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Research Article

In-silico analysis of stress tolerance and secondary metabolite production in wild *Sesamum mulayanum* compared to cultivated *Sesamum indicum*

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Abstract: Sesame (*Sesamum indicum*) is a globally cultivated oilseed crop known for its nutraceutical and pharmaceutical significance. Its rich content of antioxidant lignans, mono- and polyunsaturated fatty acids, vitamins, minerals, carbohydrates, and proteins contributes to its importance. To enhance understanding of sesame's genetic potential for crop improvement and utilization, transcriptome data from two sesame species, *Sesamum indicum* and *Sesamum mulayanum*, at two developmental stages (10 and 30 days after pollination, DAP) were analyzed using the Galaxy platform to identify differentially expressed genes. The results showed that 170 genes were up-regulated, and 46 genes were down-regulated. Gene ontology analysis revealed that up-regulated genes were involved in diverse molecular functions and biological processes related to defense response to nematode, systemic acquired resistance, abscisic acid response, and detoxification, among others. Similarly, pathway analysis revealed that the up-regulated genes were involved in pathways related to plant defense, secondary metabolite synthesis, fatty acid synthesis, and phenylalanine, tyrosine and tryptophan biosynthesis. A network analysis was also predicted for describing the interaction of secondary metabolites and stress tolerance genes. The results of the present study provide new insights into the genetic and genomic understanding of sesame, thereby helping in future crop improvement.

1. INTRODUCTION

Sesame (*Sesamum indicum*) is cultivated in different parts of the world. Countries like Myanmar, India, and China are the bulk producers of sesame. Sesame seeds are rich in antioxidant lignans mono and polyunsaturated fatty acids which make it nutritionally and pharmaceutically important oilseed crop (Dar *et al*., 2019). The oil content of sesame varies from 40% to 60%. When compared to the world's average production of sesame, productivity is low in India(Venkataravanappa, 2017). *Sesamum indicum* (Figure 1a) is a cultivated species of the genus Sesame. Sesame has nearly 26 species revised and updated by Nimmakayala *et al*., (2011) which serves as a rich source of biodiversity. Sesame seeds have a highly roasted flavor suitable for making cakes, flour, oil, and paste (Yaseen *et al*., 2021). The seed color of sesame ranges from yellow, grey, brown, black, white, red, and tan. Oil content in white seeds is greater than in other dark color seeds (Yaseen *et al*., 2021). In contrast, black color sesame seeds with

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a slightly lower oil content have a higher content of sesamin, sesamolin, total lignans, and oleic acid (Dar *et al*., 2019).

In addition to the above, sesame oil also contains vitamin E, minerals, such as copper, calcium, iron, zinc, phosphorous, carbohydrate, and proteins rich in methionine (Yaseen *et al*., 2021). Different parts of sesame have been used to treat various conditions like asthma, wound healing, ulcers, inflammations, hemorrhoids, and amenorrhea (Mili *et al*., 2021). One of the varieties of sesame Milyang 74 (M74) extract has high lignan content (17.71 mg/g) which has beneficial effects in the treatment of Alzheimer's disease (Kim *et al*., 2023). Various phytochemical compounds have been identified, isolated, and characterized from seeds, plant organs, and seed oil. Phytochemicals include polyphenols, phytosterols, lignans, phenols, naphthoquinones, anthraquinones, cerebroside, triterpenes, sugars, and other organic compounds. These components are present equally or in higher amounts in the wild varieties of sesame (Mili *et al*., 2021). Comparative genomics and transcriptomic data of wild tetraploid sesame, (*S. schinizianum*) with *S. indicum* have provided insights into lignan synthesis pathways (Wang *et al*., 2023). Indian subcontinent is rich in sesame biodiversity hence identification and exploitation of the wild relatives of sesame to improve the ruling varieties are the need of the hour.

Cultivated sesame is susceptible to most of the biotic and abiotic stresses like cold, drought, phyllody, wilt, fungal diseases, etc., (Dutta *et al*., 2020). Important reasons for the low productivity of sesame include a low harvest index, a lack of high-yielding varieties having resistance to biotic and abiotic stresses. Phyllody is the abnormal development of floral parts into a leafy structure. It is generally caused by phytoplasma or viral infections, though it may also be because of environmental factors that result in an imbalance in plant hormones. The wild species *S. mulayanum* (Figure 1b) from the same genus shows resistance to many pathogens (Dutta *et al*., 2020). It can serve as a gene pool for transferring resistant traits since interspecies crosses between these two species are possible without pre-fertilization barriers (Kulkarni *et al*., 2017). *S. mulayanum* is found to be resistant to phytoplasma with a mean incidence level of less than 5% of phyllody when artificially infected. Hybridization and back-crossing studies have revealed that phyllody resistance is governed by a single recessive and dominant gene in cultivated and wild species respectively in sesame (Singh *et al*., 2007). Many diseases are spread by insect vectors, hence insect resistance can provide increased disease resistance. Daphedar *et al*., 2024 reported that phenylalanine is one of the important secondary metabolite from the phenyl propanoidpathway which helps plants to resist various microorganisms including pathogens, and withstand biotic and abiotic stresses.

