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# Differential immunoreactivity of the root-knot nematodes, *Meloidogyne graminicola* and *Meloidogyne incognita* to polyclonal and monoclonal antibodies and identification of antigens through proteomics approach

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***Meloidogyne graminicola*** **infect graminaceous plants but have lesser tendency to infect dicotyledonous plants.** ***Meloidogyne incognita*** **is a pest of dicots and occasionally infects cereals. Evolutionary adaptation of these root-knot nematodes to their preferred hosts might have led to variability in their gene/protein profile which could contribute to their differential behaviour outside and inside the different host crops. Polyclonal and monoclonal antibodies raised against several nematode species showed cross-reactivity to antigens with different molecular weights present in the whole body homogenate of** ***M. incognita*** **and** ***M. graminicola*** **J2. This variability in antigenicity may correspond to specific functions of these molecules in** ***M. incognita*** **and** ***M. graminicola*****. Using proteomics approach possible amino acid sequence of those antigens was elucidated and showed sequence similarity with several proteins like signal recognition particle protein, galactose binding lectin, zinc finger motif, neurotransmitter gated ion channel, transmembrane protein, etc. from the genomic database of several nematode species. To investigate the function of the identified nematode genes, RNA interference could be used to reduce the expression of these selected genes and determine their importance for nematode development, survival or parasitism.**

**Key words:** Antibodies, antigens, host recognition, secreted-excreted products, surface coat.

## INTRODUCTION

Plant parasitic nematodes (PPN) are one of the major limiting factors in crop production worldwide damaging up to 10% of world's agricultural output equivalent to $157 billion annual monetary loss (Abad, 2008). PPNs are unique in their ubiquitous nature and persistence in the soil. The conflicting nature of their attack allows their presence to often pass unnoticed while crops slowly decline in vigour and yield. Rarely is any crop free from attack of these tiny and microscopic pathogens.

The molecular dialogue between PPNs and the host starts at a distance, with modifications of the surface of Infective Juveniles (J2) in response to root diffusates.

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During co-evolution with the host plant, parasitic nematodes have developed the capacity to recognize and respond to chemical signals of host origin. Signals from roots present in the rhizosphere and bulk soil can specifically influence nematode behaviour, inducing hatching, attract-tion, surface cuticle changes, root exploratory behaviour and penetration of plant roots, and involve molecular communication between the nematode and respective host plant (Curtis et al., 2011).

Once the nematode has reached the root, different secretory organs participate in the molecular interaction with the host. Feeding cell formation is presumably initiated in response to signal molecules released by the parasitic J2, but the nature of the primary stimulus is unknown, as is the host target for the presumed nematode ligand(s), which must be transduced to elicit the feeding site. The most widely held hypothesis is that the necessary metabolic re-programming of root cells is triggered by specific nematode secretions, which presumably interact with membrane or cytoplasmic receptors in the plant to switch on cascades of gene expression that alter cell development. Secretions from the cuticle build up a surface coat (SC) that is likely to hide the nematode from host perception throughout the interaction (Curtis, 2011).

The nematodes possess an elaborate nervous system which plays a great role in recognition of host roots, appropriate feeding or penetrating sites, selection of tissue for migration in root and feeding cell/site formation. Amphids are the primary structures for chemoreception of the chemical cues which orient nematodes towards food sources and lead to infection. Disruption of the sensory functions involved in nematode interactions with plants, that is, disruption of host recognition process is one of the innovative management tactics (Spence et al., 2008).

Rapid advances in protein analytical technologies, makes mass spectrometry-based interactive proteomics a method of choice for analyzing functional protein com-plexes. A combination of 2D-gel electrophoresis with micro-sequencing has led to the identification of two endoglucanases and a novel protein in the secretions of the cyst nematode, *Heterodera schachtii* (De Meuter et al., 2001).

A calreticulin and a 14-3-3 protein identified in the secretion of *M. incognita* had multiple functions including regulation of cell signaling and metabolic pathways along with the control of the cell cycle (Jaubert et al., 2002; Abad et al., 2003). An annexin gene (*Gp-nex-1*) and putative collagen gene (*gp-col-8*) were isolated from a *Globodera pallida* expression library by screening with a polyclonal and a monoclonal antibody (MAb), respect-tively, both antibodies reacted with antigens present in the amphids of *Globodera* sp. (Jones et al., 1996; Gray et al., 2001; Fioretti et al., 2001). MAb directed against amphidial secretions interfered with nematode invasion of plants and therefore secretions from the amphids might be involved in host-recognition processes (Fioretti et al., 2002).

Root-knot nematodes (*Meloidogyne* spp.) are one of the most damaging agricultural pests, attack almost every crops. A large number of host range studies have shown that some species like *Meloidogyne incognita, Meloidogyne javanica, Meloidogyne arenaria, Meloidogyne hapla* etc. characteristically prefer dicotyledonous crops and rarely infect cereals. On the contrary, another group including *Meloidogyne graminicola, Meloidogyne naasi, Meloidogyne oryzae, Meloidogyne salasi, Meloidogyne triticoryzae* etc. generally prefer cereal hosts but can also infect some dicotyledonous plants (Dutta, 2012). Preliminary attraction bioassay studies in our laboratory have shown that root-knot nematodes (*M. incognita* and *M. graminicola*) are attracted differently to good hosts and poor hosts whilst no attraction was observed for non-host plants (Reynolds et al., 2011; Dutta et al., 2011). Understanding the complexity of molecular signal exchange and response during the early stages of the plant-nematode interactions is important to identify vulnerable points in the parasitic life cycle that can be targeted to disrupt nematode host recognition. Thus, attempts were made to identify the proteins related to host recognition process of root-knot nematodes, *M. incognita* and *M. graminicola* through proteomics approach.

