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Research Article

Computational analysis of *16D10* effector peptides from root-knot nematodes and their interaction with *Mi* resistance (*R*) protein through molecular docking

Adil Zahoor[1*], Zeenat Niaz[2], Muhammad Tahir-ul-Qamar[3], Abdalmenem I. M. Hawamda[4*]

[1] *Department of Biotechnology, Chonnam National University, Yeosu 59626, South Korea.*
[2] *Graduate School of Agricultural Science, Faculty of Agriculture, Miyagi 980-8572, Japan.*
[3] *Department of Bioinformatics and Biotechnology, Government College University, 38000 Faisalabad, Pakistan.*
[4] *Department of Agricultural Biotechnology, Faculty of Agricultural Science and Technology, Palestine Technical University-Kadoorie (PTUK), Tulkarm P.O. Box 7, Palestine.*

ABSTRACT

Plant-parasitic nematodes, especially root-knot nematodes (RKNs), pose significant threats to global agriculture, affecting both the quantity and quality of crop yields. Central to the parasitism strategy of RKNs is the deployment of effector proteins, such as the *16D10* peptide, which interact with host plant resistance mechanisms. This study focuses on the computational analysis and molecular docking of *16D10* effector peptides derived from major RKN species (*M. incognita, M. arenaria, M. hapla, M. javanica, and M. chitwoodi*) to explore their interaction with the *Mi* resistance protein in tomatoes. Initial bioinformatics assessments included sequence retrieval, multiple sequence alignment, and phylogenetic analysis, utilizing tools such as CLUSTALW and the MEME Suite. Notably, our phylogenetic analysis revealed a close evolutionary relationship between *M. incognita* and *M. chitwoodi*, contrasting with other RKN species based on both DNA and protein data. Subsequent, *in silico* 3D modeling and docking studies, conducted using Chem-Sketch and MOE software, provided insights into the structural basis of the interaction between *16D10* peptides and the *Mi* protein. Our findings indicate that these interactions could play a crucial role in the modulation of plant defense mechanisms, potentially leading to effector-triggered susceptibility (ETS). This study not only enhances our understanding of nematode-host interactions but also aids in the development of novel strategies for managing RKN infections in economically important crops.

Keywords: Root-knot nematodes; *16D10* effector; Mi resistance; Molecular docking; Plant-nematode interactions.

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Correspondence
Abdalmenem I. M. Hawamda
a.hawamdeh@ptuk.edu.ps
Adil Zahoor
adilzahoor3253@gmail.com

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INTRODUCTION

Plant parasitic nematodes (PPNs) are serious pests of crop plants worldwide. They not only deteriorate the quality and quantity of agricultural products but also act as vectors or carriers of several other plant pathogens like viruses and fungi (Ali *et al.*, 2015). Most PPNs are obligate parasites; however, some categories of PPNs, during the evolutionary process, have developed a specialized kind of parasitism known as 'obligate sedentary endo-parasitism.' These important categories include cyst nematodes (CNs) and root-knot nematodes (RKNs) which are the most devastating in terms of crop losses (Ali *et al.*, 2015). Both categories of nematodes belong to the family *Heteroderidae* within the genera *Globodera* and *Heterodera* (CNs) and *Meloidogyne* (RKNs). RKNs are particularly deleterious to plants because of certain attributes like high adaptability to diverse climates, capability to infect wide variety of plant species, and their invasive strategies, including apoplastic nutrient uptake from

the plant vasculature, rapid multiplication in host roots and widespread distribution across various agro-ecological zones. This fact is obvious from the host range of *M. incognita* that infects over 3000 plant species (Abad *et al.*, 2008). In addition to these characteristics, RKNs assault is coupled with nematode secretions which contain cell wall degrading enzymes and effector proteins (Hussey *et al.*, 2002;Vanholme *et al.*, 2004).

The effector component of the nematode secretome, also known as the 'parasitome,' comprises a diverse array of parasitism proteins (Hussey *et al.*, 2002). These proteins are involved in the establishment of compatible interaction between the nematodes and plants (Ali *et al.*, 2015). Additionally, these effector proteins are involved in the transcriptional reprogramming of plant genes as they are localized in the plant cells' nuclei (Vanholme *et al.*, 2004; Bellafiore *et al.*, 2008; Rockey *et al.*, 2013).