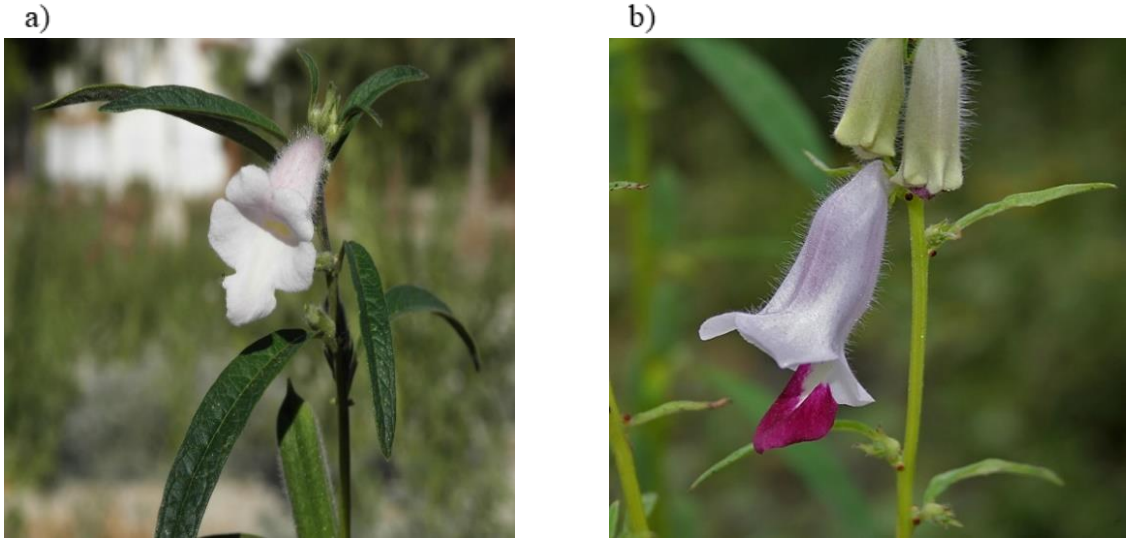

Figure 1. Comparison of plant architecture and flower color of *S. indicum* and *S. Mulayanum.*

Understanding molecular mechanisms of biotic and abiotic tolerance of sesame help not only to find the genes related to these traits but also the associated phenotypic traits that help in selection and follow speed molecular breeding approaches. Towards this end, an exclusive sesame database called Sinbase-2.0 comprising sequences, linkage maps, transcriptome, proteome, QTL, Variants, functional markers, and genes were developed (Wang *et al*., 2021). Earlier, Sesame FG was a database to get all genotypic and phenotypic data on sesame was available (Wei *et al*., 2017). Dossa *et al*., (2017) reported that the sesame crop has moved from an orphan status to a genomics resource-rich crop. This will enable the scientific community to explore the potential of the crop by integrating genomic, transcriptomic, and metabalomic data. Accordingly, to Zhang *et al*., 2021) have integrated miRNA, transcriptome, and degradome data to understand the lipid and fatty acid synthesis during seed development. In this paper, we try to integrate transcriptome data of the cultivated and wild species of sesame to to understand the genetic potential of these species for crop improvement and exploitation purposes.

2. MATERIAL and METHODS

2.1. Data Extraction

Dutta *et al*., 2022 carried out transcriptome analysis of developed seeds from two sesame types named *Sesamum indicum* (from NBPGR germplasm: IC131989) and wild *Sesamum mulayanum* (a generous gift from Mr. K Masuda, Department of Biology, Faculty of Science, University of Toyama, Japan). RNA sequencing analysis was carried out for two genotypes at 10 and 30 DAP and SRA (sequence read archives) data were submitted to the NCBI bio project (Accession: PRJNA644139). For this study, SRA data submitted by Dutta *et al*., 2022 were used. For *Sesamum indicum* 10DAP: Run- SRR12153209 & 30DAP: Run-SRR12153208 and for *Sesamum mulayanum* 10DAP: Run-SRR12153201 & 30DAP: Run- SRR12153200 were used to identify the differentially expressed genes in sesame to explore the genetic potential of these crops for crop improvement.

2.2. Identification of Differentially Expressed Genes (DEG)

To process the RNA sequences and to identify the differentially expressed genes, tools and software present in the Galaxy platform were found suitable. A flowchart adopted for RNA analysis using the Galaxy platform for the DEG identification is given in Figure 2. SRA data downloaded from NCBI was uploaded to the tool and different tools were used to find the differentially expressed genes. The quality of the SRA data was checked by using FastQC (version 0.11.8). Trimmomatic (version 0.38) was used to remove the adapters and low-quality reads in the SRA data. The reads were mapped to their reference genome by using the tool HISTAT2 (version 2.1.0).

The reference genome of *S. indicum* was used because the *S. mulayanum* genome was not yet annotated. A BAM file was generated as an output of HISAT2 which contains the aligned reads. Then the BAM file was loaded to Stringtie (version 2.1.1) which quantified the aligned reads to the reference genome. To generate non-redundancies set of transcripts in all the above RNA samples Stringtie merge was performed. As a result, Stringtie files were merged as a single file. Deseq2 (version 1.22.1) was used to estimate the variance-mean dependence in count data generated by stringtie (using a stringtie merge file). The output DEseq2 file was loaded to the tool- Annotate DESeq2/DEXSeq output tables (version 1.1.0). The resultant table file contains gene identifiers with P-value and log (FC) normalized fold change values. Results of the Galaxy contain only the gene identifiers of the differentially expressed genes. To convert the gene identifiers to gene names KEGG Mapper was used where *S. indicum* was used as an organism code.