## MATERIALS AND METHODS

### Culturing of nematodes

*M. incognita* (Kofoid & White) Chitwood and *M. graminicola* Golden & Birchfield were maintained respectively, on tomato (*Solanum lycopersicum* cv. Tiny Tim) and rice (*Oryza sativa* cv. Ballila) in a glasshouse. Egg masses were collected on a piece of 10 µ porous cloth supported on Miracloth (Calbiochem, U.K.) held by two plastic rings in a flat bottomed evaporating dish containing distilled water (Hooper, 1986). Freshly hatched second stage juveniles (J2) were used for all the experiments.

### Antigen preparation

A pellet of several thousand J2 of each nematode species were homogenized in 0.01 mM PBS pH 7, on ice using a homogenizer (Biomedix) from which whole body homogenates were obtained. Cuticle surface antigens were collected with 1% Triton X-100, Tris 0.125 M, pH 7.5. Stylet secretions were induced by adding 0.2 mg/ml of 5 methoxy-*N, N* dimethyl tryptamine (Sigma) which enhances stylet thrusting (Goverse et al., 1994; Curtis, 1996) (Figure 1). Proteins were quantified with Biorad protein assay.

### Antibodies

The polyclonal antibodies (PAbs) were raised in rabbits to several plant parasitic nematodes: IACR-PC 373 (homogenates of *M. incognita* J2), IACR-PC 374 (live pre-parasitic J2 of *M. incognita*), IACR-PC 353 (live pre-parasitic J2 of *Heterodera avenae*), IACR-PC 389 (SC extract of *M. incognita*), IACR-PC 418 (SC extract of *M. arenaria* race from Portugal) and IACR-PC 419 (SC extract of *M. arenaria*). Monoclonal antibody (MAbs) IACR-CCNj.2a.15 raised in mouse immunized 3 times intraperitoneally with whole J2 and secreted-excreted (SE) products of the cereal cyst nematode *H. avenae* (Curtis, 1996) was used in this study.

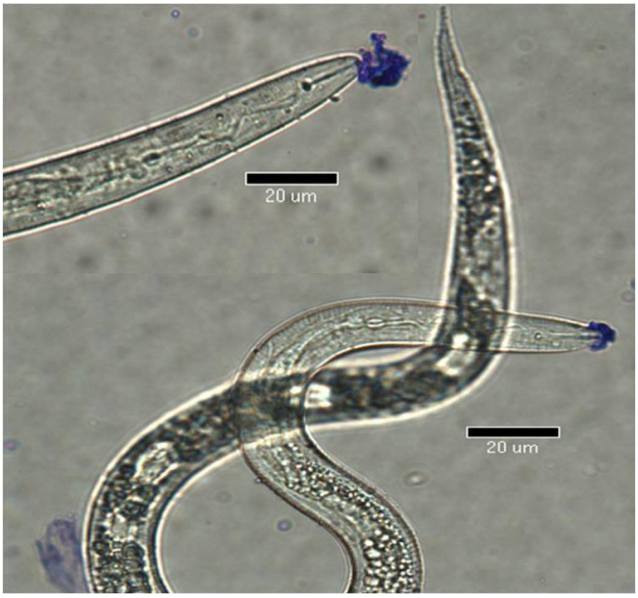


**Figure 1.** Induction of stylet secretion in *M. graminicola* J2. Nematodes treated with the neurotransmitter showed massive amounts of stylet secreted proteins around the stylet tip at 4 h of incubation. Coomassie brilliant blue R250 was added to the suspension to visualize secreted proteins.

**Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)**

One dimensional SDS-PAGE using a 15% (w/v) acrylamide in the separating gel and 4% (w/v) acrylamide in the stacking gel were performed (Laemmli, 1970). Sample buffer (10% glycerol, 5% 2-mercaptoethanol, 2.3% SDS and 0.01% bromophenol blue in 0.5 M Tris HCl pH 6.8) was added to the protein samples (40 µg protein per lane). The proteins were separated using a vertical polyacrylamide slab electrophoresis tank followed by fixed and stained with Coomassie Blue G250 (Sigma-Aldrich Chimie) or silver stained or transblotted onto 0.2 µm nitro-cellulose (NC) membrane. The molecular weight markers (Pharmacia) used was: lactalbumin 14 kDa; soybean trypsin inhibitor 20 kDa; carbonic anhydrase 30 kDa; ovalbumin 43 kDa; bovine serum albumin 67 kDa and Phosphorylase b 94 kDa. The experiment was repeated at least thrice.

