data easier. Thus, relationships among genes and pathways were recognized with less difficulty.

3. Conclusions

Soybean genes involved in glycolysis/gluconeogenesis are up-regulated during nematode feeding and several lines of evidence indicate that the gluconeogenesis is occurring. This would allow soybean cells to provide carbohydrates as an energy source to the nematode. Genes encoding enzymes involved in cell wall molding are up-regulated, including cellulases, pectinesterases and xyloglucanases. These increases in gene expression allow the development and expansion of the syncytium for nematode feeding. Genes encoding important enzymes involved in the synthesis of jasmonic acid are down-regulated in the compatible interaction. This would quench the defense response controlled by jasmonic acid and related compounds and allow the nematode to grow and develop in a compatible

reaction. In general, genes encoding pathogenesis-related proteins are more highly expressed in the incompatible interaction and a gene encoding phenylalanine ammonia lyase is much more highly expressed in the incompatible interaction of soybean roots with SCN. Phenylalanine ammonia lyase is major gateway to phenylpropanoid metabolism and to the synthesis of numerous secondary compounds involved in plant defense. All of these data indicate that there is a stronger production of transcripts of genes encoding proteins involved in the plant defense response in the incompatible interaction, while transcripts of many of these genes are lower or the genes are down-regulated leading to a weaker defense response in the compatible reaction of soybean roots to SCN. Gene expression studies performed in soybean has resulted in the understanding gene expression during infection by SCN. The challenge to scientists now is in testing the function genes to understand the molecular circuitry occurring between plants and their parasitic nematodes so new methods of nematode control can be developed.

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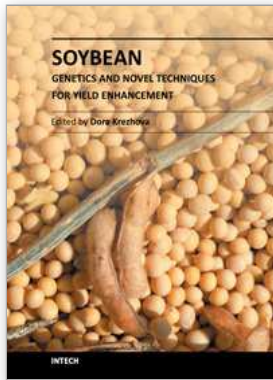
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Phone: +86-21-62489820
Fax: +86-21-62489821

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