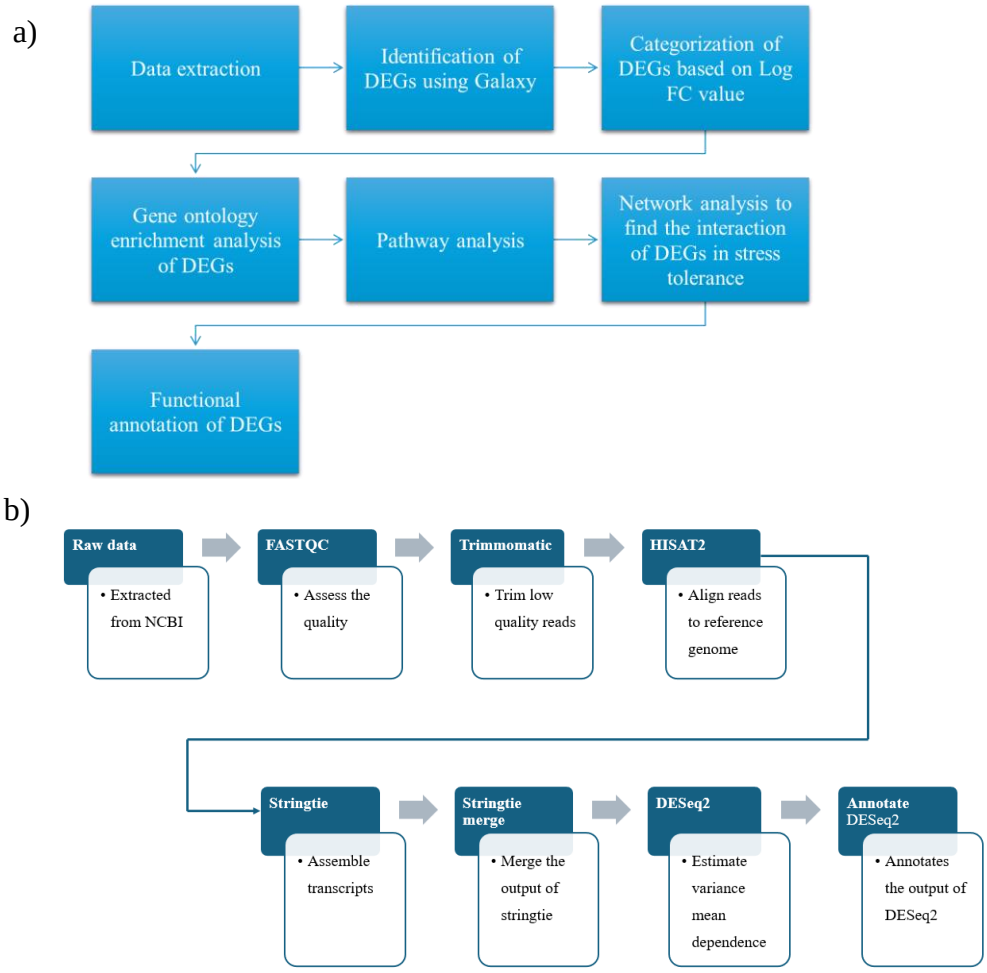

Figure 2. a) Flow chart of steps followed b) Pipeline used to find the DEGs of *S. mulayanum*.

2.3. Categorization of Degs

Based on P-value and Log FC, every gene found using KEGG Mapper was further classified. P-values less than 0.05 indicate statistical significance for a gene and based on the log Fold Change (FC) values, the gene was categorized as upregulated or downregulated. Genes having log (FC) < 0 was categorized as downregulated and log (FC) > 0 as upregulated Then, the upregulated genes were categorized into four classes into which the elevated genes were subsequently divided based on molecular functions and biological processes which are described in section 3.2.

2.4. Gene Ontology (GO) Enrichment Analysis

Gene Ontology (GO) provides an idea about the molecular function, important biological processes and potential applications of genes. Gene Ontology analysis has different categories that includes Molecular function (MF), cellular component (CC), and biological processes (BP) (Li *et al*., 2019). To understand the functions and proccesses of differentially expressed genes, GO enrichment analysis was performed by using Quick go tool (https://www.ebi.ac.uk/QuickGO/).

2.5. Pathway Analysis

Pathway analysis is the process of classifying large gene sets by the KEGG database ((Li *et al*., 2019). For doing pathway analysis different tools were referred that includes PANTHER (http://www.pantherdb.org/pathway/) and KEGG pathway database. Finally, Pathway analysis of upregulated genes was done by using the KEGG pathway database (https://www.genome.jp/kegg/pathway.html). Gene symbols were uploaded to the tool and sind (*S. indicum*) was used as an organism code.

2.6. Network Analysis of Secondary Metabolite and Stress-Related Genes

The DEGs which significantly upregulated for secondary metabolite production and stress response which includes biotic and abiotic stresses in *Sesamum mulayanum* were analyzed using (https://string-db.org/) STRING (Version -11.5) for their inter relatedness. As expected, it showed significant interconnection between the genes. Since the genome of *S. mulayanum* was not annotated yet, *Arabidopsis thaliana* was used as a reference organism.

2.7. Functional Annotation of The DEGs

The DEGs were searched and correlated with published literature to find exactly the type of stress tolerance the upregulated genes are involved in. The results of the upregulated genes involved in various types of stress tolerance mechanisms including biotic and abiotic stresses. Among the various biotic stresses, phyllody is the major disease that extremely affects the sesame growth and yield. Phyllody resistance can also be gained by insect tolerance/resistance.

3. FINDINGS

3.1. Up-Regulated *S. mulayanum* Genes During Seed Development

The hierarchical clustering heatmap shows that samples at the same developmental stages are clustered together and shown in Figure 3a. The PC1 variance is about 82% in the principal component analysis plot which means the samples have high variance in gene expression and the samples at 30 DAPs are highly separated than the samples at 10 DAPs shown in Figure 3b.