PPNs secrete *CLAVATA3* (*CLV3*)/*ESR* (*CLE*)-like effector proteins which mimic the expression of plant *CLE* peptides for compatible plant-nematode interactions (Olsen and Skriver, 2003; Wang *et al.*, 2005; Wang *et al.*, 2006; Lu *et al.*, 2009; Wang *et al.*, 2010; Replogle *et al.*, 2011; Wang *et al.*, 2011). A CLE-like effector peptide, *16D10* from RKNs shares sequence similarity to *CLE* peptides in CNs, however, the mode of action of *16D10* is different from the *CLE*-like proteins from CNs (Huang *et al.*, 2006a). The effector protein *16D10* is primarily active within the cytoplasm of plant cells following infection, where it associates with a plant-specific *SCARECROW*-like transcription factor (Huang *et al.*, 2006b). Studies employing RNA interference (RNAi) to downregulate the *16D10* effector transcripts from *M. incognita* demonstrate that this gene is crucial for the nematode's development and successful parasitism, as evidenced by hindered nematode growth (Huang *et al.*, 2006a). Furthermore, similar outcomes were observed in another experiment where dsRNA-mediated silencing of the *16D10* effector in transgenic grape hairy roots significantly reduced infection levels (Yang *et al.*, 2013).

The plants having *Mi* gene-mediated resistance have high potential to cope with the RKNs in various crop plants (Fuller *et al.*, 2008). The *Mi* gene interacts with effector proteins which lead towards the development of *R* protein-*Avr* protein interaction. A compatible *R* protein-*Avr* protein interaction may result in absolute resistance. In this study, we have performed *in silico* characterization of *16D10* effector peptides from important RKN species and examined their interactions with *Mi* gene from tomato using molecular docking techniques. This study aims to provide insights into understanding the interactions between *R* proteins and effector proteins in plant-nematode interactions.

MATERIALS AND METHODS

Computational analysis of *16D10* effector proteins from RKN species

Amino acid and DNA sequences of *16D10* effectors from different RKNs species were retrieved from nucleotide database of NCBI. The unique Genbank accession numbers for *16D10* effector proteins from *M. incognita*, *M. arenaria, M. hapla, M. javanica, and M. chitwoodi* are AAZ77751.1, ABI33932.1, ABI33933.1, ABI33931.1, and AHZ64337.1, respectively. The alignment of the five *16D10* parasitism proteins was conducted utilizing the CLUSTALW function in MEGA software version 6.0 (Tamura *et al.* 2011), adhering to the software's default settings. Subsequently, a rooted phylogenetic tree was created using the Neighbor Joining (NJ) algorithm with 1000 bootstrap replicates, applicable to both protein and DNA sequences of the *16D10* effectors. For the identification of protein motifs, MEME Suite tool version 4.9.1 (http://meme.nbcr.net/meme/cgi-bin/meme.cgi) was used to detect conserved motifs among the five *16D10* peptides. The MEME analysis was set to allow any number of repetitions, with a maximum of 10 motifs, and optimal motif widths ranging from 3 to 10 residues.

3D structure prediction and molecular docking of *16D10* effectors with *Mi* protein

After analyzing the available literature on selected *16D10* effector peptides and Mi protein from tomato, we found that their 3D structures and crystal structure of Mi protein were not available. Hence, we first designed the 3D structures of peptides using amino acid sequence in ChemSketch software. Hydrogen atoms were added to all designed peptides using MOE software to optimize them. The energy of these peptides was minimized using specific parameters: gradient of 0.05, MMFF94X Force Field, Chiral Constraint, and Current Geometry. These peptides were then stored in an .mdb database for subsequent use in docking studies. Additionally, a comparative modeling approach was employed to construct the 3D models of the Mi protein, utilizing Modeller v9.10. Once the models were generated, we assessed the Psi/Phi Ramachandran plots using PROCHECK to evaluate the backbone conformations.

For docking, the MOE software's algorithm was applied to position the designed peptides within the active site of the *Mi* protein. The docking parameters were set as follows: Re-scoring function to London dG, placement by triangle matcher, retaining the top 5 conformations, refinement by Force Field, and a second Rescoring using London dG. This approach ensures the ligand achieves a minimum energy conformation with correct bond rotations for optimal structure

flexibility. The best conformation for each peptide was chosen based on the S-score and further analyzed to examine hydrogen bonding and interactions.

RESULTS

Phylogenetic analysis of effector proteins

The phylogenetic analysis divided the RKNs into two groups based on the coding sequences (CDS) of *16D10* effector genes (Figure 1A). Interestingly *M. incognita* showed more similarity with *M. chitwoodi*, while all the other species fall in the same group based on CDS sequence of *16D10* genes. This reveals the simultaneous evolution of *M. incognita* and *M. chitwoodi* from the same progenitor. However, in the case of phylogenetic analysis based on protein sequence both species fell further apart from each other (Figure 1B). This demonstrates a high degree of variation in the use of codon coding for various amino acids by these two RKN species. The protein based phylogenetic tree grouped *M. incognita*, *M. arenaria*, *M. hapla*, and *M. javanica* in the same cluster while *M. chitwoodi* was found as an out-group due to high level of dissimilarity in the protein sequence compared to all other species. These results are well supported by multiple sequence alignment of *16D10* parasitism proteins as *M. chitwoodi* shared only 22 amino acid residues with other species which have very similar sequence of proteins among each other (Figure 1C).

