

Network-based integrative analysis of key regulatory genes involved in rice response to *Meloidogyne graminicola* in the susceptible cultivar IR64

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Abstract

Root-knot nematode infection caused by *Meloidogyne graminicola* is one of the most destructive diseases affecting rice, leading to reduced grain yield, leaf chlorosis, and ultimately plant death. In the present study, the molecular mechanisms underlying rice seedling responses to *M. graminicola* infection were investigated through transcriptome-based integrative bioinformatics analyses. Differentially expressed genes (DEGs) were analyzed using protein–protein interaction (PPI) network construction to identify key regulatory genes and major biological pathways associated with nematode stress. Hub genes were identified using the Cytoscape plugin CytoHubba based on topological network parameters, while significant subnetworks were extracted using the IPCA algorithm implemented in CytoCluster. Functional enrichment analysis revealed that the identified DEGs were predominantly involved in protein folding, ATP-dependent chaperone activity, and cellular stress response processes. Most hub genes belonged to the heat shock protein (HSP) family, suggesting their critical roles in chloroplast development, antioxidant enzyme regulation, and defense responses during pathogen-induced stress. Kyoto Encyclopedia of Genes and Genomes enrichment analysis further demonstrated that protein processing in the endoplasmic reticulum and RNA degradation pathways were significantly enriched during infection. Conserved domain analysis showed that the identified hub proteins contained functional domains associated with chaperone activity, ATP binding, and biotic stress responses, supporting their structural and regulatory roles in plant defense mechanisms. Furthermore, promoter motif analysis using Tomtom and GOMO revealed that their regulatory elements are mainly associated with plant growth, development, and responses to oxidative stress. These findings provide new insights into the molecular defense mechanisms of rice against *M. graminicola* and may contribute to the development of resistant transgenic rice cultivars.

Introduction

Rice (*Oryza sativa* L.) is one of the most important staple food crops worldwide and plays a central role in global food security, particularly in developing countries where population growth continues to increase food demand; As the primary food source for more than half of the world's population, maintaining stable and sustainable rice production is essential for future food security; However, rice cultivation is continuously challenged by multiple biotic stress factors, including fungal and bacterial pathogens, insect pests, weeds, and plant-parasitic nematodes, all of which significantly reduce yield and grain quality (Mantelin et al. 2017). Among these biotic constraints, the root-knot nematode *Meloidogyne graminicola* has emerged as one of the most destructive pathogens in rice-growing ecosystems, particularly in Asia; This sedentary endoparasitic nematode invades rice roots at early juvenile stages, migrates within root tissues, and induces the formation of specialized feeding structures known as giant cells; These hypertrophied and multinucleated cells act as strong metabolic sinks that support nematode development; Infection typically leads to root gall formation, disruption of vascular integrity, impaired water and nutrient uptake, chlorosis, reduced plant vigor, stunted growth, and, under severe infestation, plant death; Since its first major reports in China, *M. graminicola* has spread to several rice-producing regions worldwide, causing substantial economic losses (Honghai et al, 2001;

Mantelin et al. 2016; Nguyen et al. 2023). Despite significant progress in rice breeding programs, no commercially available cultivar has demonstrated complete and stable resistance to *M. graminicola*. Resistance has mainly been identified in wild or genetically distant relatives such as *Oryza glaberrima* and *O. longistaminata*, as well as in a limited number of Asian rice genotypes; However, the transfer of these resistance traits into elite high-yielding cultivars remains challenging due to genetic incompatibility and complex inheritance patterns (Cabasan et al. 2018). Recent evaluations of rice germplasm have revealed considerable variation in host responses to *Meloidogyne graminicola*, ranging from high susceptibility to strong resistance; Field and controlled experiments conducted on diverse rice varieties demonstrated significant differences in gall formation, nematode development, and disease severity; Resistant genotypes showed enhanced biochemical defense responses, including increased activities of phenylalanine ammonia-lyase (PAL), peroxidase (POX), and accumulation of total phenolic compounds, suggesting that these metabolic changes contribute to limiting nematode establishment and development (Gautam et al. 2025). Moreover, histopathological analyses revealed that resistant rice varieties restrict giant cell formation, promote callose deposition, and preserve root tissue integrity, whereas susceptible genotypes exhibit extensive giant cell development and structural damage caused by nematode feeding (Gautam et al. 2025). Genetic studies have further demonstrated that resistance and tolerance to *M. graminicola* are complex quantitative traits controlled by multiple genomic regions; QTL mapping studies using recombinant inbred lines derived from resistant and susceptible rice genotypes identified genomic loci associated with reduced nematode reproduction and improved host performance; These findings highlight the importance of exploiting natural genetic variation and molecular markers for improving resistance against rice root-knot nematode through breeding programs (Galeng-Lawilao et al. 2018). In addition to resistance, tolerance mechanisms play an important role in determining rice performance under nematode infection; Evaluation of aerobic Asian rice progenies showed considerable variation in tolerance levels, with some genotypes maintaining relatively stable growth and yield despite nematode infestation; These results indicate that host adaptation to *M. graminicola* involves not only suppression of nematode development but also the ability of plants to minimize yield losses under infection conditions (Galeng-Lawilao et al. 2016). Consequently, widely cultivated varieties such as IR64 remain highly susceptible and are frequently used as model systems for studying host–nematode interactions; Microscopic and histological studies have revealed that resistant rice genotypes often activate early defense responses characterized by localized cell death, accumulation of phenolic compounds, and enhanced production of reactive oxygen species (ROS), which collectively restrict nematode establishment and feeding site development; In contrast, susceptible interactions are characterized by successful formation of feeding sites and sustained nematode reproduction, indicating suppression or failure of early immune activation (Nguyen et al. 2022; Pandey 2020). In addition to structural defenses, nematode infection induces profound physiological and biochemical alterations in host plants; Changes in chlorophyll content, soluble sugars, proteins, phenolic compounds, and proline levels have been reported, with resistant genotypes generally exhibiting stronger activation of defense-related metabolic pathways compared to susceptible ones; These changes reflect a broad reprogramming of primary and secondary metabolism during host–nematode interaction (Pandey 2020). At the genetic level, resistance and tolerance to *M. graminicola* are quantitatively