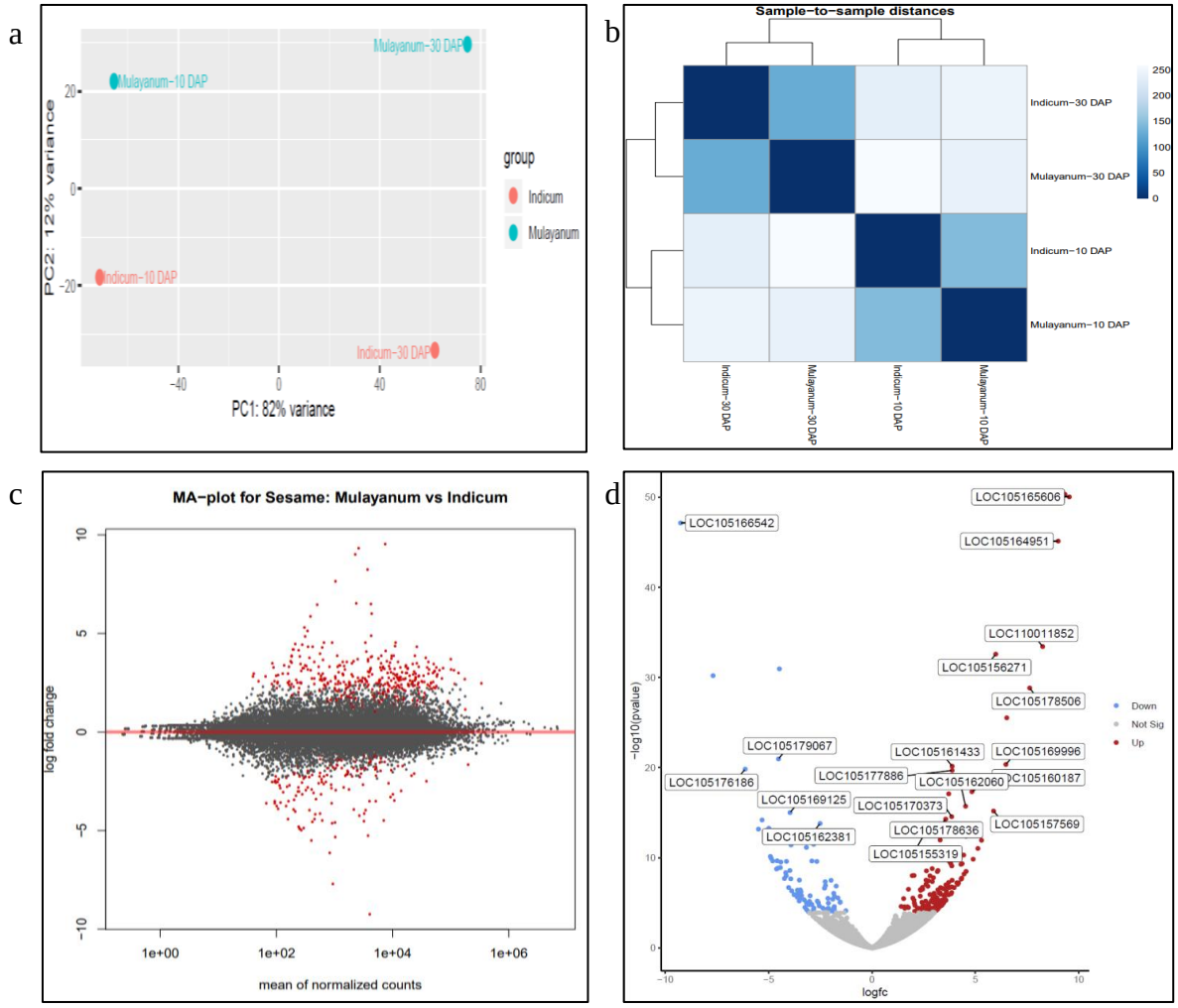

Figure 3. Visualization of DEG analysis: a) PCA plot using the log2 transformed counts b) HC heatmap of sesame sample (at 10 DAP and 30 DAP) c) MA plot representing the normalized fold change with the mean of normalized counts d) Volcano plot representing the top significant genes.

This indicates that the gene expression variation is high at 30 DAPs. In the Mean average difference plot, the red dots represent the significant genes, it shows that most of the significant genes are up regulated ones shown in Figure 3c. A volcano plot was used to highlight the top significant genes which is shown in Figure 3d. 216 genes were found to be significantly differentially regulated. Out of this,170 genes were up regulated, and 46 genes were down regulated.

The top significant genes include F-box/kelch-repeat protein At3g23880-like, MYB-like transcription factor ETC1, cytochrome P450 94C1, exocyst complex component EXO70A1-like, probable calcium-binding protein CML1, peptidyl-prolyl cis-trans isomerase FKBP15-3-like, ethylene-responsive transcription factor TINY, exocyst complex component EXO70A1, salicylate carboxy methyltransferase, 4,5-DOPA dioxygenase extradiol, 3-ketoacyl-CoA synthase 11, anthocyanidin 3-O-glucosyltransferase 2, elongation factor 1-beta-like, putative disease resistance protein RGA1, carotenoid 9,10(9',10')-cleavage dioxygenase 1-like and dicer-like protein 4 isoform X3.

The upregulated genes were then separated into 4 different categories. They are represented in the pie chart shown in Figure 4. They were categorized as annotated protein coding genes, transcription factors, putative genes (proteins having similar function) and uncharacterized genes.