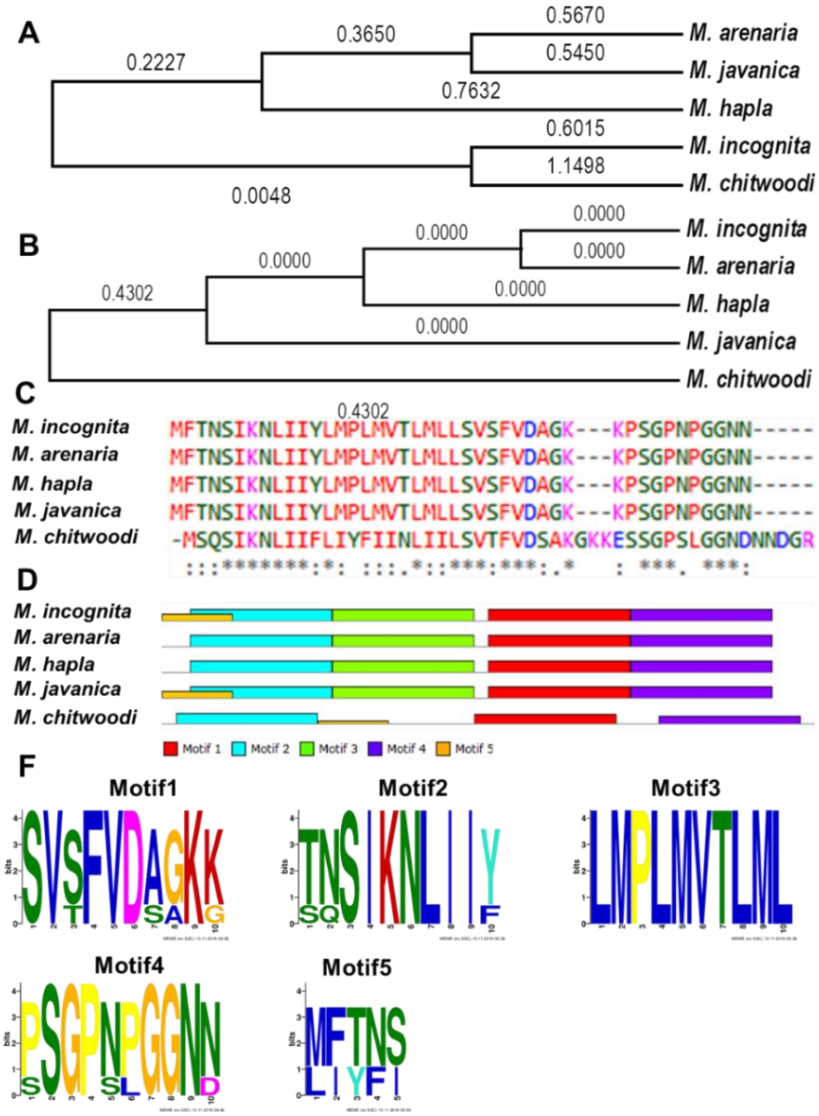

Figure 1. (A, B) Phylogenetic tree and evolutionary relationships of peptides selected for docking study. (C) Sequence similarities among selected peptides using multiple sequence alignment. (D) Comparison of different peptides based on the predicted motifs using MEME.SUITE tool. (F) Representation of predicted motifs. Hight of the letter suggests degree of conserveness for particular amino acid.

Conserved motif analysis of effector Protein Motifs in Root-Knot Nematodes

The conserved motif analysis showed only 5 conserved motifs among amino acid sequences of *16D10* peptide in 5 RKN species (Figure 1D). Motifs 1, 2 and 4 were conserved in all the 5 species, however, motif 3 was missing in *M. chitwoodi.* Similarly, motif 5 was present in *M. incognita* and *M. javanica* while absent in all other species. The sequence logos revealed that out of forty-three amino acid residues, 28 residues were highly conserved in 5 motifs (Figure 1E). All these bioinformatical analyses demonstrated a very high degree of similarity for protein sequence in different species of RKNs. Given the high degree of conservation in motifs 1, 2, and 4 across the five RKN species, these motifs likely play fundamental roles in the effector functions of the *16D10* proteins, possibly related to the initial stages of host infection or immune evasion.