**Western blotting**

A Multiphor II Nova Blotting Electrophoresis Transfer Unit (Pharmacia) was used to transfer proteins from the gel to NC membrane in transfer buffer (39 mM glycine, 48 mM Tris, 0.0375% SDS, 20% methanol). The immunolabelling was performed after blocking the NC membrane overnight at 4°C in a solution of PBS, 0.1% Triton X-100, 5% Marvel dried milk (PBSTM). The NC membranes were incubated with the primary PAbs (IACR-PC 373 1:5000; IACR-PC 374 1:5000; IACR-PC 353 1:2000; IACR-PC 389 1:2000; IACR-PC 418 1: 2000; IACR-PC 419 1:2000) for 1 h at room temperature under agitation. The membranes were then washed in PBST and incubated with horseradish peroxidase conjugated anti-rabbit secondary antibody (diluted 1: 2000 in PBS) for 45 min on a shaker in dark. After further washes in PBST the membranes were treated with 10 ml of PBS containing 0.05% w/v diaminobenzidine and 30% $H_2O_2$ until bands were suitably dark (Harlow and Lane, 1988). Negative controls were non-immune serum and secondary antibody. Membrane was incubated with MAb for 2 h, washed and treated with peroxidase-conjugated goat anti-mouse polyvalent immunoglobulins (diluted 1: 1000 in PBS) for 45 min. Immunodetection was enhanced by chemiluminescence reagent (ECL, Amersham International plc). The blot was immersed in it for 1 min and exposed to Hyperfilm-ECL for 1 min to develop the film. Negative controls consisted of blots probed with tissue culture supernatant (20D medium) and an irrelevant monoclonal antibody. Three biological and three technical replicates were taken for each of the samples.

**Protein sequencing**

The major protein spots for both the nematode species were excised from Coomassie Brilliant Blue G250 stained gels. Gel pieces were destained, reduced and alkylated, digested with trypsin (Promega, UK) and obtained peptides were sequenced through MALDI-MS and ESI-MS (Lovegrove et al., 2009) in MASCOT server. MS raw data were acquired on the Data Directed Analysis feature in the MassLynx (Micromass) software with a 1, 2, 4 duty cycle (1 s in MS mode, two peptides selected for fragmentation, maximum of 4 s in MS/MS acquisition mode). MS/MS raw data were transferred from the QTOF Micro computer to a server and automatically manipulated for generation of peak lists by employing Distiller version 2.3.2.0 (http://www.matrixscience.com/distiller.html) with peak picking parameters set at 5 for Signal-Noise Ratio (SNR) and at 0.4 for correlation threshold (CT). Peak listed data were searched by employing Mascot (http://www.matrixscience.com) version 2.3.01 against the list of protein sequences predicted for *M. incognita* and *M. graminicola* using BLASTP server for their sequence similarity to known proteins of other nematode species at the NCBI database (Altschul et al., 1990).

Comparison with homologous sequences was done with ClustalW (Larkin et al., 2007). Theoretical isoelectric point (pI) and molecular weight (mw) for the conceptually translated protein sequences were calculated by the Expasy Protparam tool available at http://expasy.org/. Gene ontology term was assigned through AmiGO BLAST. Signal peptides were predicted by the SignalP server (Petersen, 2011). Secondary structure of the protein was predicted with an *ab initio* protein modelling server I-TASSER (Roy et al., 2010). This server uses the threading technique to predict the 3D models. The server generated 5 best models based on multiple-threading alignments and iterative template fragment assembly simulations along with their confidence scores. The 5 models were visualized by the Visual Molecular Dynamics (VMD) software models, different validation techniques were used. In a similar fashion, PROCHECK (Laskowski, 1996) and VERIFY 3D (Eisenberg, 1997) were used to validate the predicted protein structures. The PROCHECK software generates ramachandran plot which nicely explains the stereochemical configuration of amino acid residues. The VERIFY 3D analyses the compatibility of an atomic model with its amino acid sequence. Finally, the better model was adopted based on the aforementioned tools.

## RESULTS AND DISCUSSION

### SDS-PAGE

All silver staining methods rely on the reduction of ionic to metallic silver to provide metallic silver images, the selective reduction at gel sites occupied by proteins as compared to non-protein sites being dependent on differences in the oxidation-reduction potentials of these

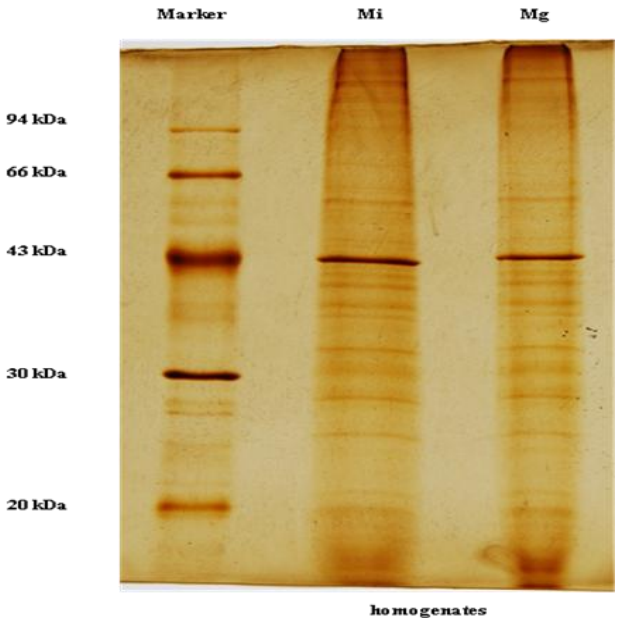


**Figure 2.** Analysis of homogenate proteins of *M. incognita* (Mi) and *M. graminicola* (Mg) by SDS-PAGE. Protein bands were visualized by silver staining.

sites; while Coomassie Blue binds strongly to arginine and lysine residues and with lower affinity to aromatic side chains (Simpson, 2003). Thus SDS-PAGE followed by silver staining was not enough to detect the differences in the secretion and homogenate proteins of both nematode species (Figure 2). But SDS-PAGE followed by Coomassie Blue staining did detect several polymorphic homogenate antigens among the two species which might have some role in the host recognition and parasitic life cycle of that two species inside different hosts (Figure 4).