inherited traits governed by multiple loci; QTL mapping studies have identified several genomic regions associated with reduced nematode reproduction and gall formation; Candidate genes involved in immune signaling, hormonal regulation, and stress adaptation—such as OsBAK1, OsDST, and OsIPT4—suggest that multiple interconnected pathways contribute to host defense regulation (Galeng-Lawilao et al. 2020; Nguyen et al. 2022). At the molecular level, infection triggers extensive transcriptional reprogramming involving major immune signaling pathways, including salicylic acid, jasmonic acid, and ethylene signaling; In addition, reactive oxygen species signaling and autophagy have been implicated in regulating resistance responses; Early activation of these pathways in resistant genotypes leads to suppression of nematode development and disruption of feeding site formation (Nguyen et al. 2022). Advances in molecular biology and biotechnology have significantly improved our understanding of plant–nematode interactions and have shifted research toward sustainable management strategies; Increasing restrictions on chemical nematicides and environmental concerns have further emphasized the importance of genetically based resistance; Modern approaches such as genome sequencing, RNA interference (RNAi), and gene editing provide powerful tools for identifying resistance-associated genes and elucidating host susceptibility mechanisms (Abd-Elgawad 2022). Natural variation in rice germplasm has revealed substantial diversity in responses to *M. graminicola*. Histological analyses of resistant cultivars such as Zhonghua 11 have shown hypersensitive-like responses, localized necrosis, phenolic accumulation, and impaired giant cell development, suggesting the involvement of major resistance genes (Phan et al. 2018). Similarly, studies in rice varieties from the Mekong Delta have demonstrated differential susceptibility, where cultivars such as Tetep exhibit moderate resistance and delayed nematode development compared with highly susceptible cultivars like IR64 (Nguyen et al. 2021). The emergence of virulent *M. graminicola* populations capable of overcoming previously effective resistance sources has further complicated sustainable disease management; These virulent pathotypes can suppress host hypersensitive responses and successfully establish infection even in resistant genotypes, raising concerns about the durability of resistance-based breeding strategies (Nguyen et al. 2023). Phenotypic evaluations across diverse rice germplasm have revealed a continuous spectrum of responses ranging from high susceptibility to partial resistance and tolerance, reflecting the polygenic nature of host resistance (Hui et al. 2023). Recent advances in transcriptomics and systems biology have enabled comprehensive investigation of host–pathogen interactions at the molecular level; Integrative bioinformatics approaches, including differential gene expression analysis, protein–protein interaction (PPI) network construction, and functional enrichment analysis, provide powerful tools for identifying key regulatory genes and pathways involved in plant defense responses; However, the molecular mechanisms underlying rice resistance to *M. graminicola* remain incompletely understood, particularly in elite susceptible cultivars such as IR64 (Zhang et al. 2023). At the systems level, plant–nematode interactions are governed by complex gene regulatory networks rather than single genes; High-throughput transcriptomic approaches combined with integrative bioinformatics analyses, including co-expression network construction and protein interaction mapping, have enabled identification of key regulatory modules and hub genes involved in defense regulation; Nevertheless, the regulatory architecture underlying susceptibility in elite cultivars remains largely unresolved; Therefore, the present study aims to identify key responsive genes, regulatory subnetworks, conserved functional

domains, and promoter regulatory motifs associated with rice defense responses against *M. graminicola* using transcriptome-based integrative bioinformatics analyses.

Materials and Methods

Identification of Differentially Expressed Genes

Transcriptome data associated with rice responses to *Meloidogyne graminicola* infection were obtained from the previously published study by Zhang et al. (2023). Differentially expressed genes (DEGs) were selected based on gene expression profiles in infected rice seedlings. Genes exhibiting expression changes with $\log_2$ fold change ($\log_2FC$) ≥ 2 or ≤ -2 were considered significantly differentially expressed and were selected for subsequent network analyses.

Construction of Protein–Protein Interaction Network

To investigate functional relationships among the selected DEGs, protein–protein interaction (PPI) analysis was performed using the online platform STRING. A minimum interaction confidence score of 0.15 was applied to identify potential functional interactions among proteins. The resulting PPI network was exported and subsequently visualized and analyzed using Cytoscape.

Identification of Hub Genes and Network Clustering

Hub genes were identified using the CytoHubba plugin implemented in Cytoscape. To improve reliability, multiple topological algorithms including Maximum Clique Centrality (MCC), Degree, Density of Maximum Neighborhood Component (DMNC), and Maximum Neighborhood Component (MNC) were employed for gene ranking and hub gene identification. To identify highly interconnected functional modules within the network, clustering analysis was performed using the IPCA algorithm implemented in the CytoCluster plugin.

Functional Enrichment Analysis

To investigate the biological functions of the identified hub genes, functional enrichment analysis was performed using the STRING database. Gene Ontology (GO) annotation was conducted to identify significantly enriched biological processes, molecular functions, and cellular components associated with the hub genes. In addition, pathway enrichment analysis was performed based on Kyoto Encyclopedia of Genes and Genomes annotations to identify major metabolic and signaling pathways involved in nematode response.

Conserved Domain Analysis

Protein sequences corresponding to the identified hub genes were retrieved from the National Center for Biotechnology Information database. Conserved domain analysis was performed using the Conserved Domain Database search tool available at NCBI. Protein sequences were submitted to the online CDD

platform, and functional domains were identified based on similarity with conserved domain profiles. Domain type, accession number, E-value, and domain positions within the protein sequences were recorded. Only domains with statistically significant E-values were considered for downstream interpretation.

Promoter Motif Analysis

To identify cis-regulatory elements associated with the hub genes, 2,000 bp upstream promoter sequences were extracted using BioMart from the Ensembl Plants database. Conserved promoter motifs were identified using Tomtom against the JASPAR CORE 2022 database. Motifs with P-values < 0.01 and E-values < 0.1 were considered statistically significant.

Results and Discussion

Identification of Hub Genes and Functional Enrichment Analysis in Response to *Meloidogyne graminicola* Infection

Based on the transcriptomic analysis previously reported by Zhang et al. (2023), a total of 565 differentially expressed genes (DEGs) were identified in the susceptible rice cultivar IR64 rice following infection with *Meloidogyne graminicola* at a significance threshold of $P < 0.05$. Among these, 318 genes were upregulated, whereas 247 genes were downregulated. Genes exhibiting expression changes of $\log_2$ fold change ($\log_2FC$) ≥ 2 or ≤ -2 were selected for subsequent protein–protein interaction (PPI) network analysis (Figure 1A). The PPI network was constructed and analyzed using Cytoscape, and hub genes were identified using the CytoHubba plugin based on four topological algorithms, including MCC, Degree, DMNC, and MNC. The selected hub genes were subsequently used for subnetwork construction to evaluate their interactions with neighboring proteins (Figure 1B). The resulting hub-centered subnetwork revealed strong functional connectivity among stress-responsive proteins, indicating coordinated molecular responses during nematode infection.