Tertiary structure and molecular docking of effectors with *Mi-1* gene of tomato

3D structures of all *16D10* peptides from different nematode species were constructed using amino acid sequences extracted from the NCBI database, handled in Chem-Sketch software (Figure 2). On the other hand, the 2D structure of the *Mi* protein was derived from its sequence available on the Uniprot database, which was subjected to a PSI-BLAST search against the PDB database. The structure with the highest identity was selected to predict the 3D structure of *Mi* proteins using Modeller v9.10, a Python-based software that employs a homology modeling approach. The resulting structure is depicted in Figure 3A. Subsequently, the accuracy of this 3D structure was confirmed by PROCHECK, which showed that 98% of residues were in the most favorable regions of the Ramachandran plot (Figure 3B). This validated 3D structure is instrumental in elucidating protein interactions and holds significance for future research.

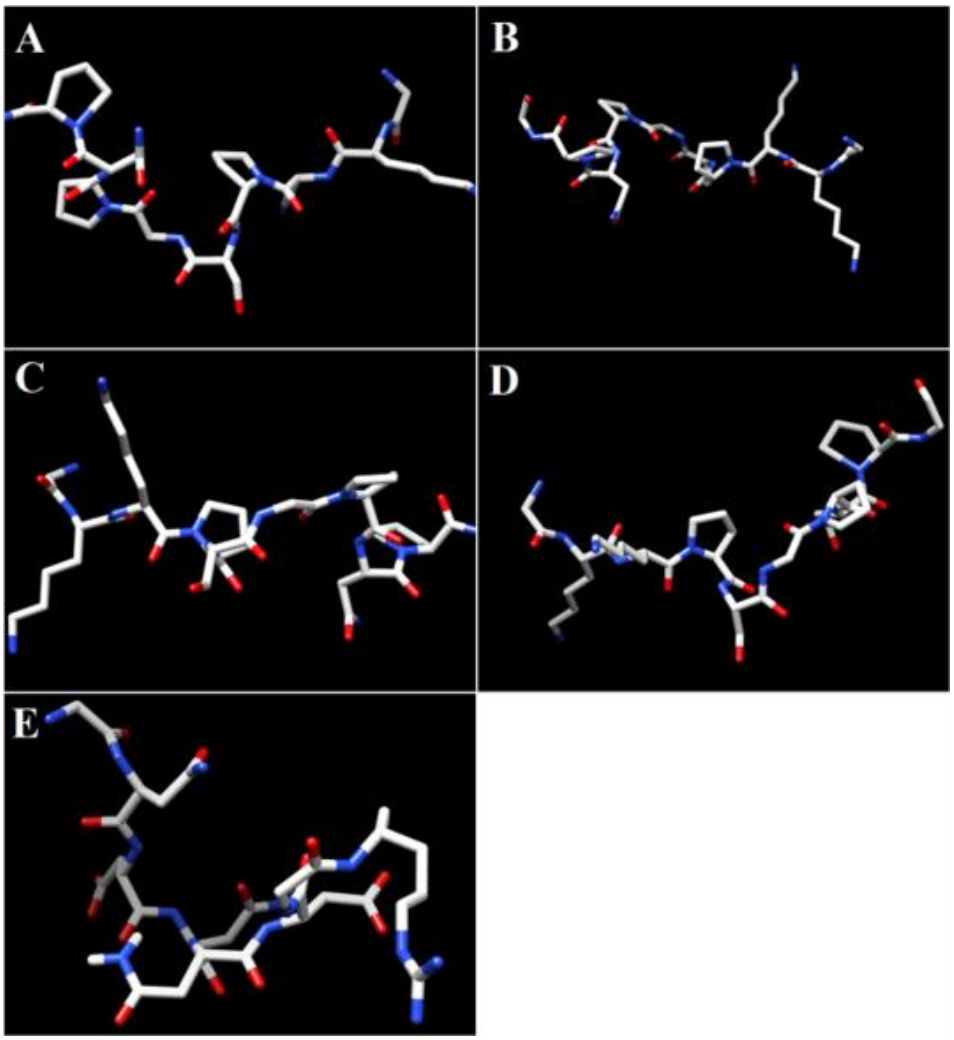

Figure 2. Illustrates the predicted tertiary structures of the RKN peptides under study, derived from computational modeling. Each Figure (A-E) displays a distinct peptide structure, visualized to emphasize the folding patterns and spatial configurations.

In docking studies using the MOE molecular docking program, five conformations for each peptide were generated and ranked according to their S scores. The top-ranking conformation for each peptide, identified by the lowest S score, was analyzed further. Notably, a peptide sequence from *M. incognita* was determined to have the best conformation, followed by other peptides (Table 1). Further analysis of the top conformation of each peptide was performed to identify molecular interactions, visualized using PYMOL and presented in Figure 4.