## Western blots

PAb raised against SC extract and SE products of *Meloidogyne* spp. showed antigenic cross-reactivity with the different molecular weight antigens of *M. incognita* and *M. graminicola* stylet exudates. Antigens from *M. incognita* and *M. graminicola* proteins shared some proteic epitopes and also reacted with the antibody at different bands when the blot was probed with the PAb raised against live pre-parasitic J2 of *M. incognita* (Figure 3a and b). It can be speculated that some of the antigens of *M. incognita* and *M. graminicola* recognized by several antibodies are continuously shed from the nematode SC and may also originate from SE products of nematodes. The origins of surface-associated antigens on nematodes may differ for various antigens. These non-structural proteins originate from gland cells such as excretory cells, pharyngeal glands, amphids and phasmids as well as from the hypodermis and rectal glands (Blaxter and Robertson, 1998; Hu et al., 2000). Glycosylated peptides have been reported to be present in abundance on the SC and SE products of several parasitic nematodes (Robertson et al., 1989; Schallig et al., 1994). These antigens may participate in the infection process by binding to proteins/receptors on the plant cell plasma membrane or modulate changes via signal transduction. Little is known about the roles of the surface antigens of plant-parasitic nematodes in pathogenicity. A more dynamic role as an elicitor in the determinative phase of nematode-plant interaction has been postulated for these surface molecules (Kaplan and Davis, 1987). Cuticular exudations appear to correlate with feeding periods of the nematode, implying that they might play a more sophisticated role in the infection process (Endo, 1993). Several proteins from the cuticle and amphids have previously been identified using antibodies (Atkinson et al., 1988; Davis et al., 1992; Stewart et al., 1993; Curtis, 1996; De Boer et al., 1996a, b). A putative role has been suggested for an amphid-secreted protein, which might be involved in the early steps of recognition between (resistant) plants and (avirulent) nematodes (Semblat et al., 2001).

PAb raised against whole body homogenates of *M. incognita* showed very strong reaction with the antigens of both *M. incognita* and *M. graminicola* in several similar and different band positions (Figure 3c). While, MAb raised against whole J2 and SE products of *H. avenae* showed very high level of cross reactivity with a couple of specific antigens of *M. incognita* proteins but surprisingly did not show any reaction with the *M. graminicola* stylet secreted proteins (Figure 3d). This might indicate that the common antigens (sharing proteic epitopes) perform the same function in the larval stages of *M. incognita* and *M. graminicola* during host recognition, invasion and development inside the cereal and dicotyledonous crops. While antigenic differences (recognized by antibodies at different molecular weight) among the two nematode species might account for adaptations of the proteins to allow parasitism in different hosts. This variability in antigenicity may also correspond to specific functions of these antigenic molecules in *M. incognita* and *M. graminicola*. Variability in antigenic properties of isoforms might be important for the survival of the parasite in the host (Overath et al., 1994).

## Protein sequencing

Attempts were made to determine the molecular size of the antigen of the homogenates of *M. incognita* and *M. graminicola* recognized by western blot. Polymorphic bands were identified in Coomassie Blue stained gel with both the nematode species (Figure 4). 5 prominent bands of 87, 41, 32, 28 and 18 kDa specific to *M. incognita* and 3 distinct bands of 87, 41 and 33 kDa specific to *M. graminicola* was picked out for proteomics study. Several of the individual peptides obtained from the MALDI-MS

