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IDENTIFICATION AND COMPUTATIONAL ANALYSIS OF BIOTIC STRESS TOLERANT GENES FROM *Piper nigrum* GENOME

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ABSTRACT:

Black pepper (*Piper nigrum* L.) is a woody perennial spice crop that is essential for both traditional medicine and cuisine. The major disease 'Quick wilt' that severely threatens *Piper nigrum* is found to be caused by oomycete *Phytophthora capsici*, which affects the leaf, stem, spike, collar, and root. Plants use a wide range of defensive mechanisms to protect themselves against biotic and abiotic stress that includes the accumulation of stress related genes and its respective protein counterparts. Through computational analysis, six biotic stress tolerant genes that belong to TPR motif were identified from *Piper nigrum*. Using MEME, the conserved motifs of stress related genes were found. Predictions based on secondary and three-dimensional structural studies of stress related genes were also made, suggesting a potential functional connection to *P. nigrum*. Their role in the defense responses was predicted by the GO and KEGG functional annotations. The structural details phylogenetic relationships and conserved sequence motifs of stress related genes were further identified through subsequent computational analysis.

KEYWORDS: *Phytophthora capsica*, *Piper nigrum*, biotic stress, Quick wilt, TPR motif

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INTRODUCTION

Black pepper (*Piper nigrum* L.), belonging to *Piperaceae* family considered as the 'King of Spices' (Indu et al, 2022; Damanhour and Ahmad, 2014). *P. nigrum* is traded as black and white peppercorns, respectively, for its dried fruits and seeds. Due to their different aromas and potent qualities, both are utilized as cuisine ingredients. Additionally, pepper plants especially the roots are employed in many traditional medicines around the world due to their well-documented therapeutic and restorative properties (Fan et al, 2020). Originating in South India, *P. nigrum* is now grown in many tropical and subtropical parts of Asia, Africa, and Latin America due to its medicinal, economical, nutritional, and therapeutic values (Fan et al, 2022).

Though pepper is a highly valuable vegetable crop that is grown all over the world, it is vulnerable to pests and diseases, particularly to the oomycete pathogen *Phytophthora capsici*, a soil borne pathogen which causes quick wilt disease (Jin et al, 2016). Quick wilt, also known as foot rot disease is a deadly black pepper disease that can damage plant growth at any stage and drastically reduce the number of plants (28) (Nysanth et al, 2022). High mortality of cuttings and significant financial losses are brought on by *Phytophthora* foot rot. Every portion of the vine is infected and the plant is vulnerable to the fungus, thus the severity of the disease relies on the portion of the plant that is affected and the extent of damage (Anandaraj et al, 1994).

When this disease infects plants, they die in two to three weeks in the rain and infect other plants in one to two months. It is possible to explain *Piper nigrum*'s rapid demise by the development of *Phytophthora* spores into the roots and stems, particularly the collar, which causes foot rot. Phloem and xylem are destroyed when a collar rots, which stops water and nutrients from moving from the roots to the plant's aerial branches. As a result, the plant withered halfway and showed signs of abrupt leaf wilting and falling (Johnson and Kochuthressia, 2020).

A wide variety of perennial plants are also infected by the pathogen, which is a worst-case scenario for farmers due to its wide host range, long-lived dormant sexual spores, high genetic diversity, and explosive asexual disease cycle. The spores spread easily by water and accumulates on the soil surface (Asha and Soniya, 2016). The pathogen reproduces by a variety of sexual and asexual mechanisms. The three asexual spores that mycelia produce are sporangia, zoospores, and chlamydospores. One of the main sources of infection is zoospores (Pérez-Jiménez, 2008). The overwintering inoculum of the pathogen is produced by each oospore that develops into a male and female gametangium, also referred to as antheridium and oogonium. Furthermore, in conditions of adequate free moisture on the surface of plants or saturated soil, sporangia discharge both motile and biflagellate zoospores (Hausbeck et al, 2004). In free water, a single sporangium can yield 20–40 motile zoospores (Mchau and Coffey, 1995). Furthermore, zoospores and sporangia are secondary inocula that have the ability to proliferate frequently throughout the growth season, leading to a fast progression of infection (Nam, 2012).

The pathogen's life cycle is one of the factors that keeps it alive and helps it spread. For both chlamydospores and oospores, the ideal temperature range is 20°C to 28°C and moisture content is above 80%. Oospores survive for six years in the soil and two to three rainy seasons in

decomposing plant matter (Nambiar and Sarma, 2017). And most crucially, inoculum does not require host plants to live in the soil for up to 19 months (Kueh et al, 1993). This disease can result in the complete destruction of individual holdings and is highly detrimental to all pepper-growing nations. Therefore, the development of a *Phytophthora* foot-rot-resistant cultivar is one of the most preferred methods for controlling this disease.