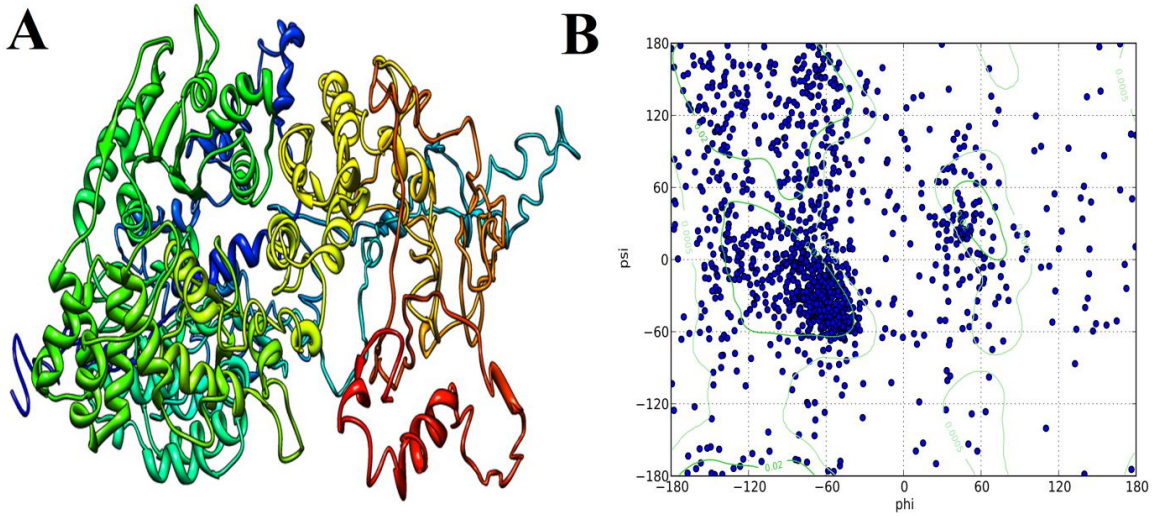

Figure 3. (A) 3D molecular structure of Mi-1 protein from tomato plant showing presence of alpha helix and beta-sheets. (B) illustrates the Ramachandran plot generated for the predicted 3D structure of the *Mi* protein, modeled using Modeller v9.10. The plot displays the phi (φ) and psi (ψ) angles of the amino acid residues in the protein structure. Notably, 98% of the residues are located in the most favorable regions, indicating a high-quality model with proper backbone conformation.

Table1. Molecular docking of different peptides and predicted residues for interaction.

Sr. No.	Peptides	S-Score	RMSD Value	Interacting Residues
1	*M. incognita*	-23.41	2.10	Ile97, Leu904, Asp909, Trp1154, Asp866, Ile993, Glu1016, Asp1011, Met1156, Glu1153, Arg870, Ile872
2	*M. javanica*	-22.37	2.13	Arg895, Glu1153, Pro869, Asn115, Glu1152, Phe1170, Asp909, Asp866, His881, Glu1034
3	*M. arenaria*	-21.23	2.84	Asp913, Asp909, Asp866, Arg870, Pro34, Leu1032, Lys833, Arg1083, Ser835, Cys834
4	*M. hapla*	-19.20	2.32	Glu1016, Asp1011, Asn885, Ser914, Met1156, Glu1153, Arg870, Ile872
5	*M. chitwoodi*	-18.39	2.62	Leu904, Gln910, Phe1170, Asp909, Asp866, His881, Glu1034

DISCUSSION

PPNs use different strategies to parasitize plants including the secretion of cell wall degrading enzymes, effector proteins and peptides and transcriptional activators or repressors (Ali *et al.*, 2017). Effector proteins interact with resistance (*R*) proteins from the plants to define compatible or incompatible interaction with the plants (Ansari and Saleem, 2023). *16D10* is one of the *CLE* effector peptides which are important mediators of nematode parasitism in plants. RNAi based *in planta* gene silencing using of the *16D10* effector gene established broad resistance in potato against all Meloidogyne species (Dinh *et al.*, 2015). However, suppression of *16D10* transcripts did not interfere with the attraction and invasion of *M. incognita* second-stage juveniles towards potato roots. This proposed that this effector peptide may interact with the R proteins from the plants and establish nematode infection in plants through effector triggered susceptibility (ETS) (Pires *et al.*, 2022). The results have shown high conservation of *16D10* peptides in important species of RKNs. However, the divergence observed in protein sequences between *M. incognita* and *M.*

chitwoodi, as compared to their closer alignment at the CDS level, possibly reflects different selective pressures or adaptive responses to their host environments (Geffersa *et al.*, 2023). This kind of variation suggests that these species have undergone significant adaptive divergence, potentially in response to host immune pressures or in their parasitic strategies, despite a common evolutionary origin. Previous research indicates that host-specific adaptation often leads to accelerated evolution process in the effector proteins of pathogen (McDonald and Stukenbrock, 2016). Further examination of the ecological niches and host ranges of these nematodes could provide deeper insights into the evolutionary mechanisms shaping these interactions.