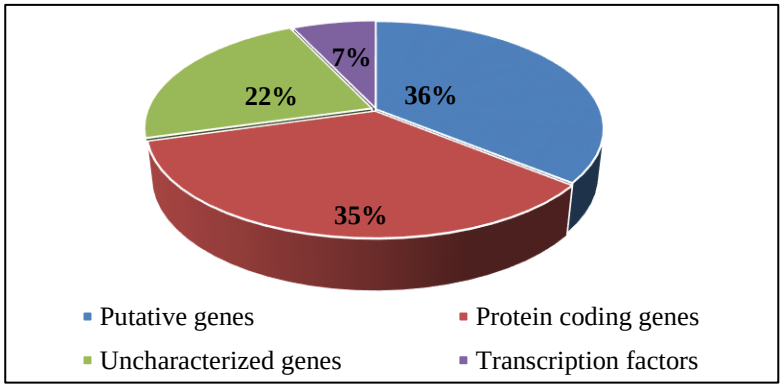

Figure 4. Categories of Differentially expressed genes (DEGs) in *S. mulayanum* Vs *S. indicum.*

3.2. Augmentation of Stress-Responsive Genes in *S. mulayanum*

Gene ontology analysis showed that the DEGs were involved in various molecular functions as well as diverse biological processes. The upregulated genes(170) were represented by 36 different molecular functions and 38 different biological processes and for the (46) downregulated genes, 14 different molecular functions and 15 different biological processes were involved. The Predominant biological functions of upregulated genes related to resistance are defense response to nematode (GO:0002215), systemic acquired resistance and salicylic acid-mediated signaling pathway (GO:0009862), response to abscisic acid (GO:0009737), detoxification of zinc ion (GO:0010312), salicylic acid biosynthetic process (GO:0009697), cellular oxidant detoxification (GO:0098869), response to ethylene (GO:0009723). The important biological processes of differentially expressed genes related to resistance and the corresponding expression level of the genes were represented by the bar graph shown in Figure 5.

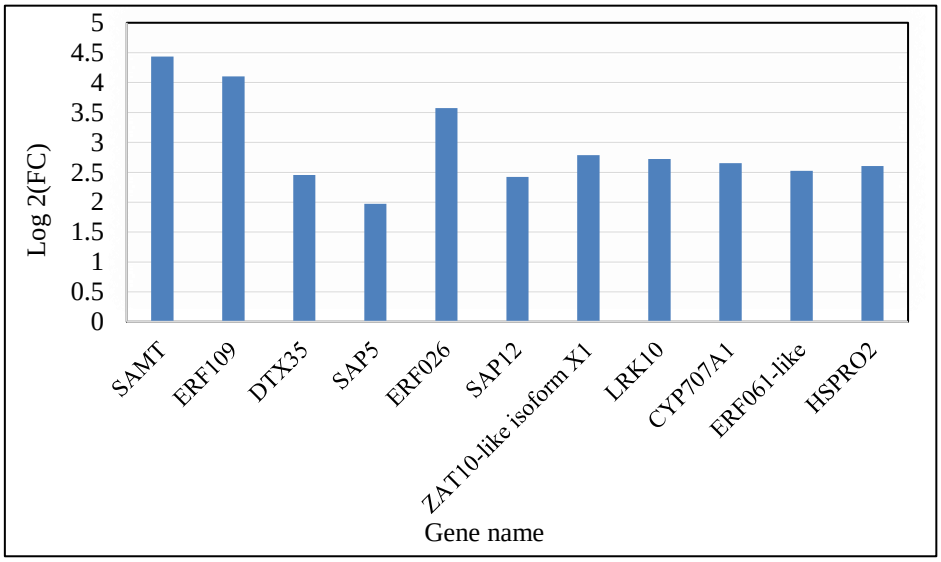

Figure 5. Stress-related *S. mulayanum* genes and their fold changes with reference *S. indicum* expression levels.

As a result, of comparative analyses, several upregulated genes were found to be involved in various biotic and abiotic stresses in different plant species. There are 23 biotic stress tolerance genes, and 26 abiotic stress tolerance genes were found. Upregulated genes involved in biotic stress tolerance were identified through literature analyses are shown with its log (FC) value in Table 1. Similarly, genes related phyllody disease resistance were specifically analyzed.

Table 1. Upregulated genes of *S. mulayanum* in biotic stress responses along with its Log2(FC) value.

Genes	Log2(FC) value
Probable calcium-binding protein CML18	3.88
Anthocyanidin 3-O-glucosyltransferase 2	3.57
Salicylate carboxymethyl transferase	4.44
4,5-DOPA dioxygenase extradiol	2.91
Polyubiquitin	1.95
3-ketoacyl-CoA synthase 11	3.71
Probable calcium-binding protein CML18	3.58
Mitogen-activated protein kinase kinase kinase NPK1	3.86
Syntaxin-121	3.31
Glutathione S-transferase F12	3.82
Probable protein phosphatase 2C 63	3.14
Putative calcium-binding protein CML23	3.34
Calmodulin-binding protein 60 C isoform X2	1.79
Calcium-dependent protein kinase 11 isoform X1	2.38
Zinc finger AN1 domain-containing stress-associated protein 12	2.42
F-box/kelch-repeat protein At3g23880-like	9.55
Elongation factor 1-beta-like	2.50
Calcium-binding allergen Ole e 8-like	2.99
Ethylene-responsive transcription factor ERF109	4.10
Nematode resistance protein-like HSPRO2	2.61
Glutathione S-transferase F12	3.82
Peroxidase 25-like	2.72
Linoleate 13S-lipoxygenase 3-1, chloroplast	2.71

Biosynthetic process and other includes L-phenylalanine biosynthetic process (GO:0009094), thiamine biosynthetic process (GO:0009228), chorismate biosynthetic process (GO:0009423), glutathione biosynthetic process (GO:0006750), coenzyme A biosynthetic process (GO:0015937), protein targeting to ER (GO:0045047), CAAX-box protein maturation (GO:0080120), intermembrane lipid transfer (GO:0120009), ethylene-activated signaling pathway (GO:0009873), regulation of exocyst localization (GO:0060178), cell wall modification (GO:0042545), mitochondrial calcium ion transmembrane transport (GO:0006851), xyloglucan metabolic process (GO:0010411).