Research on control strategies for *Phytophthora* foot-rot has lasted for many years. These strategies include chemical treatment, crop rotation, grafting resistant *Piper* species as the rootstock, breeding and selecting resistant varieties, and applying autogenous bacterins to the rhizosphere (Hao et al, 2016). Even so, no successful varieties resistant to the *Phytophthora* footrot disease have been produced right now. The primary method of managing diseases has been the use of fungicides, which are hazardous, harm the environment, raise production costs, and have a poor history of success. Understanding resistance mechanisms and how to use them in resistance breeding programs can be aided by the natural genetic variety of resistance traits. Succeeding in breeding resistant variants of black pepper requires an understanding of the variations in *P. capsici*-plant interactions between susceptible and resistant species of *Piper*, as well as the molecular mechanisms behind the resistant. Plants have evolved complex defense mechanisms in response to pathogenic attack during their co-evolution with pathogen infestation (Mou et al, 2017). Defense mechanisms, include inducible innate immunity and physiological, biochemical, molecular, and cellular processes (Hou et al, 2018). The primary objective of this work is to elucidate the defense mechanisms of some stress related genes related to TPR motif. TPR (tetratricopeptide repeat) genes are a class of genes found in plants that are extensively distributed in nature and have conserved TPR motifs (Zhou et al, 2021). TPR features an amphipathic channel and is frequently seen as a right-handed supercoil shape. This channel's presence allows TPR to adjust and enhance the target protein interaction area (Goebel et al, 1991). TPR is made up of 34 amino acid conserved motifs, each of which is made up of two antiparallel α -helix subunits (helixA, helixB). Since TPR repeats were initially discovered in yeast, approximately 20,000 functionally distinct TPR genes have been found (Hirano et al, 1990). Biochemical activities like cell cycle regulation, gene expression, protein transport, and protein breakdown are all greatly influenced by this gene family (Groshong et al, 2014). Many plant species also have TPR repeats, which can mediate interactions between genes and chaperones. According to studies, numerous TPR has avital role in stress response. In this study, the stress-related genes from *P. nigrum* were identified, and a comprehensive genome-wide analysis was carried out to enhance understanding of the defence mechanisms underlying the stress role in this species. This work provides a ground work for further investigation in the role of pepper genes in plant defense mechanisms against *P. capsici* infection.

2. MATERIALS AND METHODS

2.1 Identification and analysis of stress related genes from *Piper nigrum* genome.

The pepper (*Capsicum annum* L.), which is grown all over the world, is a valuable vegetable crop that is susceptible to pests and diseases, particularly *Phytophthora* blight, which is brought on by the pathogen *Phytophthora capsici* (*P. capsici*). Recent studies have highlighted the

significance of SBP-box (Squamosa-promoter binding protein) genes, specifically CaSBP08, in the plant's defense mechanisms against *P. capsici* infection. Silencing CaSBP08 has been shown to enhance the plant's resistance to this pathogen, underscoring its critical role in stress response pathways (Zhang et al, 2020). This finding justifies the use of *Capsicum annuum* as a model for studying stress-related genes in *Piper nigrum* (black pepper).

To identify potential stress-related genes in *Piper nigrum*, sequences of CaSBP08 from *Capsicum annuum* were retrieved from the NCBI database (<http://ncbi.nlm.nih.gov>). These sequences were then used in a tBLASTn search against the *Piper nigrum* genome assembly. This comparative analysis yielded over 150 unique matches, indicating the presence of multiple potential stress-related genes in *Piper nigrum*. These matched sequences were subjected to further analysis to predict their functions and classify them into appropriate protein families.

The functional prediction of these sequences was carried out using ScanProsite, (<https://prosite.expasy.org/scanprosite/>) a web-based tool that identifies PROSITE signature matches in protein sequences. This tool helps predict the functions of proteins and assign family identities, which is crucial for understanding the roles of these proteins in stress responses. The identification of PROSITE signatures provides insights into the potential biological functions of the stress-related genes identified in *Piper nigrum*. It is necessary to conduct searches against signature databases, sometimes referred to as secondary databases (Attwood and Parry-Smith, 2003), in order to forecast protein function, determine family identity, or find distant homologues. A web interface can be obtained by ScanProsite to find protein matches against signatures from the PROSITE database (Teufel et al, 2021).

To translate and align the coding sequences, a multiple sequence alignment was performed with the BioEdit(<https://bioedit.software.informer.com/7.2/>)Sequence Alignment Editor (Hall, 1999).Using Mega 11(<https://www.megasoftware.net/>), the conservation of nucleotide and protein sequences for each stress related gene was examined.The programme known as Molecular Evolutionary Genetics Analysis, or MEGA, is intended for the comparative study of stress gene sequences from various species, with a focus on discovering patterns of DNA and protein evolution as well as evolutionary relationships. This analysis helps determine the conservation of stress-related genes and their evolutionary significance.

To understand the structural aspects of the identified genes, domain structure prediction was performed using the NCBI-Conserved Domain Database (CDD)(<https://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml>) (Marchler-Bauer et al, 2015). This database helps identify conserved domains within the protein sequences, providing insights into their functional domains and potential roles in stress response. Additionally, the molecular weight and isoelectric point (pI) of the stress-related genes were determined using the ExPASy ProtParam tool. (<https://web.expasy.org/protparam/>), which analyzes the basic physicochemical properties of proteins.