Functional enrichment analysis of the identified hub genes was performed using the STRING platform, and the results are presented in Figure 2. Gene Ontology enrichment analysis classified the significantly enriched terms into three major categories, including molecular function (MF), biological process (BP), and cellular component (CC). In the molecular function category, the most significantly enriched terms were associated with ATP binding, ATP-dependent chaperone activity, unfolded protein binding, and protein refolding (Figure 2B). In the biological process category, the identified hub genes were significantly enriched in protein folding, protein stabilization, response to heat, and protein refolding (Figure 2C). In the cellular component category, the hub genes were predominantly associated with the cytosol, cytoplasm, and stress-associated cytoplasmic regions (Figure 2A). Furthermore, Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis identified two significantly enriched pathways, including protein processing in the endoplasmic reticulum and RNA degradation (Figure 2D). These findings indicate that rice defense responses against *Meloidogyne graminicola* are strongly

associated with protein quality control systems, cellular stress adaptation, and post-transcriptional regulatory mechanisms.

Functional Characterization of Key Hub Genes

Twelve hub genes were identified as key regulatory genes in the susceptible rice cultivar IR64, including Os03g0787300, Os08g0500700, Os12g0514500, Os04g0107900, Os05g0562300, Os02g0247200, Os10g0462900, Os03g0143400, Os02g0758000, Os02g0493050, Os01g0840100, and Os02g0770800. Among these, Os03g0787300, encoding the DnaJA5 protein, belongs to the Heat Shock Protein 40 family and functions as a molecular co-chaperone involved in multiple cellular processes. DnaJA5 is primarily localized in chloroplasts, where it contributes to protein folding, maturation, and maintenance of chloroplast development. Similar to the YDJ1 homolog in yeast, DnaJA5 regulates the ATPase activities of Heat Shock Protein 70 and Heat Shock Protein 90 proteins and participates in the recognition and processing of misfolded proteins under stress conditions (Li et al., 2021; Zhu et al., 2015; Fernández et al., 2024). Interestingly, this gene exhibited significant downregulation in the susceptible cultivar ($\log_2FC = -2.367$), suggesting that suppression of its transcription may impair cellular stress adaptation and defense responses during nematode infection; A similar expression pattern was observed for Os05g0562300, indicating possible functional conservation between these two genes (Zhang et al., 2023). Another important hub gene, Os08g0500700, encodes OSHSP82A, a heat shock protein involved in enhancing antioxidant enzyme activities and improving stress tolerance in rice; Previous studies have demonstrated that overexpression of this gene improves plant establishment, grain yield, and agronomic performance under stress conditions (Wang et al., 2020). However, the observed downregulation of this gene in IR64 ($\log_2FC = -2.65$) may reduce stress tolerance and contribute to increased susceptibility to nematode infection; In addition, Os12g0514500, together with Os02g0247200 and Os04g0107900, was associated with the expression and regulation of Heat Shock Protein 90 proteins. HSP90 proteins are highly abundant cytoplasmic molecular chaperones that account for approximately 1–2% of total cellular proteins and play essential roles in signal transduction, protein folding, transcription factor regulation, kinase activation, and cellular homeostasis under stress conditions (Lindquist et al., 1988; Pratt et al., 2001; Wang et al., 2004; Manchado et al., 2008). Therefore, the reduced expression of these HSP-associated hub genes may represent one of the major molecular determinants underlying the susceptibility of rice cultivar IR64 to *Meloidogyne graminicola* infection; Previous studies have demonstrated extensive structural and functional remodeling of root cells during nematode parasitism, accompanied by enhanced endocytic activity, highlighting the dynamic cellular reprogramming that occurs during host–pathogen interactions (Verstraeten et al., 2021). The gene Os10g0462900 (OsHsp60-1) encodes a member of the Heat Shock Protein 60 (HSP60) family in Rice and has been identified as part of the Conserved Drought Tolerance (CDT) gene set through comparative transcriptomic analyses; According to Baldoni et al. (2021), OsHsp60-1 is evolutionarily conserved across cereal species and is associated with multiple abiotic stress responses, including drought and oxidative stress; HSP60 proteins function as essential molecular chaperones localized primarily in chloroplasts and mitochondria, where they facilitate protein folding, prevent the accumulation of misfolded proteins, and maintain cellular proteostasis under stress conditions. In this context, Jensen et

al. (2007) demonstrated that Photosystem I (PSI) represents one of the most stress-sensitive components of the photosynthetic electron transport chain. Because PSI integrity is critical for efficient electron transfer and photosynthetic performance, chloroplast-localized chaperones such as HSP60 are likely to play a protective role in preserving the stability of photosynthetic protein complexes under adverse environmental conditions. Accordingly, OsHsp60-1 may indirectly contribute to the maintenance of photosynthetic efficiency through protection of chloroplast protein complexes, including PSI-associated components; Similarly, Os03g0143400 also belongs to the chaperonin-60 (HSP60-like) protein family in rice and exhibits strong structural and functional similarity to mitochondrial chaperonins; This gene is likely involved in essential cellular processes including protein folding, prevention of protein aggregation, and maintenance of proteome stability under stress conditions (Baldoni et al., 2021). The gene Os02g0758000 was identified in transcriptomic analyses by Qing et al. (2024) as a multi-abiotic stress-responsive gene in rice; This gene exhibited significant upregulation under both submergence stress and combined submergence-cold stress conditions, suggesting its involvement in adaptive stress-response pathways; Previous studies have also demonstrated its responsiveness to cadmium stress, indicating a broader role in environmental stress adaptation; The present findings further expand its potential functional relevance beyond heavy metal responses, suggesting possible involvement in flooding- and cold-responsive signaling pathways; Nevertheless, the precise molecular function of Os02g0758000 remains largely uncharacterized, and additional functional studies, including gene knockout and biochemical characterization, are required to elucidate its biological role (Qing et al., 2024). Os02g0493050 encodes an ankyrin repeat-containing protein harboring both ankyrin and tetratricopeptide repeat (TPR) domains (Chakrabarty and Parekh, 2014). This conserved domain architecture strongly suggests its involvement in protein–protein interactions and cellular signaling regulation; Proteins containing these domains commonly function as regulatory scaffolds in signal transduction, developmental regulation, and protein complex assembly; Therefore, Os02g0493050 is likely involved in coordinating intracellular signaling networks and growth-related regulatory processes in rice (Zhao et al., 2025). The gene Os01g0840100 encodes a member of the HSP70 family in rice, functioning as a central component of cellular protein homeostasis under stress conditions; As an ATP-dependent molecular chaperone, HSP70 participates in protein folding, prevention of protein aggregation, and maintenance of protein quality control; In addition, HSP70 proteins are involved in diverse stress-associated processes including metabolic regulation, immune responses, photosystem repair, and signal transduction; These proteins also interact with transcriptional regulators such as HsfA6a, as well as components of the ubiquitin-mediated degradation pathway, thereby modulating the expression of key stress-responsive genes such as Hsp101 (Jung et al., 2013; Singh et al., 2021). These findings suggest that Os01g0840100 serves as a core regulatory component of the rice stress-response network; Furthermore, Os02g0770800 was identified through integrated transcriptomic and metabolomic analyses as a candidate aluminum-responsive gene in rice; This gene was classified among differentially expressed genes (DEGs) and was associated with the phenylpropanoid biosynthesis pathway, a critical metabolic pathway involved in plant defense and oxidative stress adaptation under aluminum toxicity; However, its precise molecular function has not yet been experimentally validated and requires further functional investigation (Wang et al., 2023). Overall, these findings suggest that the rice