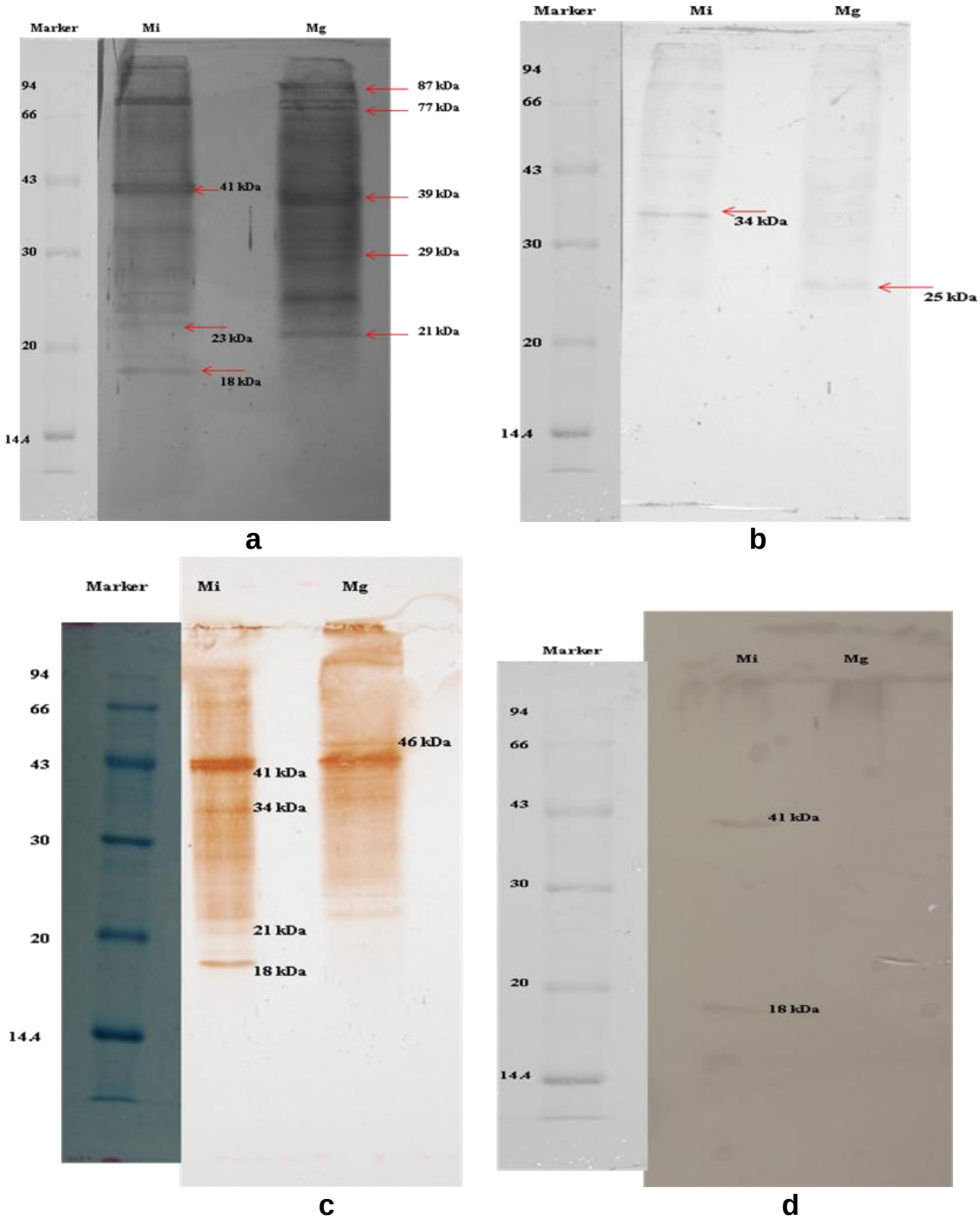


**Figure 3.** Western blot of *M. incognita* (Mi) and *M. graminicola* (Mg) stylet secreted proteins probed with a) PAb IACR-PC 374, b) PAb IACR-PC 389, c) PAb IACR-PC 373, d) MAb IACR-CCNj.2a.15.

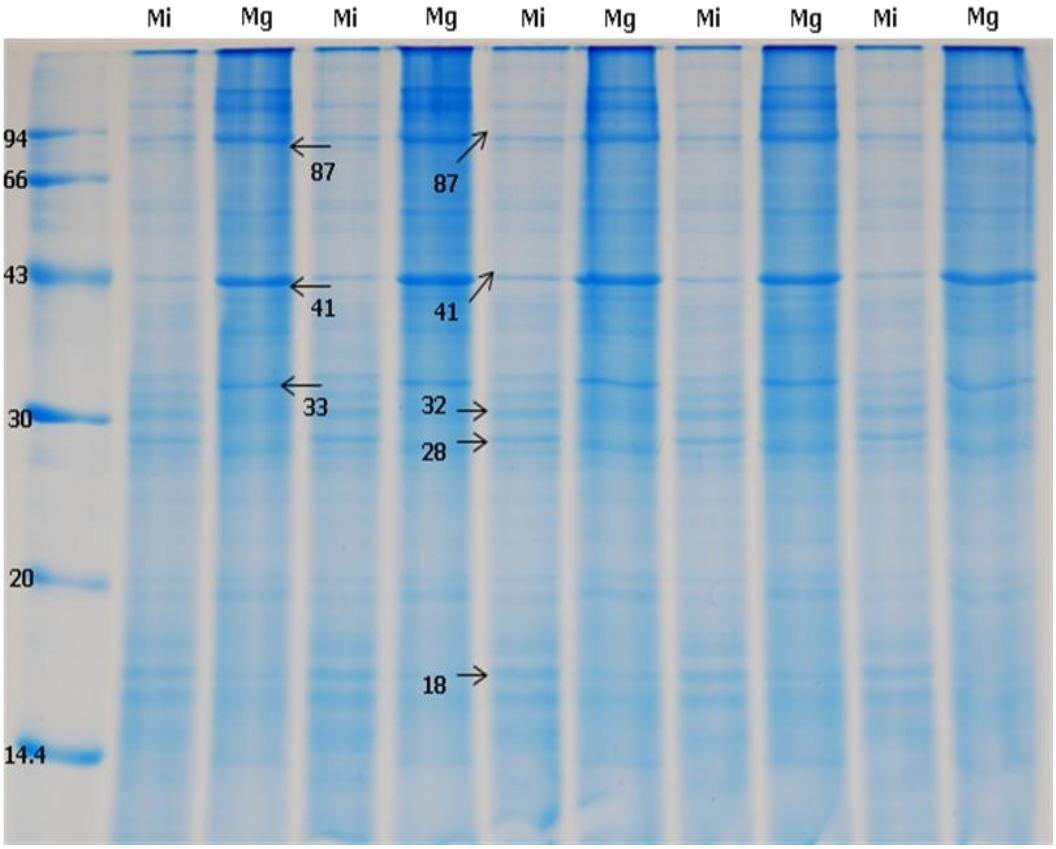


**Figure 4.** Analysis of homogenate proteins of *M. incognita* (Mi) and *M. graminicola* (Mg) by SDS-PAGE. Protein bands were visualized by Coomassie blue G250.