A SignalP 6.0 server was also used to predict the cleavage sites and potential signal peptideregions.(<https://services.healthtech.dtu.dk/services/SignalP-6.0/>)Short amino acid sequences known as signal peptides (SPs) regulate the translocation and secretion of proteins in all living organisms (Teufel et al, 2021). Identifying these peptides helps understand the regulatory mechanisms of protein translocation and secretion in stress responses.

Then, using MEME (Multiple Em for Motif Elicitation), the conserved motifs of stress related genes were predicted. (<http://meme-suite.org/tools/meme>) (Bailey et al, 2015).The identification and characterization of these motifs is crucial for understanding molecular interactions within cells, particularly those involving the control of gene expression, as they encode a wide range of biological functions (Bailey et al, 2015).This comprehensive analysis of stress-related genes in *Piper nigrum*, informed by insights from *Capsicum annuum*, highlights the importance of these genes in plant defense mechanisms. The use of various bioinformatics tools and databases provides a detailed understanding of the roles and characteristics of these genes. Such studies are essential for developing strategies to enhance crop resistance to diseases and environmental stresses, ultimately improving agricultural productivity and sustainability.

2.2 GO and KEGG analysis

Gene Ontology (GO) database used as gene annotation system describes biological domains in three aspects that are cellular components, molecular functions and biological processes. The stress related genes in the *Piper nigrum* were analyzed for their role in GO using PANNZER2 web server. (<http://ekhidna2.biocenter.helsinki.fi/sanspanz/>) (Törönen et al, 2018). PANNZER2 offers functional annotations derived from protein sequences, providing valuable information about the roles these genes play in molecular functions, cellular structure, and various biological processes This analysis helps elucidate the specific functions and pathways that these stress-related genes are involved in, thereby enhancing our understanding of their roles in the plant's response to stress.

For the individual characterization of gene functions a KEGG (Kyoto Encyclopedia of Genes and Genomes) tool and BlastKOALA (KEGG Orthology and Links Annotation) webserver were used. (<https://www.kegg.jp/blastkoala/>) (Kanehisa et al, 2016).

By associating genes with certain biological networks and pathways, KEGG analysis offers a pathway-based knowledge of gene activities. BlastKOALA assigns KEGG Orthology (KO) terms to the genes, facilitating the identification of their roles in various metabolic and signaling pathways. This combined approach allows us to identify the precise pathways by which these genes linked to stress function, providing a comprehensive understanding of their functional effects in *Piper nigrum*.

2.3 Secondary and tertiary prediction

SOPMA (Self-Optimized Prediction Method with Alignment) Server is used to predict secondary structure of stress related genes in the *Piper nigrum* (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_sopma.html). SOPMA is a potent tool that predicts the secondary structure of proteins by analyzing their amino acid sequences and generating a profile that includes alpha-helices, beta-strands, and random coils. This prediction is crucial for

understanding the structural aspects of the proteins encoded by these genes, which in turn helps infer their functional properties and interactions.

2.4 Sequence Alignments and Phylogenetic Analysis

Multiple sequence alignment was performed for the amino acid sequences using Clustal W software(<https://www.genome.jp/tools-bin/clustalw>) to examine the evolutionary relationships among the stress-related genes. Clustal W aligns numerous protein sequences in order to determine conserved regions and sequence similarities which are crucial for comprehending the functional and evolutionary linkages between the genes. Following the alignment, the maximum likelihood approach with 1000 bootstrap repeats was used to create phylogenetic trees using the MEGA 11 programme. Using bootstrap values to show the stability of each branch in the tree, this method creates phylogenetic trees that show the evolutionary ancestry and relationships of the genes. The stress-related genes in *Piper nigrum* have undergone divergence and evolution over time, as revealed by the phylogenetic analysis, which sheds light on their evolutionary history. To improve our understanding of the roles and relevance of the stress-related genes in *Piper nigrum*, this comprehensive breakdown offers a clear and organised description of the approaches employed for their functional annotation, structural prediction, and evolutionary analysis.

3. RESULT

3.1 Identification of genes and analysis of stress related genes in *Piper nigrum*

Two potential stress-related genes containing TPR motifs were identified in *Piper nigrum* using the ScanProsite tool. Subsequent BLAST analysis of these two genes revealed four additional stress-related genes, resulting in a total of six stress-related genes. Table 1 lists the result of Scanprosite. Table 2 lists the fundamental characteristics of the stress related genes, such as their molecular weight, isoelectric point, and length of protein sequence. These genes have lengths varying from 127 to 357 amino acid residues and molecular weights from 14.38 and 38.49 kDa. Most of the stress related genes were classified into acidic categories using the theoretical isoelectric point (pI) data. The extremes of acidity may influence the unique roles that each stress related gene plays. Moreover, signal peptide regions and cleavage sites of all the stress related genes were predicted (Table 3).

