Transcriptomic Dissection of the AMF-*Solanum melongena* Interaction: Unveiling Molecular Secrets

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Key words:

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Abstract:

Fungus-based biofertilizers, in particular, have been shown to improve plant growth and health by providing essential nutrients and improving soil health. Arbuscular mycorrhizal fungi (AMF) form mutualistic symbiotic relationships with plants, including those in the Solanaceae family. They colonize the root systems of plants and aid in enhancing nutrient absorption efficiency, particularly in phosphorus-deficient soils. The present study was carried out to study the interaction between *Solanum melongena* L. with *Glomus macrocarpum* and *Funneliformis mosseae* (formely known as *Glomus mosseae*) at transcript level. In this study a total of 365 transcripts were upregulated (>1.5x) folds in *S. melongena* in response to both the fungi, while 44 transcripts were upregulated only in response to *G. macrocarpum* and 28 transcripts were upregulated only in response to *F. mosseae*. Similarly, 49 transcripts were downregulated less than -1.5 folds in response to both the fungi while 78 were downregulated only in response to the *G. macrocarpum* and 36 were downregulated only in response to *F. mosseae*.

KEGG pathway analysis of *S. melongena* treated with *G. macrocarpum* revealed carbon metabolism, cofactor biosynthesis and Endocytosis as the dominant metabolic pathway, while analysis of the *F. mosseae* treatment revealed Glycerophospholipid and Endocytosis metabolism as dominant metabolic pathways.

Key words : Arbuscular Mycorrhizal Fungi, Biofertilizer, *Funneliformis mossae*, *Glomus macrocarpum* and Transcriptomics, *Solanum melongena*,

Introduction

Plants play a significant role in our ecosystem and human survival, and agriculture is the cornerstone of global food security. Investigations into the environmentally-friendly and sustainable methods are needed to boost crop yields in order to meet the growing global demand for food.

In the recent years, studies on the role of microorganisms in plant growth and sustainable agriculture has increased. Fungus-based biofertilizers, in particular, have been shown to improve plant growth and health by providing essential nutrients and improving soil health. Particularly, the advantageous effects of arbuscular mycorrhizal fungi (AMF) on a variety of crops, such as cereals, legumes, and vegetables, have been extensively studied (Chen et al., 2018; Begum et al., 2019; Khan et al., 2022; Sui et al., 2022).

Most AMF species are members of the phylum Mucoromycota's sub-phylum Glomeromycotina. (Spatafora et al., 2016). This sub-phylum contains 25 species and four orders of AMF, namely Glomerales, Archaeosporales, Paraglomerales, and Diversisporales (Redecker et al., 2013). They use lipids and products from plant photosynthetic processes to complete their life cycle since they are obligatory biotrophs (Bago et al., 2000, Jiang et al., 2017).

Glomus macrocarpum and *Funneliformis mosseae* are both species of arbuscular mycorrhizal fungi (AMF) that form mutualistic symbiotic relationships with plants, including those in the Solanaceae family. They colonize the root systems of plants and aid in enhancing nutrient absorption efficiency, particularly in phosphorus-deficient soils (Aziz et al., 2011). These fungi develop structures in the root cells of plants called arbuscules, which expand the root system's surface area and enable a direct interchange of nutrients between the fungus and the plant. This causes the plant to absorb more nutrients, which improves plant development and productivity.

The vegetable crop brinjal (*Solanum melongena* L.), which is widely grown in tropical and subtropical regions, may be a potential candidate for the use of fungus-based biofertilizers. The impact of these biofertilizers on the proteins in brinjal is not well understood, though. More effective and environmentally friendly agricultural practices might come from a better understanding of the molecular mechanisms by which fungus-based biofertilizers interact with and alter the physiology of brinjal.

Systems biology is a cross-disciplinary area of research that brings together numerous fields, including biology, mathematics, computer science, and engineering, to comprehend complex biological systems. In order to develop a thorough understanding of biological processes, the system biology entails the integration of high-throughput experimental data with computer models.

Systems biology approaches have fundamentally changed how we understand the way plants grow and develop as well as the way they react to environmental stress. Plant systems biology is the study of the structure, function, and interactions of plant molecules and networks using high-throughput technologies. These technologies include genomics, transcriptomics, proteomics, and metabolomics. The system biology has become a potent tool for comprehending intricate biological processes in plants, and it is anticipated to be crucial in the advancement of sustainable agriculture

The purpose of the research is to employ the system biology to understand the mechanism via which the fungal biofertilizers affect the transcriptome of brinjal. The investigation would clarify the molecular mechanisms and pathways underpinning the effects of fungus-based biofertilizers on brinjal.

Materials and Methods

Study site and Sampling

The study was conducted at botanical garden, department of botany, Banaras Hindu University, Varanasi, India located at 25° 16' 4.3608" N and 82° 59' 25.7784" E with an altitude of 81 metres above the sea level.

Inoculation of *Solanum melongena* L. plants

For the purpose, pot culture experiment was set up. Control and both the treatments were set up in triplicates. Pure culture of Funneliformis mosseae and Glomus macrocarpum was obtained by monoculturing both these isolates of AMF. For biofertilizer experiment brinjal seeds disinfected with 1% sodium hypochloride was placed on sterilized sand-soil substrate(1:1v/v) in 200 ml disposable plastic containers. The germination set up were kept in B.O.D incubator at 25□ under white fluorescent tubes (Photoperiod 12 hr) and watered whenever required to keep the soil mixture moist. After one month, germinated seedlings of S.melongena were transferred in 8 x 7.6 inch clay pots containing sterilized sand-soil substrate(1:1 v/v) with 10g each of both the inoculants. Uninoculated(control) plants were also maintained. AMF colonisation was ascertained by advanced microscopy.