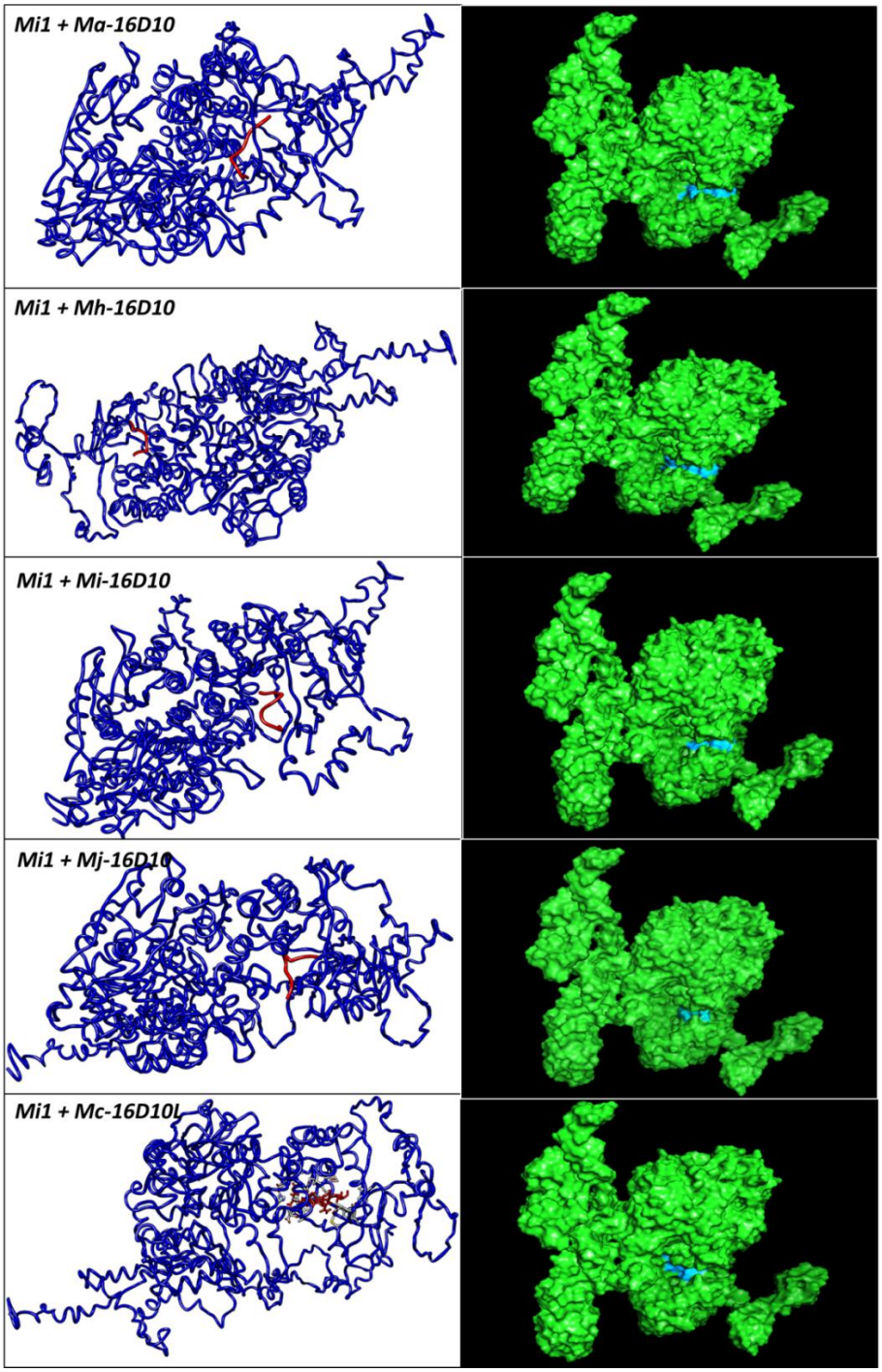

Figure 4. Molecular Docking of Mi-1 Protein with Different Peptides from Plant Parasitic Nematodes. The Figure presents the results of molecular docking between the Mi-1 protein from tomato and various effector peptides sourced from different species of root-knot nematodes (RKNs). Each box in the Figure corresponds to a distinct peptide-protein interaction, showcasing the top-ranked conformation based on the minimum S score. The peptides are shown in red and blue color on the left and right side of the Figure respectively.