Table 1. Scan prosite result



Gene name	Scan Prosite result	Link address
PnTPS1		https://prosite.expasy.org/cgi-bin/prosite/nicedoc.pl?PS50005
PnCPS		https://prosite.expasy.org/cgi-bin/prosite/nicedoc.pl?PS50005

Table 2. Sequence characteristics and physio-chemical properties of the stress related genes in *Piper nigrum*.

Gene	Accession numbers	Protein length (AA)	Molecular weight	Theoretical pI
PnTPS1	>A0A1V0E492.1	559	64935.7	5.5
PnTPS2	>A0A1V0E4A6.1	562	64564.05	5.44
PnTPS3	>A0A2R4QKX7.1	561	64519.94	5.46
PnCPS	>KU953957.1	559	64935.7	5.5
PnCO	>KU953958.1	562	64564.05	5.44
PnGDS	>MF104556.1	561	64519.94	5.46

Table 3 Signal peptide region predicted in stress genes

Genes	Protein type	Other	Signal Peptide (Sec/SPI)	Lipoprotein signal peptide (Sec/SPII)	TAT signal peptide (Tat/SPID)	TAT Lipoprotein signal peptide (Tat/SPII)	Pilin-like signal peptide (Sec/SPIII)
PnTPS1	Likelihood	1	0	0	0	0	0
PnTPS2	Likelihood	1.0001	0	0	0	0	0
PnTPS3	Likelihood	1.0001	0	0	0	0	0
PnCPS	Likelihood	1	0	0	0	0	0
PnCO	Likelihood	1.0001	0	0	0	0	0
PnGDS	Likelihood	1.0001	0	0	0	0	

3.2 Sequence conservation of stress related genes

The MEME server was used to identify ten conserved motifs in total. There were ten conserved motifs found in all. With consensus sequences, every color denotes a distinct motif. Motif 1 to 10 were conserved in all protein sequences (Figure 1). The consensus sequences of the motifs were

determined, with each motif being conserved across all protein sequences examined. Considering their high level of conservation, these motifs play a crucial role in maintaining the proteins functional integrity and linked to stress responses in *Piper nigrum*.

Name	<i>p</i> -value	Motif locations
PnTPS1	2.88e-87	
PnTPS2	3.26e-57	
PnTPS3	6.36e-55	
PnCPS	3.26e-57	
PnCO	2.88e-87	
PnGDS	6.36e-55	

Figure 1 Conserved motifs identified from *Piper nigrum* stress related genes

3.3. GO and KEGG Pathway Analysis stress related genes

Fifteen biological processes, seven molecular function and two cellular components were identified by Gene ontology analysis. This analysis revealed several biological processes, including the metabolism of sesquiterpenes, the diterpenoid biosynthesis process, the sesquiterpene biosynthesis process, and the sesquiterpenoid biosynthesis process. Most plant secondary metabolites are called terpenoids, and both their manufacture and control are very complex. Terpenoids are important components of defense responses and interactions among animals, microbes, and plants. Terpene chemicals are extremely important for the ecology and the plants themselves (Li et al, 2023). It possess terpene synthase activity, magnesium ion binding, beta-element synthase activity, alpha-copaene synthase activity and response to stimulus in terms of molecular function. And, in terms of cellular components it had plastid and membrane activity. (Table 3). All of these activities were related to role in defense response. According to a KEGG pathways analysis, it plays a part in secondary metabolite synthesis such as sesquiterpenoid and triterpenoid biosynthesis producing high level of (-)-germacrene D. The biosynthesis of sesquiterpenoid and triterpenoid KEGG pathways (map00909) explains how farnesyl pyrophosphate (FPP) is converted by enzymes into a variety of terpenoid chemicals that are important for plant responses to biotic stress. Sesquiterpenoids,

which are produced from FPP by cyclization and modification reactions, occur in an array of forms, including cyclic ones like bisabolene, germacrene, and humulene types, and acyclic ones like (E,E)-farnesol and (E,Z)-farnesol. These transformations are catalysed by enzymes such as β -bisabolene synthase (EC 4.2.3.55) and caryophyllene synthase (EC 4.2.3.57), which result in chemicals that are implicated in plant defence mechanisms against pathogens and herbivores. Triterpenoids are derived from squalene (produced by squalene synthase, EC 2.5.1.21), which is then transformed into 2,3-oxidosqualene by squalene epoxidase (EC 1.14.14.17) and oxidosqualene cyclase enzymes to produce different skeletons such lanosterol, dammarane, oleanane, and lupane types.