Biological processes which are highly enriched by downregulated genes include fatty acid beta-oxidation (GO:0006635), ethylene mediated signaling pathway (GO:0009866), carotenoid biosynthetic process (GO:0016117), translational termination (GO:0006415), induced systemic resistance (GO:0009682), phosphatidylinositol dephosphorylation (GO:0046856), trans sulfuration (GO:0019346), induced systemic resistance and jasmonic acid mediated signaling pathway (GO:0009864).

3.3. Secondary Metabolite and Stress Tolerance Pathways are Linked

Pathway analysis of the upregulated genes indicated that the genes are involved in various pathways like Shikimate pathway, Betalain biosynthesis, Terpene biosynthesis, Carotenoid biosynthesis, MAPK signaling pathway, SNARE interactions in vesicular transport, Inositol phosphate metabolism, Fructose and mannose metabolism, pentose phosphate pathway and Monolignol biosynthesis. The secondary metabolite-producing gene's pathway and its applications are given in Table 2. Some of the genes of *S. mulayanum* involved in different pathways which directly (plant pathogen interaction, secondary metabolites, and pest resistance) and indirectly (synthesis of amino acids, phenylalanine, tyrosine synthesis and fatty acid synthesis) involved in plant defense against biotic and abiotic stresses shown in Figure 6.

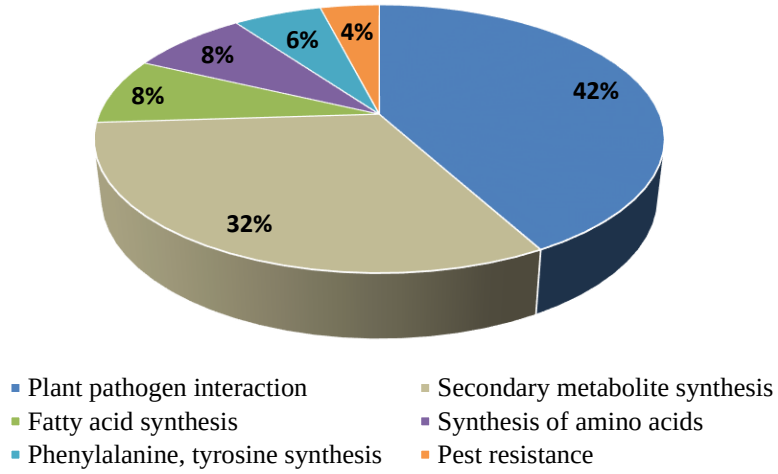

Figure 6. Upregulated genes of *S. mulayanum* involved in pathways connected to plant defense.

A network analysis was predicted for relating the interaction of secondary metabolites and stress tolerance genes. The network is generated between the secondary metabolites producing genes (PAL, LDOX, CM1, CYP707A1) with biotic (UFGT, KCS1, GSTF12, At4g33920) and abiotic stress tolerance genes (PUMP5, RDUF2, CCD4, GOLS1) are shown in Figure 7. Genome of *S. mulayanum* has not been annotated yet. Hence, *Arabidopsis thaliana* was used as a reference. The network generated was found to have 12 nodes and 10 edges with an average node degree of about 1.67. PPI enrichment P value is 2.53e-11. This shows that the network has significantly more interactions than expected. Totally, there are 16 secondary metabolite producing genes were found by correlating the literatures. Out of 16 genes, only a few formed the network with the biotic and abiotic genes. This is because the reference organism (*Arabidopsis thaliana*) does not possess certain genes that were present in *S. mulayanum.*

Table 2. Major secondary metabolite-producing genes of *S. mulayanum* and their application.

Genes involved in secondary metabolite synthesis	Products of the pathway	Applications
Probable carotenoid cleavage dioxygenase 4	Carotenoids - isoprenoids, terpenoids or terpenes	Antioxidants, light-harvesting pigments, and attractants for pollinators and seed dispersers. Response of plants to environmental stresses. Act in defense mechanisms (phytoalexins)
S-adenosylmethionine synthase 3	Ethylene	Plant growth regulation
Chorismate mutase 1, chloroplastic-like	Chorismate, shikimate	The synthesis of aromatic amino acids, p-aminobenzoic acid, folate
4,5-DOPA dioxygenase extradiol	Betalamic acid	Strong antioxidant activity, anticancer, hypolipidemic, hepatoprotective, anti-inflammatory, and antidiabetic activities
Geranylgeranyl pyrophosphate synthase, chloroplastic	Terpenoids	Signal molecules to attract the insects of pollination
	Polyketides	Anti-viral, anticancer, antifungal, and anti-microbial agents and neuroprotective
	Geranyl diphosphate	Gibberellin biosynthesis
Type I inositol polyphosphate 5-phosphatase 2 isoform X1	Phytic acid	The main storage form of phosphorus in the seeds
Fructokinase-2	D fructose 6 phosphate	Precursor of mucopolysaccharides (polysaccharides with nitrogen-containing components)
UDP-glucuronate 4-epimerase 1	Ascorbate	Antioxidant protects plants against oxidative damage
Phenylalanine ammonia-lyase	Monolignol	Biosynthesis of both lignans and lignin, regulating plant development, pigmentation, and UV protection
	Flavanone	Controlling plant development through their action in cell wall synthesis, and in defense against fungal pathogens.
Leucoanthocyanidin dioxygenase-like	Flavonoids	Important plant pigments for flower coloration, UV filtration, symbiotic nitrogen fixation,
Trans-cinnamate 4-monooxygenase-like	Quinone	Anti-proliferation and anti-metastasis effects
	Stilbenoid	Phytoalexins, which are antimicrobial compounds produced de novo in plants to protect against fungal infection and toxins
	Diarylheptanoid	A small class of plant secondary metabolites
	Gingerol	Powerful anti-inflammatory and antioxidant effects
Shikimate O-hydroxy cinnamoyl transferase-like	Cutin and suberin	Cell-wall associated glycerol lipid polymers,
	16 feruloyloxi palmitic acid	Antioxidants
Abscisic acid 8'-hydroxylase 1-like	Beta carotene	Protects plant cells against the destructive effects of ultraviolet light
	Abscisic acid	Role in various physiological processes of plants, such as stomatal closure, cuticular wax accumulation, leaf senescence, bud dormancy, seed germination, osmotic regulation etc..,
Linoleate 13S-lipoxygenase 3-1, chloroplastic	Oxo ode (octa decadienoic acid)	PPAR α agonist to decrease triglyceride accumulation in mouse primary hepatocytes
Peroxidase 25-like	Phenylpropanoid	Precursors for a wide range of secondary metabolites
Allene oxide cyclase, chloroplastic-like	Jasmonic acid	Endogenous growth-regulating substance