response to *Meloidogyne graminicola* involves a highly integrated regulatory network encompassing protein quality control, effector-mediated host manipulation, RNA-mediated regulation, and intracellular trafficking pathways; A deeper understanding of these molecular mechanisms may facilitate the identification of novel genetic targets for improving nematode resistance in rice.

Subnetwork Analysis and Functional Module Identification

To improve the reliability of hub gene identification, four topological algorithms implemented in the CytoHubba plugin, including Maximum Neighborhood Component (MNC), Density of Maximum Neighborhood Component (DMNC), Degree centrality, and Maximum Clique Centrality (MCC), were employed for hub gene ranking. Based on these algorithms, twelve candidate hub genes were consistently identified as key regulatory genes involved in the rice response to *Meloidogyne graminicola* infection (Table 1), indicating their potential functional significance within the stress-responsive interaction network (Li et al., 2017). To further investigate the modular organization of the protein–protein interaction (PPI) network, clustering analysis was performed using the IPCA algorithm implemented in the CytoCluster plugin. The results identified four highly interconnected functional modules within the network.

Table 1. Ranking of functional gene clusters identified within the protein–protein interaction subnetworks of rice seedlings in response to *Meloidogyne graminicola* infection using the IPCA algorithm implemented in the CytoCluster plugin in Cytoscape (version 3.10.2).

Gene ID	Function	Method	Rank
Os03g0787300	Similar to DnaJ homolog	MCC, DMNC	1, 2, 3, 4, 5, 7
Os08g0500700	HSP81-1	MCC, MNC, DEGREE	1, 2, 3, 4, 5, 7
Os12g0514500	Q0IN14_ORYSJ - Hsp90 protein	MCC, MNC, DEGREE	1, 2, 3, 4, 5, 7
Os04g0107900	Similar to Heat shock protein 82	MCC, MNC, DEGREE	1, 2, 3, 4, 5, 7
Os05g0562300	Similar to DnaJ-like protein	MCC,	1, 2, 3, 4, 5, 7
Os02g0247200	6K3R8_ORYSJ - T-complex protein 1 subunit gamma.	DMNC,	1, 2, 3, 4, 5, 7
Os10g0462900	Q8H903_ORYSJ/Belongs to the chaperonin (HSP60) family	DMNC,	1, 2, 3, 4, 5, 7
Os03g0143400	Q10RW9_ORYSJ - Chaperonin CPN60-1	DMNC,	1, 2, 3, 4, 5, 7
Os02g0758000	Heat shock protein, mitochondrial; Belongs to the small heat shock protein (HSP20) family.	DMNC,	7
Os02g0493050	Ankyrin repeat domain containing protein	MNC, DEGREE	1, 2, 3, 4, 5
Os01g0840100	Belongs to the heat shock protein 70 family	MNC,	1, 2, 3, 4, 5, 7

Cluster 1 and Cluster 2 showed the highest interaction density, each containing 22 nodes and 144 edges. Functional enrichment analysis revealed that these clusters were significantly associated with bacterial secretion system-related pathways and protein processing in the endoplasmic reticulum. Cluster 3 and Cluster 4 each contained 21 nodes and 141 edges and were significantly enriched in pathways related to bacterial secretion systems, protein processing in the endoplasmic reticulum, endocytosis, RNA degradation, and spliceosome-associated processes (Figure 3).

These findings suggest that rice seedlings undergo extensive molecular network reprogramming in response to *M. graminicola* infection; The enrichment of highly connected modules involved in protein processing in the endoplasmic reticulum indicates activation of cellular protein quality control mechanisms and possible induction of the unfolded protein response (UPR) during nematode infection; Since the endoplasmic reticulum functions as a major cellular center for protein folding, maturation, and secretion, its activation under biotic stress likely contributes to host defense regulation; Previous transcriptomic studies have shown that *M. graminicola* infection induces substantial changes in the expression of genes associated with protein metabolism and defense-related pathways, reflecting extensive cellular reprogramming in the host plant; Moreover, the formation of nematode-induced feeding cells requires major metabolic and structural reorganization within host root tissues (Ji et al., 2013). Interestingly, enrichment of bacterial secretion system-related pathways may reflect the functional activities of nematode-secreted effector proteins; Root-knot nematodes secrete a diverse repertoire of effectors into host cells, where they manipulate host signaling pathways and suppress

immune responses to establish successful parasitism; These effectors share functional similarities with microbial secretion systems and play critical roles in host manipulation (Haegeman et al., 2012). In addition, the enrichment of RNA degradation and spliceosome-associated pathways in Clusters 3 and 4 highlights the importance of post-transcriptional regulation during nematode infection. Previous studies have demonstrated that non-coding RNAs, including miRNAs and siRNAs, play crucial roles in regulating defense-related genes during plant–nematode interactions; Changes in RNA stability, alternative splicing, and gene silencing mechanisms may therefore represent essential regulatory components of host defense responses (Kyndt et al., 2012). Furthermore, the enrichment of endocytosis-related pathways suggests that vesicle trafficking and membrane remodeling are actively involved in rice responses to nematode infection. Endocytosis regulates receptor internalization, signal transduction, and cellular morphological changes, processes that are essential for the formation and maintenance of nematode feeding structures.