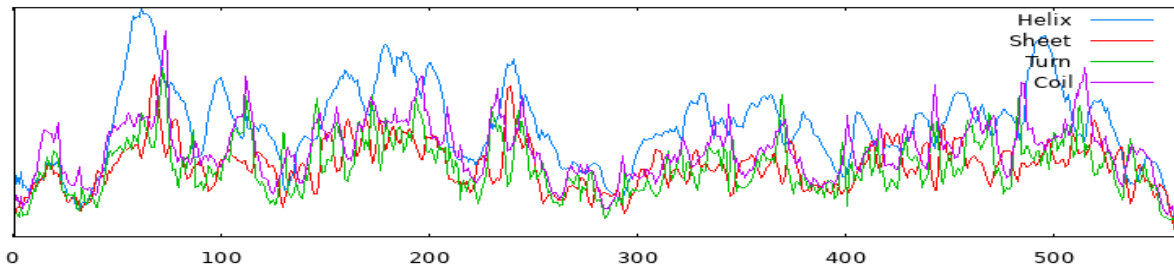
Table 3 Gene ontology term distribution of stress related genes in *Piper nigrum*

GO ID	GO Domain	Function Description
GO:0016102	Biological process	diterpenoid biosynthetic process
GO:0051762	Biological process	sesquiterpene biosynthetic process
GO:0010597	Biological process	green leaf volatile biosynthetic process
GO:1901935	Biological process	beta-caryophyllene metabolic process
GO:1901926	Biological process	cadinene metabolic process
GO:0016106	Biological process	sesquiterpenoid biosynthetic process
GO:0045339	Biological process	farnesyl diphosphate catabolic process
GO:0016099	Biological process	monoterpenoid biosynthetic process
GO:1901362	Biological process	organic cyclic compound biosynthetic process
GO:0120251	Biological process	hydrocarbon biosynthetic process
GO:0051761	Biological process	sesquiterpene metabolic process
GO:0009835	Biological process	fruit ripening
GO:0009723	Biological process	response to ethylene
GO:1901931	Biological process	alpha-copaene biosynthetic process
GO:0050896	Biological process	response to stimulus
GO:0010333	Molecular function	terpene synthase activity
GO:0000287	Molecular function	magnesium ion binding
GO:0102889	Molecular function	beta-elemene synthase activity
GO:0102877	Molecular function	alpha-copaene synthase activity
GO:0009975	Molecular function	cyclase activity
GO:0102905	Molecular function	valencene synthase activity
GO:0016740	Molecular function	transferase activity
GO:0009536	Cellular Component	plastid
GO:0016020	Cellular Component	membrane

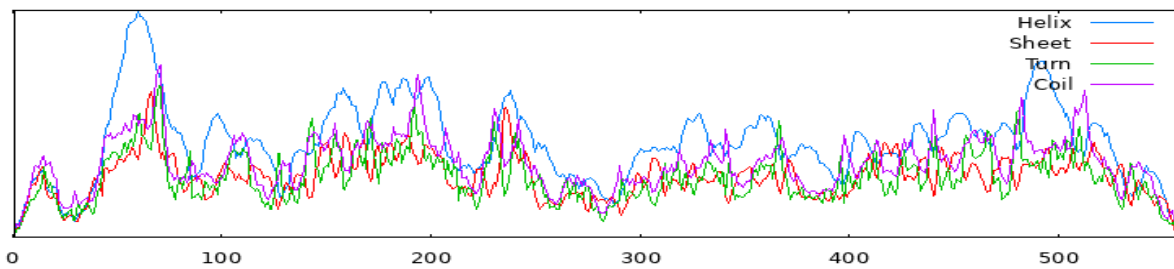
3.4. Secondary and 3D Structure of the stress related Protein

The stress related genes had varying amounts of α -helices (67.38–69.77%), extended strands (3.21–4.28%), β -turns (2.85–3.76%), and random coils (22.90–25.49%). Different stress genes had different relative proportions of the structural characteristics. In comparison to the other equivalents, MF109556.1 displayed a greater number of random coils and less α -helix structures. 3-D structures of these stress genes are depicted in figure 2