and ESI-MS data showed very high level of sequence similarity to the signal recognition particle protein, transmembrane protein, zinc finger motif, galactose binding lectin, neurotransmitter gated ion channel proteins, cellulose binding precursor and FMRFamide-like peptides of several nematode species using protein Blast search in Genbank (Table 1).Galactose binding lectin or Galectin of *M. incognita* (Minc03540) which is 308 amino acids long were chosen for further study as it may play the imperative role during the host recognition process. Theoretical isoelectric point (pI) and molecular weight (mw) for the conceptually translated protein (Minc03540) was calculated by the Expasy Protparam tool showing mw of 35490 dalton and pI of 5.33. The instability index (36.73) classified it as stable protein. SignalP result predicted 21 residue long signal peptide at N-terminal end, suggesting the protein has excellular function like signal transduction. Galectin protein sequences of closely related species were retrieved from Genbank database using protein Blast Search. Best hits were obtained with *Caenorhabditis elegans*, *Caenorhabditis briggsae* and *Brugia malayi*. The homologous sequences aligned using multiple sequence alignment (MSA) suggested that galectins are highly conserved across the nematode genera regardless of amino termini or carboxyl termini (Figure 5).

The predicted secondary structure of Minc03540 revealed that it has more number of beta sheets as compared to alpha-helices revealing the protein is folded properly (Figure 6). The predicted C-score (-0.69) indicated the model is of higher confidence and TM-score (0.63) signifying the model is in correct topology. To analyze the stereochemical quality of the predicted structure PROCHECK software was used. According to PROCHECK results, the first model (Table 2) seems to be most appropriate one because it has most of the amino acid residues present in the core and allowed regions (95.9%) while only 3.0% of the total amino acids were found in the generous region as indicated by Ramachandran plot (Figure 7). Further, the quality was assessed by the VERIFY 3D server which verified the 3D structural distribution of amino acids as compared to the 1D distribution of amino acid residues. According to the results provided by the DoBo server (http://sysbio.rnet.missouri.edu/dobo) the N-terminal domain stretches from amino acid 1-46 and the C-terminal domain stretches from amino acid 192-308.

The model has shown structural similarity with several

**Table 1.** Individual peptides obtained from MALDI-MS and ESI-MS data showed sequence homology to the target proteins of several nematode species from the genomic database.

| Protein spot | Micro-sequence | Homology with known proteins (% of identical amino acids) |
|---|---|---|
| *M. incognita* | | |
| Spot 1. | MSHPGWIMVSFLTELLSQSSK | 100% neurotransmitter gated ion channel protein of *C. elegans* |
| 87 kDa | WSFYLTSLSEYFDEDVNIDQP | 90% neurotransmitter gated ion channel protein of *C. elegans* |
| Spot 2. | MANKLIPVLLIFFLSVPQFDAD | 90% galectin (Minc03540) protein of *M. incognita* |
| 41 kDa | LEVDGTFELHTVTINNAR | 80% galectin (Minc03540) protein of *M. incognita* |
| Spot 3. | MFFVLILLFSFPPFCFPNKFSSK | 100% zinc finger motif (Minc02576) of *M. incognita* |
| 32 kDa | RFQHERDLYYFTMSHLGNLG | 85% zinc finger motif (Minc02576) of *M. incognita* |
| Spot 4. | MSGCLDQIRCNCTFDLEGRRN | 75% transmembrane protein of *C. briggsae* |
| 28 kDa | SSTILGVYFPFVALFLFRFI | 70% transmembrane protein of *C. briggsae* |
| Spot 5. | MVLADLGRKIRNAISKL | 80% signal recognition particle protein of *Brugia malayi* |
| 18 kDa | LQNMMKQLQGASSSLGNRRN | 95% signal recognition particle protein of *Brugia malayi* |
| *M. graminicola* | | |
| Spot 6. | ASFFYLLIISVSLLILANADDA | 88% cellulose binding precursor of *M. javanica* |
| 87 kDa | VENRDIGVVYNDVPEPLPTI | 60% cellulose binding precursor of *M. javanica* |
| Spot 7. | LALFGFVVLIVGQMSVLGA | 95% FMRFamide-like peptides of *M. incognita* |
| 41 kDa | SSGGNKGNNFLRFGR | 65% FMRFamide-like peptides of *M. incognita* |
| Spot 8. | MSIFLTSALLIISLIAMTEG | 60% msp1 gene of *M. incognita* |
| 33 kDa | VDFKIVPTDKKISPKACTMKM | 85% msp1 gene of *M. incognita* |