Conserved Domain Analysis of Hub Genes

To further investigate the molecular functions of the identified hub genes, conserved domain analysis was performed using the Conserved Domain Database available at the National Center for Biotechnology Information; The identified protein domains revealed that the hub genes encode proteins involved in an integrated molecular network associated with chaperone-mediated protein folding, stress signaling, nitrogen metabolism, and immune regulation in the susceptible rice cultivar IR64 (Table 2). Conserved domain analysis was performed for all identified hub genes, and the complete results are presented in Table 2. To provide a clearer structural visualization of domain organization, three representative hub genes, including Os08g0500700, Os12g0514500, and Os04g0107900, were selected based on their consistent identification across three independent topological algorithms (MCC, MNC, and Degree) (Figure 4). The selection of these genes reflects their robust network centrality and potential functional significance within the stress-responsive protein interaction network.

The identified domains included highly conserved members of the molecular chaperone families, including Heat Shock Protein 90, Heat Shock Protein 70, Heat Shock Protein 40, small heat shock proteins (sHSPs), GroEL/Hsp60, and the eukaryotic chaperonin complex CCT/TRiC. In addition, domains related to co-chaperone regulation, such as SGT1, signaling-associated Ankyrin repeat motifs, nitrate reductase catalytic domains, and stress-associated α -crystallin domains were identified. Collectively, these domains suggest that the identified hub genes function within an interconnected molecular network responsible for maintaining cellular homeostasis under biotic stress. At the center of this network, ATP-dependent chaperone systems composed of HSP90 and HSP70, together with specialized co-chaperones such as DnaJ/HSP40 and SGT1, appear to play essential roles in protein folding, stabilization of immune receptors, and regulation of defense signaling pathways. Previous structural and functional studies have demonstrated that HSP90 undergoes ATP-dependent conformational transitions that enable its interaction with co-chaperones and client proteins, thereby regulating multiple stress-responsive signaling pathways (Jackson, 2012; Cajo et al, 2006; Solana et al, 2022). Similarly, the HSP40/HSP70 chaperone system acts as an ATP-dependent molecular machinery responsible for

maintaining protein quality control and preventing aggregation of unfolded proteins (Liu et al., 2020; Pan et al., 2024). The co-chaperone SGT1 has been reported to interact with HSP90 and plays a critical role in the stabilization and activation of nucleotide-binding leucine-rich repeat (NLR) immune receptors, thereby contributing directly to plant defense responses (Shirasu, 2009; Meldau et al., 2011). In addition, the eukaryotic chaperonin complex CCT/TRiC, identified in several hub proteins, is known to mediate ATP-dependent folding of essential cytoskeletal proteins such as actin and tubulin, thereby contributing to cellular organization and stress adaptation (Kabir et al., 2011; Shen and Willardson, 2025). Similarly, GroEL/Hsp60 family chaperonins facilitate correct protein folding and prevent irreversible protein aggregation under stress conditions (Thomson, 1994; Bross, 2015). Small heat shock proteins containing the α -crystallin domain were also identified; These proteins function as ATP-independent molecular chaperones and protect denatured proteins from irreversible aggregation under environmental stress conditions (Paul et al., 2016; Sarwar et al., 2019). Furthermore, proteins containing Ankyrin repeat domains were detected, suggesting their involvement in protein–protein interactions, signal transduction, and immune signaling pathways; Alterations in the expression of ankyrin-containing proteins have previously been associated with disruption of cellular signaling networks during stress responses (Huang et al., 2009; Sharma and Pandey, 2016; Chakrabarty and Parekh, 2022). Interestingly, nitrate reductase-related domains were also identified among the hub genes; Nitrate reductase is recognized not only as a key enzyme in nitrogen metabolism but also as an important contributor to Nitric Oxide production, which plays a central role in plant defense signaling. Reduced activity of this enzyme may impair NO-dependent defense signaling pathways during pathogen attack (Ampudia-Chamizo et al., 2017; Gupta et al., 2025).

Table 2. Conserved domain analysis of the identified hub genes using the Conserved Domain Database from the National Center for Biotechnology Information.

Gene ID	Domain name	Domain accession	Description	Interval	E-value
Os03g0787300	PTZ00272	PTZ00272	Heat shock protein 83 kDa (Hsp83)	38-694	0
Os08g0500700	HSP90	pfam00183	Hsp90 protein	1-172	9.07×10^{-56}
Os12g0514500	HSP90	pfam00183	Hsp90 protein	1-70	5.21×10^{-32}
Os04g0107900	PTZ00037	PTZ00037	DnaJ-C chaperone protein	5-362	4.13×10^{-146}
Os05g0562300	chap_cct_gamma	TIGR02344	T-complex protein1, gamma subunit	6-531	0
Os02g0247200	GroEL	cd03344	GroEL_like type1 chaperonin	35-555	0
Os10g0462900	GroEL	cd03344	GroEL_like type1 chaperonin	35-555	0
Os03g0143400	ACD_sHsps-like	cd06464	Alpha-crystallin domain (ACD) of alpha-crystallin-type small(s) heat shock proteins (Hsps)	124-197	3.19×10^{-14}
Os02g0758000	ANKYR	COG0666	Ankyrin repeat [Signal transduction mechanisms]	73-270	3.70×10^{-37}
	Ank_2	pfam12796	Ankyrin repeat (3 copies)	17-118	8.08×10^{-12}
	PLN03088	PLN03088	SGT1, suppressor of G2 allele of SKP1	308-406	4.37×10^{-5}
Os02g0493050	PLN02252	PLN02252	Nitrate reductase [NADPH]	1-889	0
Os01g0840100	PTZ0009	PTZ0009	Heat shock 70 protein kDa protein	4-684	0