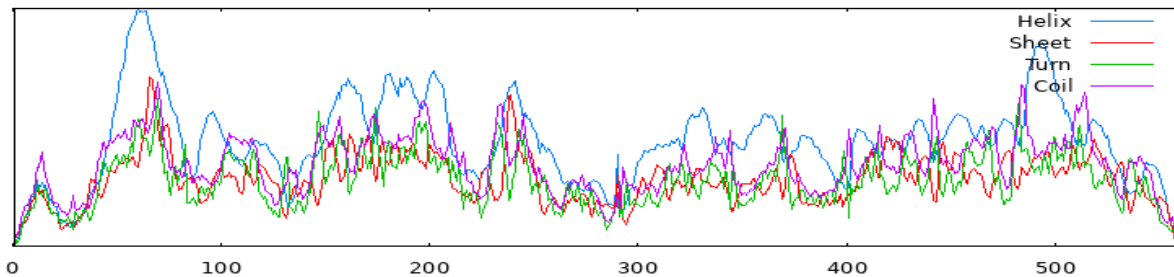
PnTPS1



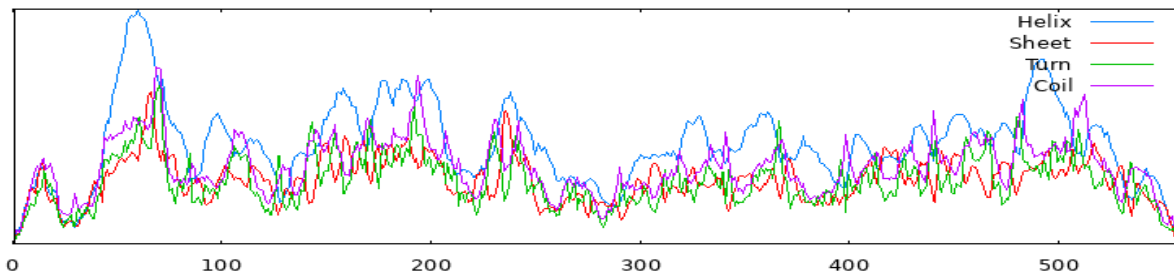
PnTPS2



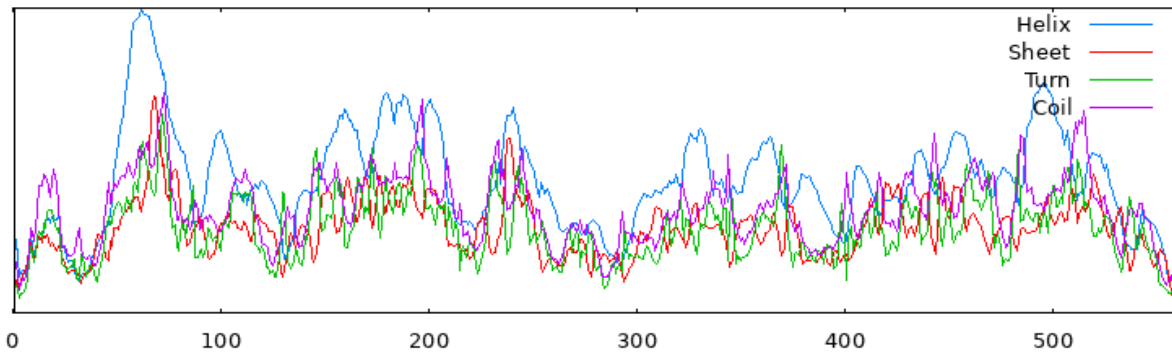
PnTPS3



PnCPS



PnCO



PnGDS

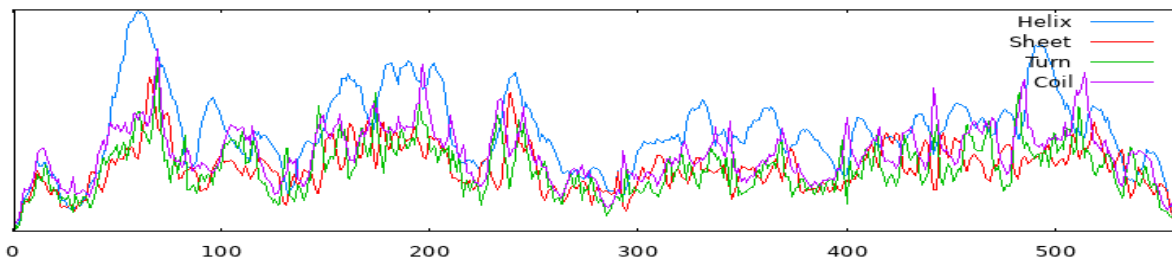


Figure 2. 3-D structures of stress related genes in *Piper nigrum*

3.4 Phylogenetic analysis

To understand the evolutionary relationships between stress genes in *Piper nigrum*, a phylogenetic tree was constructed using the maximum likelihood algorithm with six stress genes (Figure 3a). Figure 3b indicates phylogenetic relationship between PnTPS genes with other plant species. PnTPS genes appear to share an evolutionary pathway with defense-related genes such as LOC126699464 and LOC110645764. Strong evolutionary links are indicated by the high bootstrap values close to PnTPS gene clusters, which supports the genes' function in plant stress responses. The PnTPS genes exhibit conserved characteristics when compared to other genes in the tree, indicating potential roles for these genes in providing resistance against biotic stresses.