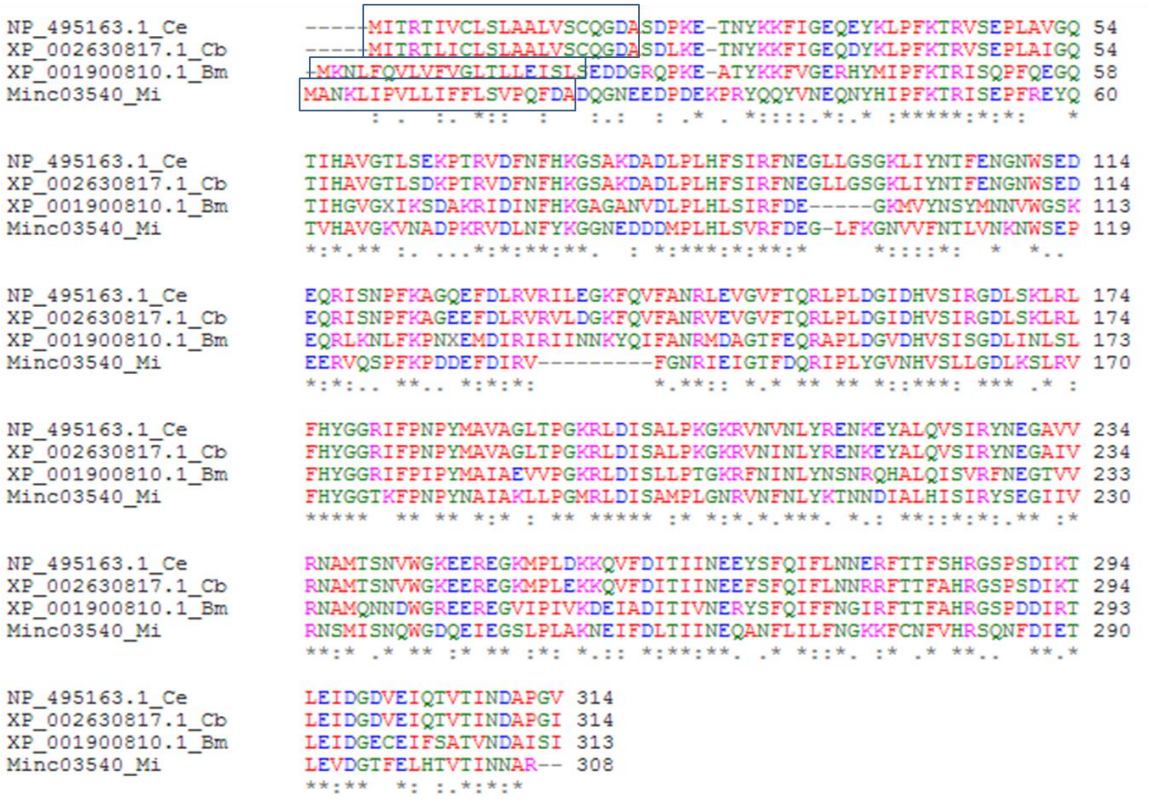


**Figure 5.** MSA of the predicted amino acid sequence of galectin of *M. incognita* with *galectin* sequences from other nematodes. The sequences are denoted by their Genbank identifier followed by the species abbreviation. Ce, *Caenorhabditis elegans*; Cb, *Caenorhabditis briggsae*; Bm, *Brugia malayi*; Mi, *Meloidogyne incognita*. * and **:** signs indicates conserved and similar amino acids respectively. Boxed region represents signal peptide.

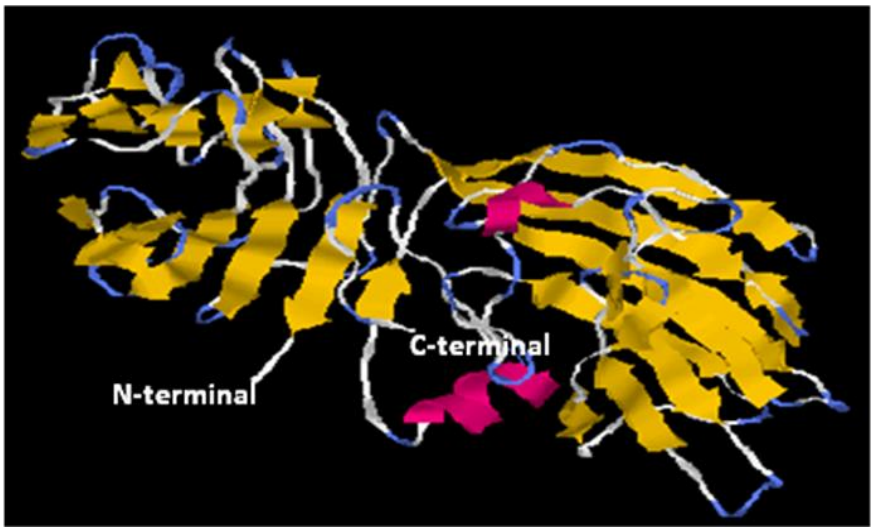


**Figure 6.** Predicted secondary structure of Minc03540 protein of *M. incognita* using *ab initio* protein modelling server I-TASSER (http://zhanglab.ccmb.med.umich.edu/I-TASSER). Red representing alpha helix, yellow representing beta sheet and blue representing loop in the model.

**Table 2.** Evaluation results of the I-TASSER models of the tertiary structure by PROCHECK and VERIFY 3D. *****These models had an average 3D-1D score >0.2.

| Parameter | | Model 1 (%) | Model 2 (%) | Model 3 (%) | Model 4 (%) | Model 5 (%) |
|---|---|---|---|---|---|---|
| PROCHECK | Core Region | 80.0 | 72.2 | 77.0 | 70.0 | 69.6 |
| | Allowed Region | 15.9 | 18.9 | 16.3 | 20.7 | 22.6 |
| | Generous Region | 3.0 | 6.3 | 5.2 | 4.8 | 4.8 |
| | Disallowed Region | 1.1 | 2.6 | 1.5 | 4.4 | 3.0 |
| *VERIFY3D | | 67.0 | 64.0 | 71.0 | 65.0 | 70.0 |

concanavalin A-like lectins/glucanases from the PDB database. Further, gene ontology terms suggested that the protein has carbohydrate binding affinity (GO: 0005529). The protein has more number of positively charged residues (aspartate and glutamate) as compared to negatively charged ones which is suspected to interact with the cations emanating from rhizosphere during host recognition process.