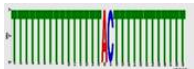
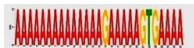
Taken together, these findings indicate that the identified hub genes operate as components of an integrated molecular network coordinating chaperone activity, immune signaling, and cellular metabolism; The simultaneous downregulation of these genes in the susceptible cultivar under nematode stress suggests a systemic disruption of cellular protection mechanisms, impairment of proteostasis, and weakened defense capacity; These results further support the hypothesis that susceptibility to *Meloidogyne graminicola* is not caused by dysfunction of a single gene, but rather by partial collapse of a complex chaperone–signaling–metabolic regulatory network; Motif MA1831.1 corresponding to WRKY75 indicates the involvement of WRKY-mediated signaling pathways; WRKY

transcription factors are key regulators of plant immunity and have been implicated in jasmonic acid and salicylic acid signaling, reactive oxygen species regulation, and senescence-associated defense responses (Guo et al., 2017; Chen et al., 2021). In addition, motif MA2024 corresponding to the Basic Leucine Zipper family suggests integration of multiple developmental and stress-related signaling pathways; Members of the bZIP family are known to regulate plant growth, metabolic homeostasis, and adaptive responses through homo- and heterodimer formation, enabling efficient signal integration under stress conditions (Dröge-Laser et al., 2018; Zhao et al., 2021). Collectively, these results demonstrate that the identified hub genes are regulated by a highly complex and multilayered transcriptional network integrating calcium signaling, hormonal regulation, circadian control, epigenetic mechanisms, nutrient homeostasis, and immune signaling. Notably, the presence of multiple DOF family motifs suggests that this transcription factor family may play a particularly important role in coordinating the balance between growth, metabolism, and defense responses during *M. graminicola* infection.

Promoter Motif Analysis and Identification of Regulatory Transcription Factors

To further investigate the transcriptional regulatory mechanisms underlying the response of the susceptible rice cultivar IR64 to *Meloidogyne graminicola* infection, 2,000 bp upstream promoter regions of the identified hub genes were retrieved using BioMart from the Ensembl Plants database. Conserved cis-regulatory motifs were identified using the MEME Suite, and the predicted biological functions of these motifs were further annotated using GOMO (Table 3).

Table 3. Conserved promoter motifs and their associated transcription factors identified in the upstream regulatory regions of hub genes responsive to *Meloidogyne graminicola* infection using the MEME Suite.

Motif	Logo	Function
MA0970.1		CC: mitochondrion
MA1267.1		MF: transcription factor activity CC: nucleus CC: plasma membrane BP: regulation of transcription, DNA-dependent
MA1268.1		MF: transcription factor activity CC: nucleus CC: plasma membrane BP: regulation of transcription, DNA-dependent MF: kinase activity
MA1274.1		MF: transcription factor activity CC: nucleus CC: plasma membrane BP: regulation of transcription MF: kinase activity
MA1380.1		MF: transcription factor activity BP: response to ethylene stimulus, response to abscisic acid stimulus, response to salt stress, response to water deprivation
MA1827.1		CC: ribosome, chloroplast
MA1831.1		MF: nucleotide bilding, RNA binding CC: mitochondrion, CUL4 RING ubiquitin ligase complex BP: translation



Promoter analysis revealed that the identified hub genes contain a diverse repertoire of transcription factor binding sites associated with defense signaling, environmental adaptation, and developmental regulation. Motif enrichment analysis identified cis-regulatory elements corresponding to several major transcription factor families, including CAMTA, DOF, CDF, ERF, WRKY, bZIP, and CPP, indicating that these genes are regulated by a multilayered transcriptional network involved in plant immunity and stress adaptation. Among the identified motifs, motif MA0970.1 corresponding to CAMTA3 suggests the involvement of calcium-dependent signaling pathways in regulating the expression of the identified hub genes. Members of the CAMTA family are recognized as major mediators of Ca^{2+} signaling in plants and play essential roles in pathogen-triggered immunity, reactive oxygen species (ROS) accumulation, and activation of defense-associated transcriptional reprogramming; Functional studies in *Arabidopsis thaliana* have shown that loss of CAMTA3 function leads to enhanced defense gene expression, increased ROS accumulation, and improved resistance against pathogens (Konishi et al., 2015; Chao et al., 2022). Motif MA1267.1, corresponding to the DOF transcription factor Dof5.8, suggests that members of the DOF family may contribute to the transcriptional regulation of the identified hub genes; Previous studies in *Arabidopsis thaliana* demonstrated that Dof5.8 is regulated by auxin-dependent signaling pathways and plays important roles in vascular differentiation and developmental regulation (Galon et al., 2008). The presence of this motif suggests that the identified rice genes may participate in coordinating defense responses with developmental processes; Similarly, motif MA1268.1 corresponding to CDF5 indicates possible regulation by circadian rhythm-associated transcriptional networks, suggesting that the identified genes may integrate defense responses with environmental adaptation and physiological timing mechanisms (Martin et al., 2020). Motif MA1274.1, corresponding to OBP3, further supports the involvement of DOF family transcription factors in the regulatory network. OBP3 has been associated with iron homeostasis and nutrient signaling; Since iron availability strongly influences reactive oxygen species production and plant immune responses, the presence of this motif suggests functional cross-talk between nutrient metabolism and defense signaling pathways (Xu et al., 2025). Interestingly, motif MA1380.1 corresponding to TCX6 indicates potential epigenetic regulation of hub gene expression. TCX-related transcription factors have been associated with chromatin remodeling, DNA methylation, and transcriptional repression complexes, suggesting that transcriptional regulation of these genes may also involve higher-order chromatin dynamics (Colin et al., 1987). The identification of motif MA1827.1 corresponding to ERF105 suggests activation of ethylene-responsive signaling pathways and environmental stress adaptation; Members of the ERF family are widely recognized for their roles in abiotic and biotic stress responses, including cold stress adaptation and pathogen defense (Bolt et al., 2017; Illgen et al., 2020).