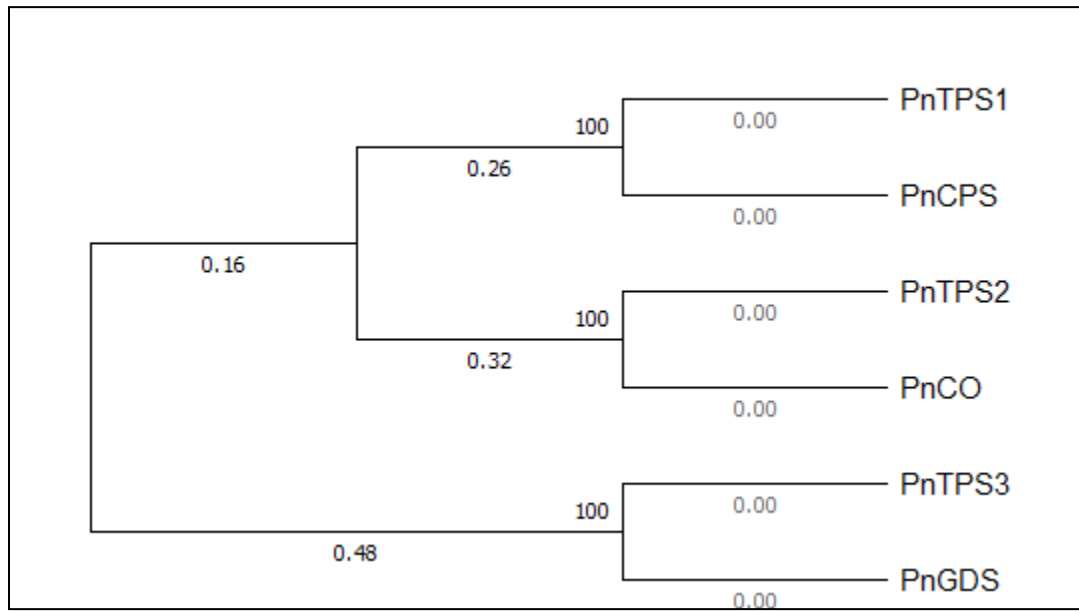


Figure 3a. *Phylogenetic analysis of biotic stress genes in Piper nigrum*

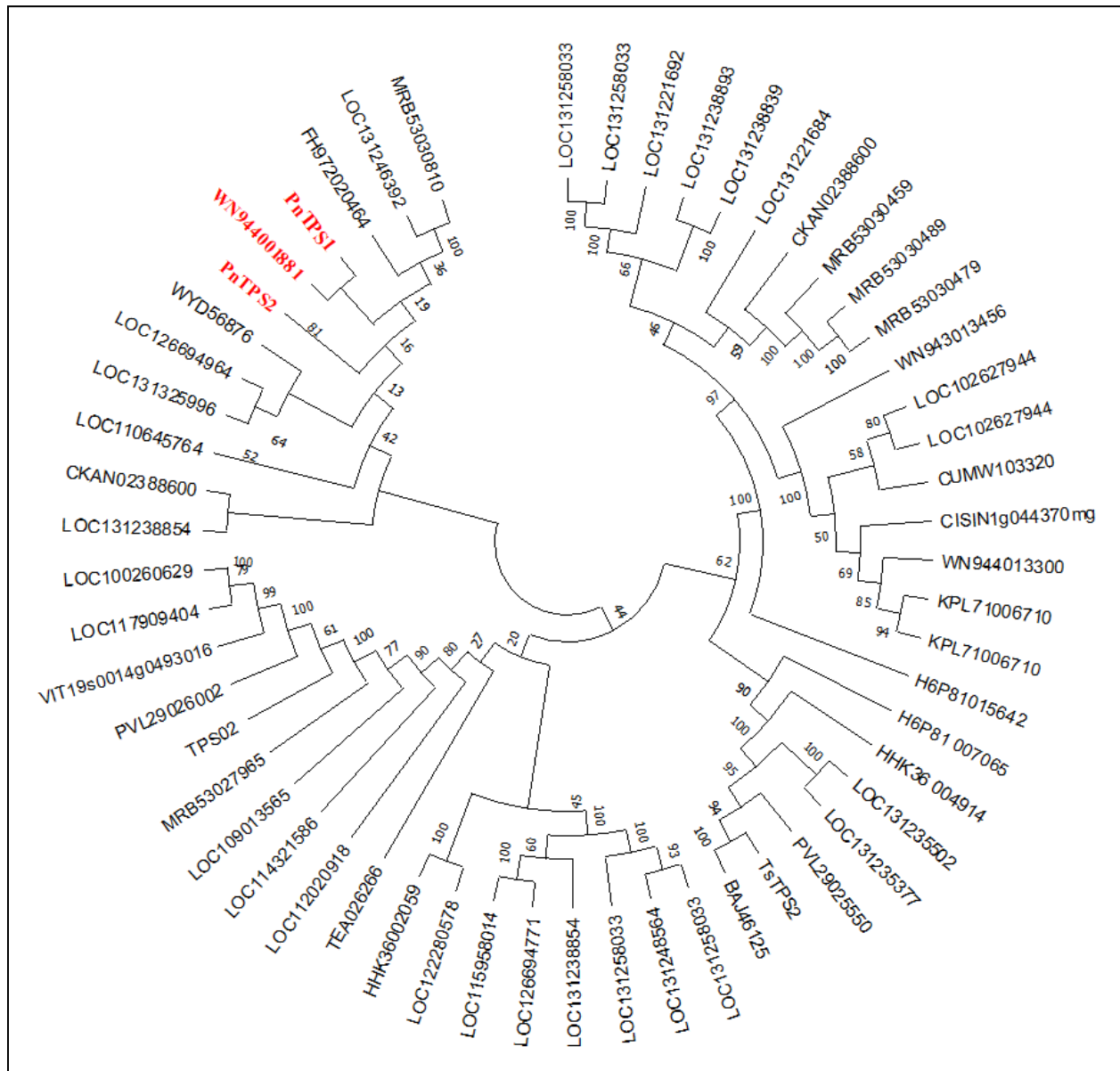


Figure 3b. Phylogenetic analysis of pepper with other plants.