## Conclusion

The antigens identified in this study might represent potential targets for nematode control, since proteins from SC and SE products are the first molecules to have contact with their hosts, and, therefore, might play an important role in the host-parasite interaction including host recognition, invasion and development processes. Problems have been encountered in obtaining resistance sources against a particular pest/pathogen and emergence of resistance breaking pathogen races or biotypes. These problems can now be overcome by using modern day cellular and molecular approaches to plant biotechnology, e.g. RNAi based transgenics which can facilitate the transfer of existing sources of nematode resistance across conventional barrier to reproduction into other related or even unrelated crop species.

Furthermore, molecular analysis of nematode/host interaction and molecular dissection of nematode systems such as neurobiology, sensory perception or moulting, may now allow the construction and expression in plant of novel broad spectrum form with synthetic resistance. This investigation was an attempt to generate more information at molecular level with respect to interactions of nematodes and host plants in the areas of nematode host finding.

Work is in progress to characterise the identified proteins other than galectin and their role in plant-nematode interaction to be established by knocking out those genes by RNA interference.

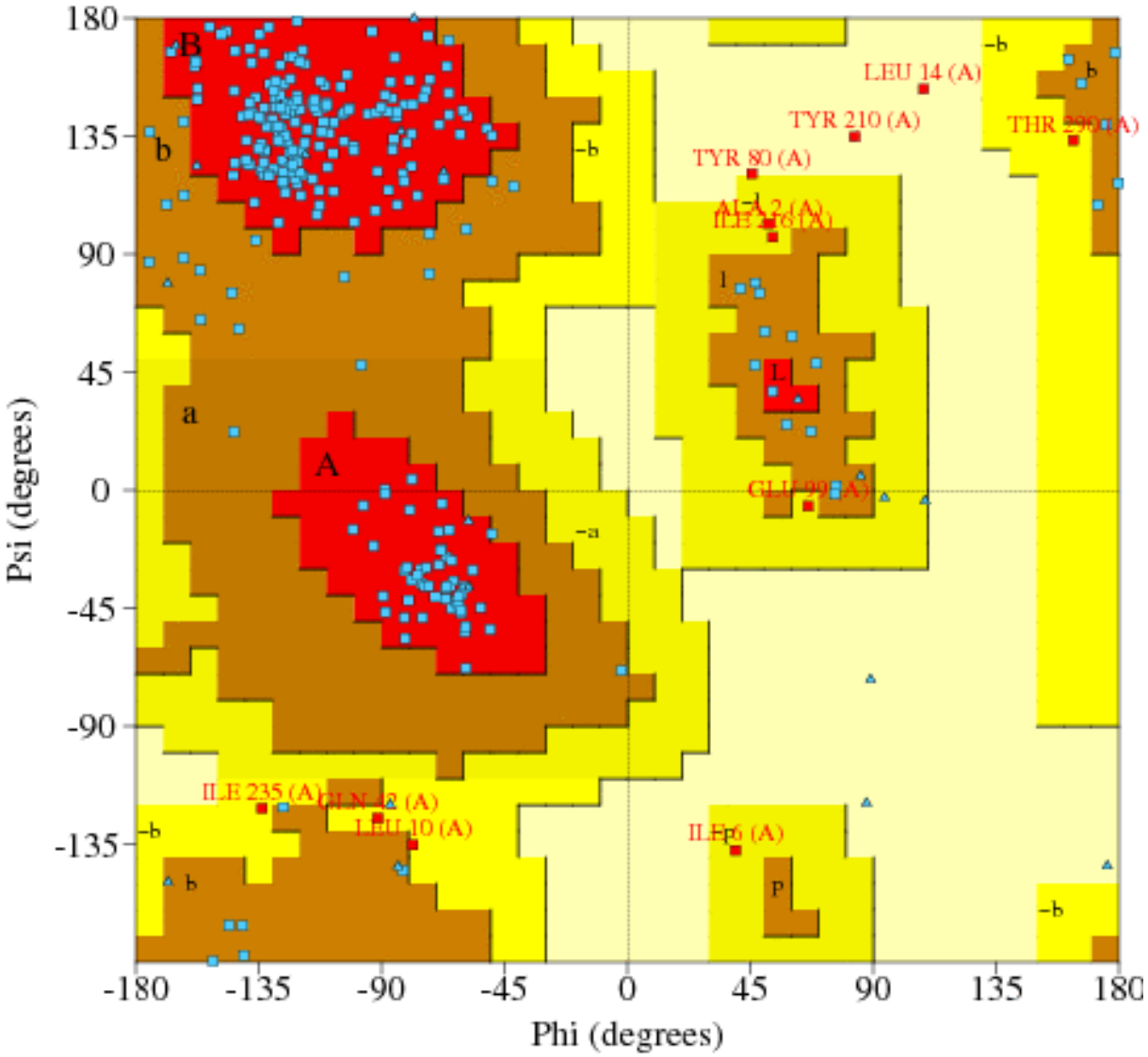


**Figure 7.** Ramachandran plot of the predicted model of Minc03540 protein of *M. incognita* showing Psi and Phi angles.

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