The conserved motif analysis was performed using MEME Suite tool which displayed high degree of conservation of different motifs of *16D10* peptides in different nematode species with a little bit of divergence as well. The results exhibited the absence of motif 3 in *M. chitwoodi* and motif 5 in all species except *M. incognita* and *M. javanica* which suggests probable functional diversification of *16D10* in these species. One of the possible explanations of this divergence could be an adaptation to different host ranges or immune responses, allowing these nematodes to optimize their parasitic strategies within specific hosts (Khan *et al.*, 2023; Kumar *et al.*, 2022).

The results of molecular docking of *16D10* peptides and *Mi* protein showed the top-ranking conformation for each peptide, that was identified by the lowest S score. In addition to having minimum S score, *16D10* peptides from all the nematode species also showed interactions with *Mi* protein, which advocated that the effector peptides are likely to play a crucial role in the modulation of the host's immune response, providing a potential target for the development of novel nematode-resistant crop varieties (Ali *et al.*, 2015; Lin *et al.*, 2016). On the basis of these results, the future experiments should aim to validate these *in silico* findings through *in vivo* or *in vitro* assays to further elucidate the biological significance of these interactions and their impact on plant health and resistance traits.

CONCLUSION

The current study investigated the interaction between *16D10* effector peptides from various root-knot nematode (RKN) species and the *Mi* resistance protein present in tomato. Bioinformatics tools like sequence retrieval, alignment, phylogenetic analysis, and molecular docking were used for this purpose. The results indicated a close evolutionary relationship between *M. incognita* and *M. chitwoodi*. *In silico* 3D modeling and docking studies elucidate the structural basis of *16D10* peptide-*Mi* protein interaction, suggesting a potential role in modulating plant defense mechanisms, possibly leading to effector-triggered susceptibility (ETS). The study contributes to the understanding of nematode-host interactions and informs strategies for managing RKN infections in crops.

AUTHOR CONTRIBUTIONS

All authors contributed equally to this research.

COMPETING OF INTEREST

The authors declare that they have no competing interests.

REFERENCES

Abad, P., Gouzy, J., Aury, J. M., Castagnone-Sereno, P., Danchin, E. G. J., Deleury, E., ... & Wincker, P. (2008). Genome sequence of the metazoan plant-parasitic nematode *Meloidogyne incognita*. Nature Biotechnology, 26, 909-915.

Ali, M. A., Abbas, A., Azeem, F., Javed, N., & Bohlmann, H. (2015). Plant-nematode Interactions: From Genomics to Metabolomics. International Journal of Agriculture and Biology, 17, 1071-1082.

Ansari, T., & Saleem, M. (2023). Plant Parasitic Nematodes: A Silent Threat to Agricultural Output and Sustainable Approaches for Their Management. In Climate-Resilient Agriculture, Vol 1: Crop Responses and Agroecological Perspectives (pp. 799-819). Cham: Springer International Publishing.

Bellafiore, S., Shen, Z. X., Rosso, M. N., Abad, P., Shih, P., & Briggs, S. P. (2008). Direct Identification of the Meloidogyne incognita Secretome Reveals Proteins with Host Cell Reprogramming Potential. Plos Pathogens, 4.

Fuller, V. L., Lilley, C. J., & Urwin, P. E. (2008). Nematode resistance. New Phytologist, 180, 27-44.

Geffersa, A. G., Burdon, J. J., Macfadyen, S., Thrall, P. H., Sprague, S. J., & Barrett, L. G. (2023). The socio-economic challenges of managing pathogen evolution in agriculture. Philosophical Transactions of the Royal Society B, 378(1873), 20220012.

Huang, G. Z., Allen, R., Davis, E. L., Baum, T. J., & Hussey, R. S. (2006a). Engineering broad root-knot resistance in transgenic plants by RNAi silencing of a conserved and essential root-knot nematode parasitism gene. Proceedings of the National Academy of Sciences of the United States of America, 103, 14302-14306.

Huang, G. Z., Dong, R. H., Allen, R., Davis, E. L., Baum, T. J., & Hussey, R. S. (2006b). A root-knot nematode secretory peptide functions as a ligand for a plant transcription factor. Molecular Plant-Microbe Interactions, 19, 463-470.