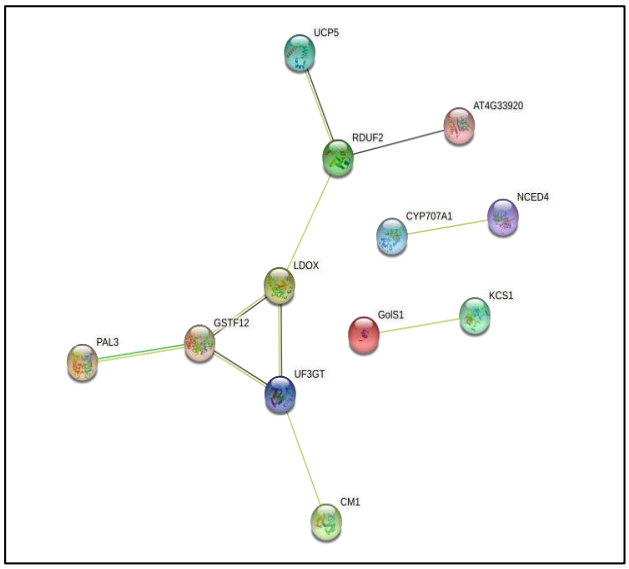

Figure 7. Interaction between secondary metabolites and stress tolerance (biotic and abiotic) genes of *S. mulayanum.*

4. DISCUSSION and CONCLUSION

4.1 Discussion

Transcriptome profiling is a comprehensive approach to understand the disease resistance pathways in sesame. In rice, sheath blight disease is studied by comparing the transcriptome profiles of resistant (CR1014) and susceptible (Swarna-sub-1) varieties (Bal *et al*., 2022). The molecular response, genetic basis and candidate genes of sheath blight was identified from differentially expressed genes. Molecular mechanism of phyllody resistance in sesame is poorly understood. Singh *et al*., (2007) have earlier reported the involvement of two independent non-allelic genes in phyllody resistance. In this study, we have compared the transcriptomic profiles of S. *indicum*, a phyllody susceptible, and *S. mulayanum*, a tolerant species to identify differentially expressed genes that are related with disease resistance. Transcriptome analyses of charcoal rot challenged *S. mulayanum* plants were used to identify defense related genes and pathways involved during plant- pathogen interaction (Dutta *et al*., 2020). In this study, among the DEGs, eleven S. *mulayanum* upregulated genes which are related to defense or stress tolerance are observed to have 2-4.5-fold increased expression than S. *indicum* and shown in Figure 5. A similar study in pepper root knot nematode had broadened the single loci concept to several interrelated pathways in transcriptome profiling (Hu *et al*., 2020). The gene responsible for exhibiting the systemic acquired resistance was up regulated and the gene responsible for induced systemic resistance was down regulated because the SRA data of *S. mulayanum* was obtained during seed development. This gene may be upregulated and shows its resistance whenever the plant is challenged with the pathogen. Induced systemic resistance (ISR) can be induced during any interactions between the plant and pathogens. Once it is activated, it elicits a set of localized responses in and around the infected host cells (Heil & Bostock, 2002). In the current transcriptome analyses S. *mulayanum* has revealed 42% of pathogenesis related genes which are up regulated than *S. indicum* are shown in Figure 6. This suggests that these genes and their pathways might contribute to the disease tolerance nature of *S. mulayanum.*

Plant secondary metabolites confer several functions to the plants. They improve the innate immunity of plants besides contributing to the growth, development, and nutritive value of the plant. The disease tolerance is recently proven by transgenic rice overproducing flavonoids exhibiting bacterial leaf blight resistance (Jan *et al*., 2021). Similarly, in sesame, greater accumulation of secondary metabolites is correlated with increased charcoal rot disease resistance (Chowdury *et al*., 2021). Hoda *et al*., 2024 reported that sesame seeds irradiated with

low doses of gamma rays showed an increased production and activity of peroxidase and polyphenol oxidase, phenolic, and lignan contents after germination when compared to non-irradiated sesame seeds. The increased production of secondary metabolites increased the resistance for charcoal rot disease caused by *Macrophomina phaseolina*. In this investigation, S. *mulayanum*, a disease tolerant wild species shows 32% of the upregulated genes to be involved in secondary metabolite production compared to the susceptible species which is shown in Figure 6. Genes like Peroxidase 25-like and phenylalanine ammonia lyase significantly upregulated in *Sesamum mulayanum* involved in the production of phenylpropanoid and monolignols which are the precursors for lignan, and phenolic compounds shown in Table 2. These increased secondary metabolites production made *Sesamum mulayanum* a natural tolerant species to various biotic stresses. Carotenoids, Betalamic acid, Ascorbate, Gingerol, and 16 feruloyloxi palmitic acid have strong antioxidant property. Antioxidants are present in higher amounts in the wild sesame species compared to cultivated gene pool (Pathak *et al*., 2020). Under stress conditions whether it may be biotic or abiotic, the production of reactive oxygen species (ROS) was increased in plants. This in turn induces oxidative stress. The increased oxidative stress, plants increase the production of lower molecular and higher molecular antioxidants. These in turn help to reduce the oxidative stress in the plant (Kasote *et al*., 2015).