Conclusion

In this study, an integrative bioinformatics framework was employed to elucidate the molecular response of the susceptible rice cultivar IR64 to infection by *Meloidogyne graminicola*. By integrating differential gene expression analysis, protein domain characterization, functional annotation, and promoter cis-regulatory element profiling, this study provided a comprehensive systems-level understanding of host–pathogen interactions in Rice. The results revealed that the identified hub genes exhibited significant transcriptional modulation under nematode stress and encoded proteins enriched in highly conserved functional domains associated with molecular chaperone activity, protein folding, intracellular signaling, and stress adaptation. Structural and functional annotation further suggested that several candidate genes, particularly members of the HSP60, HSP70, and ankyrin-related protein families, may contribute to maintenance of cellular proteostasis and regulation of stress-responsive signaling pathways during nematode infection. Promoter analysis demonstrated that these genes are regulated by diverse cis-regulatory elements associated with major phytohormone signaling pathways, including salicylic acid, jasmonic acid, and ethylene, indicating that transcriptional regulation of defense-associated genes is mediated through a complex hormone-dependent regulatory network. Collectively, these findings suggest that susceptibility of the IR64 cultivar to *Meloidogyne graminicola* is not determined by a single genetic factor, but rather by coordinated perturbations across interconnected regulatory layers involving transcriptional control, protein quality maintenance, and hormonal signaling. The identified hub genes and associated regulatory motifs represent promising molecular candidates for future functional validation and may provide valuable targets for the development of nematode-resistant rice cultivars through molecular breeding and genome engineering strategies.

Declarations

Author Contribution

A.D. conceived the study, performed the bioinformatics analyses, interpreted the data, prepared the figures, and wrote the original manuscript. M.M. supervised the research, contributed to the study design, reviewed and edited the manuscript. All authors read and approved the final manuscript.

Data Availability

The transcriptome datasets analyzed during the current study were obtained from the previously published study by Zhang et al. (2023), which are publicly available in the NCBI Gene Expression Omnibus (GEO) repository. In the present study, these publicly available RNA-seq datasets were reanalyzed using an integrative bioinformatics approach to identify differentially expressed genes and their associated regulatory networks. No new experimental datasets were generated during this study. All processed data supporting the findings are included in this published article, and additional information is available from the corresponding author upon reasonable request.

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Figures

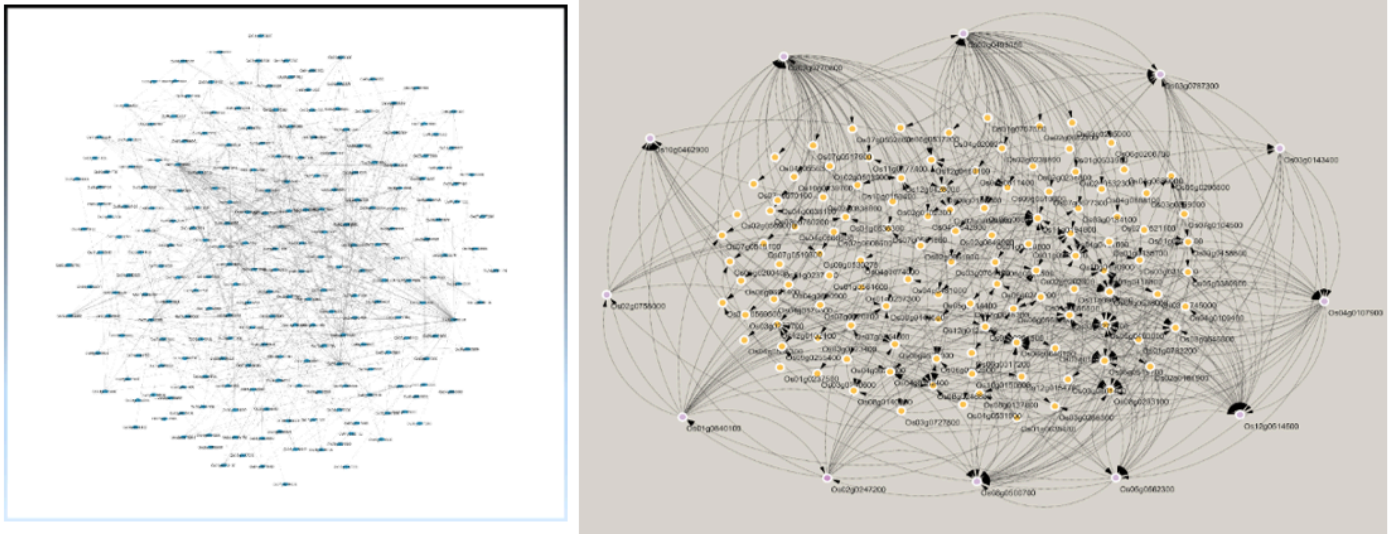


Figure 1

Protein–protein interaction network analysis of differentially expressed genes in the susceptible rice cultivar IR64 rice in response to *Meloidogyne graminicola* infection. (A) Global protein–protein interaction network of differentially expressed genes. (B) Subnetwork of the identified hub genes and their interacting neighboring genes. Gray nodes represent hub genes, whereas orange nodes indicate their interacting neighboring genes.