Hussey, R. S., Davis, E. L., & Baum, T. J. (2002). Secrets in secretions: genes that control nematode parasitism of plants. Brazilian Journal of Plant Physiology, 14. (3) https://doi.org/10.1590/S1677-04202002000300002

Khan, A., Khan, A., Ali, A., Fatima, S., & Siddiqui, M. A. (2023). Root-knot nematodes (*Meloidogyne* spp.): Biology, plant-nematode interactions and their environmentally benign management strategies. Gesunde Pflanzen, 75(6), 2187-2205.

Kumar, A., Joshi, I., Changwal, C., Sirohi, A., & Jain, P. K. (2022). Host-delivered RNAi-mediated silencing of the root-knot nematode (*Meloidogyne incognita*) effector genes, *Mi-msp10* and *Mi-msp23*, confers resistance in Arabidopsis and impairs reproductive ability of the root-knot nematode. Planta, 256(4), 74.

Lin, B., Zhuo, K., Chen, S., Hu, L., Sun, L., Wang, X., ... & Liao, J. (2016). A novel nematode effector suppresses plant immunity by activating host reactive oxygen species-scavenging system. New Phytologist, 209(3), 1159-1173.

Lu, S. W., Chen, S. Y., Wang, J. Y., Yu, H., Chronis, D., Mitchum, M. G., & Wang, X. H. (2009). Structural and Functional Diversity of *CLAVATA3/ESR (CLE)*-Like Genes from the Potato Cyst Nematode *Globodera rostochiensis*. Molecular Plant-Microbe Interactions, 22, 1128-1142.

McDonald, B. A., & Stukenbrock, E. H. (2016). Rapid emergence of pathogens in agro-ecosystems: global threats to agricultural sustainability and food security. Philosophical Transactions of the Royal Society B: Biological Sciences, 371(1709), 20160026.

Olsen, A. N., & Skriver, K. (2003). Ligand mimicry? Plant-parasitic nematode polypeptide with similarity to *CLAVATA3*. Trends in Plant Science, 8, 55-57.

Pires, D., Vicente, C. S., Menéndez, E., Faria, J. M., Rusinque, L., Camacho, M. J., & Inácio, M. L. (2022). The fight against plant-parasitic nematodes: Current status of bacterial and fungal biocontrol agents. Pathogens, 11(10), 1178.

Replogle, A., Wang, J. Y., Bleckmann, A., Hussey, R. S., Baum, T. J., Sawa, S., ... & Mitchum, M. G. (2011). Nematode CLE signaling in Arabidopsis requires *CLAVATA2* and *CORYNE*. Plant Journal, 65, 430-440.

Rockey, D., Shinya, R., Morisaka, H., Kikuchi, T., Takeuchi, Y., Ueda, M., & Futai, K. (2013). Secretome Analysis of the Pine Wood Nematode *Bursaphelenchus xylophilus* Reveals the Tangled Roots of Parasitism and Its Potential for Molecular Mimicry. Plos One, 8, e67377.

Vanholme, B., De Meutter, J., Tytgat, T., Van Montagu, M., Coomans, A., & Gheysen, G. (2004). Secretions of plant-parasitic nematodes: A molecular update. Gene, 332, 13-27.

Wang, J., Hewezi, T., Baum, T. J., Davis, E. L., Wang, X., & Mitchum, M. G. (2010). Trafficking of soybean cyst nematode secreted CLE proteins in plant cells. Phytopathology, 100, S132-S132.

Wang, J., Replogle, A., Wang, X., Davis, E. L., & Mitchum, M. G. (2006). Functional analysis of nematode secreted CLAVATA3/ESR (CLE)-like peptides of the genus *Heterodera*. Phytopathology, 96, S120-S121.

Wang, J. Y., Replogle, A., Hussey, R., Baum, T., Wang, X. H., Davis, E. L., & Mitchum, M. G. (2011). Identification of potential host plant mimics of *CLAVATA3/ESR (CLE)*-like peptides from the plant-parasitic nematode *Heterodera schachtii*. Molecular Plant Pathology, 12, 177-186.

Wang, X. H., Mitchum, M. G., Gao, B. L., Li, C. Y., Diab, H., Baum, T. J., Hussey, R. S., & Davis, E. L. (2005). A parasitism gene from a plant-parasitic nematode with function similar to *CLAVATA3/ESR (CLE)* of *Arabidopsis thaliana*. Molecular Plant Pathology, 6, 187-191.

Yang, Y., Jittayasothorn, Y., Chronis, D., Wang, X., Cousins, P., & Zhong, G. Y. (2013). Molecular characteristics and efficacy of *16D10* siRNAs in inhibiting root-knot nematode infection in transgenic grape hairy roots. Plos One, 8, e69463.