4. DISCUSSION

Soil-borne fungus and nematodes are the primary causes of disease-related losses in black pepper production. Each and every segment of the vine is infected with *P. capsici*. The portion of the plant afflicted and the degree of damage determine the disease's severity. The term "quick wilt" refers to the destruction of a vine in a couple of days if the collar becomes affected. Still, if the infection is limited to feeder roots, it causes a gradual decline (Anandaraj, 2000). The initial sign of the disease is a dark black, water-soaked patch on leaves with a distinctive fimbriate border. Eventually, the entire leaf lamina becomes covered. Heavily defoliation occurs as a result of a dark brown lesion that forms on the stem (Kifelew and Adugna, 2018).

Infection with *P. capsici* in black pepper is polycyclic and polyetic, and it depends on the weather. From season to season, the inoculum is transferred. When the weather is good, the airborne spread happens quickly (Anandaraj and Sarma, 1995). Numerous biotic stress conditions can affect plants. When a plant experiences stress, a complex network of morphological, physiological, and biochemical pathways get activated, initiating a series of molecular and cellular processes (Ben Rejeb et al, 2014). Complex sensory mechanisms have been established by plants to recognize biotic invasion and counteract its detrimental effects on growth, productivity, and survival (Rizhsky et al, 2004). From this study it was identified that these stress genes are related to TPR motif. TPRs have been linked to hormone signaling and plant stress. Recent studies in tomato found that TPR motif responds to various biotic stress (Zhou et al, 2021). These are defense-related signalling molecules that phytopathogens produce and which are essential for preventing the invading pathogen from entering the host. Based on their characteristics like as amino acid sequence, molecular weight, and function, biotic stress genes have been grouped into many different families (AGRIOS, 2005). Different studies in tomato and tobacco proved that it contains 11 families of biotic stress genes (Van Loon et al, 1987; Van Loon et al, 1994). Biotic Stress genes can be acidic or basic based on its nature. Acidic stress genes are generated extracellularly and are only activated by particular stress signals, basic stress genes are found intracellularly in the vacuole areas, where they are also constitutively expressed to some extent (Memelink et al, 1990). In the present work it was identified that majority of stress related genes are acidic in nature and it has some role in biotic stress response. Similar to this 12 biotic stress response genes were identified in rice (Mitsuhara et al, 2008). In contrast to this a higher percentage of stress genes are identified in *S. lycopersicum* under conditions of biotic stress (Akbulak et al, 2020). Further analysis have done with GO and KEGG analysis and identified these biotic stress genes mapped to the sesquiterpenoid and triterpenoid biosynthesis. Sesquiterpenoids and terpenoids are secondary metabolites have role in plant defense such as biotic stress. Low-molecular-weight chemicals called phytoalexins are produced by plants as a part of their defence mechanisms. Sesquiterpenes and diterpenes function as phytoalexins in a few number of plant species. For example, 14 diterpene phytoalexins from *Oryza sativa* have been investigated (Singh and Sharma, 2015). It was identified that these stress related genes have antifungal activities, antimicrobial and insecticidal properties.

With a special emphasis on *Piper nigrum*, a thorough phylogenetic study was carried out to clarify the evolutionary links and conserved defence responses among different plant species. The study discovered two genes in *Piper nigrum*, LOC126699464 and LOC110645764 that showed remarkably similar defence mechanisms. The oak plant *Quercus robur* produced the gene LOC126699464, which was particularly interesting because it showed a similar defence response against fungus infections (Escandón et al, 2021). Interestingly, these genes were involved in the defence cascade because they triggered the production of secondary metabolites such as terpenoids during pathogen invasion. These results demonstrate the evolutionary history and adaptation mechanisms that plants share, and also provide information about possible ways to improve fungal resistance in *Piper nigrum* and related species.

So far only few research has been done on stress protein function in *P. nigrum* or any similar *Piperaceae* species. The current investigation makes a substantial contribution to our knowledge of the molecular role played by stress genes in *P. nigrum*. It is found that some stress genes play a critical role in defense mechanisms against fungal pathogens. Thus, more research on identifying putative *P. capsici* effectors in conjunction with functional studies of stress genes will determine the detailed processes underlying the defense signal amplification and anti-oomycete characteristics of these mysterious genes in *P. nigrum*

5. CONCLUSION

In this study we identified six stress genes in *Piper nigrum* and analyzed them through different computational analysis methods. A thorough insilico analysis revealed that some stress genes are induced in *piper nigrum* plants at the time of Pathogen infection. This suggests that the stress gene is involved in the defense mechanism of plants against *P. capsici*. We believe that the results from our experiment supports more research on the impact of the stress gene on pathogen infection.

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