The key genes related to environment stress in *S. mulayanum* are salicylate carboxy methyltransferase, ethylene-responsive transcription factor ERF025, protein DETOXIFICATION 35, zinc finger A20 and AN1 domain-containing stress-associated protein 5, rust resistance kinase Lr10-like and abscisic acid 8'-hydroxylase 1-like. Among those key genes, salicylate carboxy methyltransferase exhibits systemic acquired resistance and salicylic acid-mediated signaling pathway. Salicylic Acid (SA) plays an important role in both systemic and local defense responses in plants. Systemic acquired resistance (SAR) is a salicyclic acid (SA) dependent response that elicits long distance signaling mechanism. As a result, it provides broad spectrum and long-lasting resistance to secondary infections of the entire plant (Gao *et al*., 2015). Of the up regulated genes, salicylate carboxy methyl transferase was found to be the topmost gene (4.4-fold up regulated in *S. mulayanum* as compared to the *S. indicum*). This suggests the plausible role of such secondary metabolite genes in conferring disease resistance to *S. mulayanum*.

In our study, an exhaustive literature analysis was performed and a total of 16 secondary metabolite producing genes, 23 biotic stress tolerance genes and 26 abiotic stress tolerance genes were further analyzed for interaction. Out of this, four secondary metabolite producing genes (PAL, LDOX, CM1, CYP707A1) were found to interact with biotic and abiotic genes to form a significant network. Phenyl alanine ammonia lyase (PAL) is one of the major key enzymes which are involved in the phenyl-propanoid pathway. Major products of the pathway and product's applications are mentioned in Table 2. Major products are lignans and flavonoids which are involved in resistance against charcoal rot disease (Hoda *et al*., 2024). LDOX, one of the secondary metabolites producing genes which formed significant interaction with stress tolerance genes was involved in anthocyanin biosynthesis. Upon cold stress this gene was found to be expressed more in purple black carrot and helps the plant to withstand the stress (Dar *et al*., 2022). CYP707A1 is the loci where histone deacetylase HDA9 will bind and induce drought resistance of the plant. This mechanism was studied in Cai *et al*., 2022. Drought induced long intergenic noncoding RNA DANA1 interacts with DANA1-INTERACTING PROTEIN 1 (DIP1) which further increases the binding of HDA9 to CYP707A1 loci and increases the drought resistance of the plant. Few genes were removed and the reasons behind this could be that the novel secondary metabolite producing genes from *S. mulayanum* that were identified in this study were not included in the *A. thaliana* PPI network in STRING previously.

The secondary metabolites involved in plant growth and development are induced by compounds like ethylene, and gibberellic acid (GA) etc., GA plays an important role in seed

germination, internode elongation, flower initiation, development, and abscisic acid (ABA) is involved in maintenance of dormancy. Gibberellins (GA) plays a vital role in embryo development. GA is one of the important constituents that regulates temporal organization of maturation phase (Gupta & Chakrabarty, 2013). Secondary metabolites accumulation during seed development in sesame species is correlated to their transcriptomic profile. In *S. radiatum*, another wild species, novel secondary metabolites, jan sesangolin and episesantalin accumulation was shown to be correlated with their corresponding transcript levels at 35 DAPs (Harada *et al*., 2020).

In another study, secondary metabolites of wild sesame, *S. angustifolia* a potential vegetable crop, is found to have higher nutritive value that satisfies daily nutritional requirements of vitamins and micronutrients (Maina *et al*., 2019). Similarly, the dried leaves of *S. radiatum* have been shown to possess significant amounts of micro and macro nutrients, proteins, phenolic compounds, and antioxidants (Catarino *et al*., 2019). Sesame wild species, *S. lanciniatum, S. radiatum,* and *S. indicum* subsp. *malabaricum* have higher phenolic contents and antioxidants (Pathak *et al*., 2020). Accordingly, *S. mulayanum* also might also have several secondary metabolites correlating to their gene expression during seed development. Correspondingly, sesamin and total lignan content in *S. mulayanum* were found to be higher than the other cultivated Indian varieties (Pathak *et al*., 2015). The above observations suggest that *S. mulayanum* could be a potential oilseed crop that can be exploited for its valuable oil, secondary metabolites, and disease tolerance nature.

4.2 Conclusion

The farmed *S.indicum* contains a significant number of secondary metabolites in addition to its high oil content. However, a number of diseases, including phyllody, leaf spot, powdery mildew, and root and stem rot, can affect planted sesame and severely reducing its oil yield productivity. Improved disease tolerance commercial varieties urgently need to be substituted. In order to achieve this, this study assessed and analyzed a wild sesame *S.mulayanum*'s genetic potential for the synthesis of lignans and other secondary metabolites. It was found that nearly 74% of the upregulated genes in *S.mulayanum* are either involved in plant pathogen interaction or secondary metabolite production which interact with stress tolerance genes. The secondary metabolites of *S. mulayanum* can be directly used for nutraceutical or pharmaceutical purposes and the genetic potential of the crop can be exploited in breeding programs to improve the domesticated indicum varieties.

Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

Selvi Subramanian: Supervision, validation of results, original draft revision, resources. **Dharanidharan Manivannan**: Investigation, methodology, visualization, software, formal analysis, and writing -original draft.

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