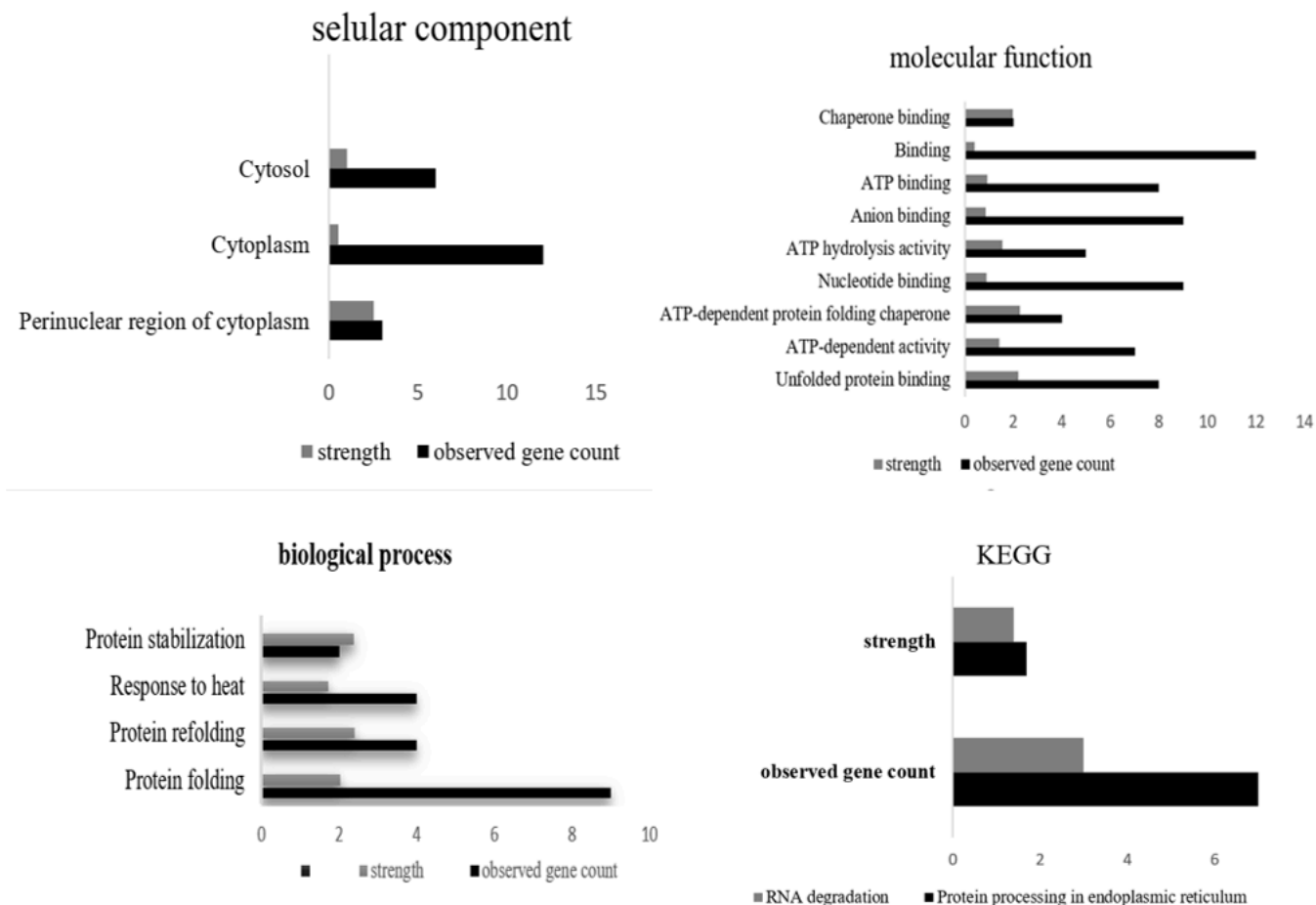


Figure 2

Functional enrichment analysis of the identified hub genes in the susceptible rice cultivar IR64 in response to *Meloidogyne graminicola*. (A) Cellular component (CC), (B) Molecular function (MF), (C) Biological process (BP), and (D) KEGG pathway enrichment analysis performed using the STRING database.

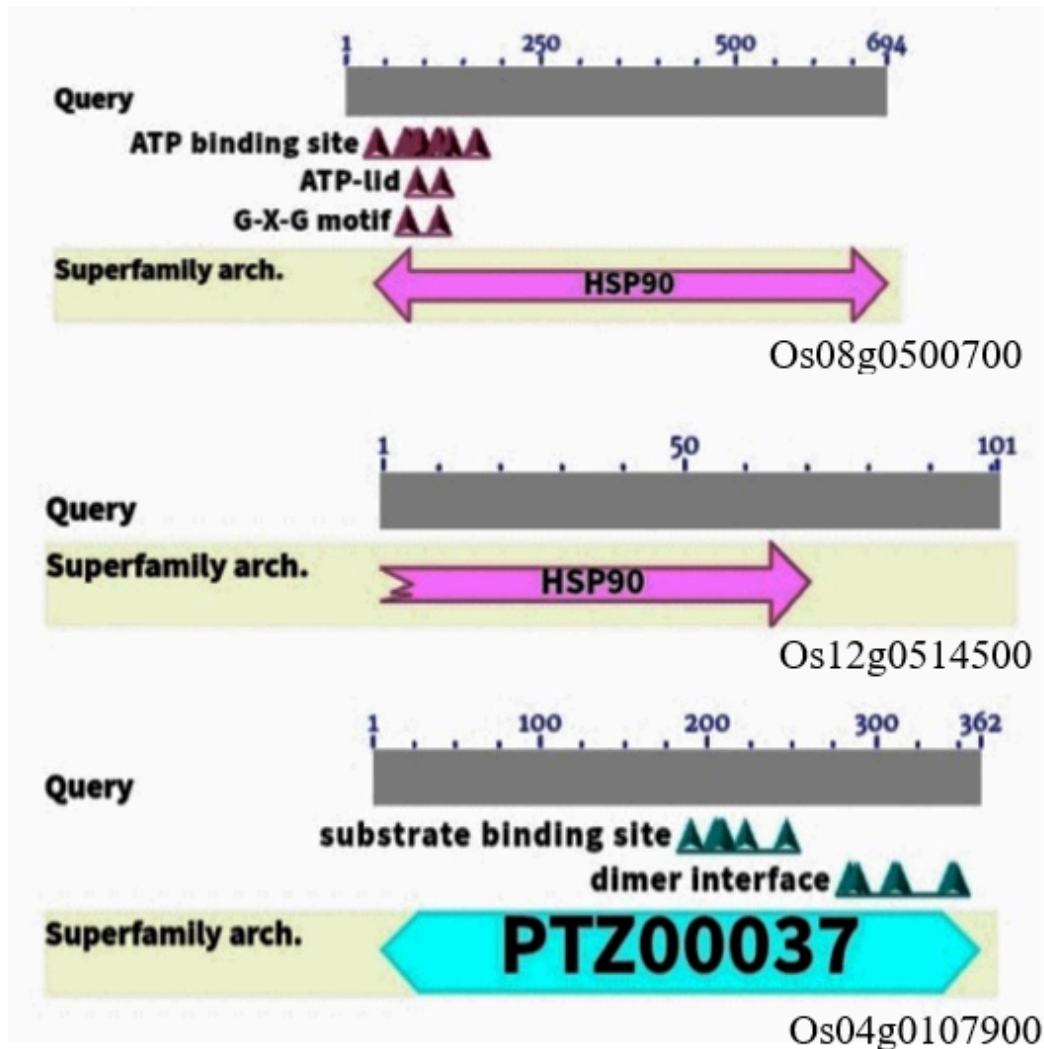


Figure 4

Conserved domain architectures of three representative hub proteins identified in the susceptible rice cultivar IR64 in response to infection by *Meloidogyne graminicola*. Although conserved domain analysis was performed for all identified hub genes (Table 2), these three proteins (Os08g0500700, Os12g0514500, and Os04g0107900) were selected for structural visualization based on their consistent identification across three independent topological algorithms (MCC, MNC, and Degree), indicating their central regulatory roles in the protein–protein interaction network.