Sample collection and RNA extraction:

The leaves of *S. melongena* plants treated without or either with *Glomus macrocarpum* or *Funneliformis mosseae* AMF were collected and grounded in Trizol reagent (Invitrogen). The RNA was extracted as per the manufacturer's instructions.

Transcriptome analysis

The RNA quantity and quality was determined by 260:280 ratio on eppendorf nanodrop as well as tapestation respectively. Only contamination-free, high quality of RNA (having RNA integration number >6) was used in the study. The sequencing library was prepared using a NEBNext® Ultra™ II RNA Library Prep Kit for Illumina® (#E7770L) kit essentially following the manufacturer's instructions. Adapters were ligated to the cDNA fragments and the libraries were then size-selected and PCR amplified. The paired-end 2*150 bp libraries were then sequenced using Illumina Novaseq 6000 high-throughput sequencer at Redcliffe Research India Pvt Ltd facility. 40 million reads were generated to achieve the desired depth of the coverage.

Data analysis:

Performed quality control on the raw sequencing data and performed adapter and quality trimming using Cutadapt tool (GitHub).The transcripts were assembled de novo. Quantification of gene expression level was done and differential expression analysis was performed.

The raw sequences obtained via RNA seq were subjected to the quality check using FastQC via base quality score distribution, average base content per read, GC distribution in the reads. The raw files were found to be of good quality. The good quality reads were analysed by de novo transcriptome assembly.

Transcriptome assembly using De novo approach

Trinity software was used to assemble the clean reads with default options.

Estimation of Gene Expression

Alignment of the reads was done using Bowtie 2 program .The expression of each transcript and its abundance was estimated using RSEM software and normalized using FPKM (fragments per kilobase per million mapped reads) method.

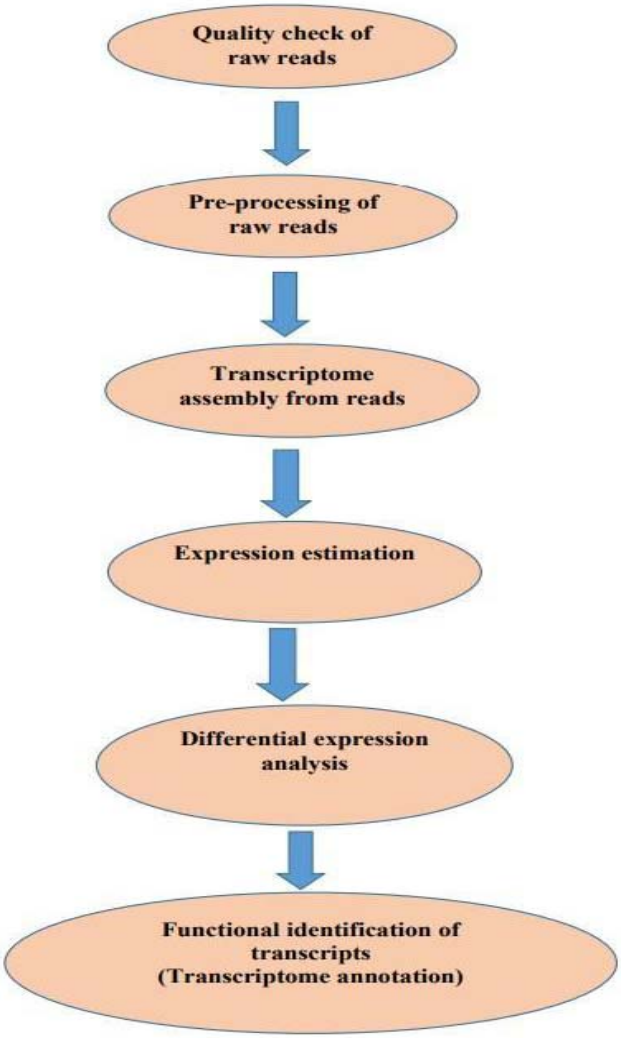

Figure 1. Pipeline of bioinformatics analysis: De novo transcriptome analysis involves the following steps.

Differential gene expression analysis

Bioconductor package edgeR (R Studio) was used for differential analysis of genes (p-values □0.05, significant threshold). Genes with positive and negative log fold change values (logFC) ≥ │1│ was described as up-or down-regulated genes, respectively.

Transcriptome annotation

The assembled unigenes were annotated against Uniprot protein resource (UNIPROT) using BLASTX programme. Matches with E-value cut- off of 10-5 and % identity of 50% retained 47592 Uniprot IDs for further annotation. DAVID software was used for pathway KEGG (Kyoto Encyclopedia of Genes and Genomes) and GO (Gene ontology) analysis. Unigenes

was classified into 3 ontological categories namely; biological process, cellular components and molecular functions.

qRT PCR

same RNA extracted for RNAseq was used to validate the differential regulated gene using qRT PCR. cDNA of 3 samples were synthesized using Bio-Rad iScript cDNA synthesis Kit Cat. no. 1708891. A 20 µl reaction was set for each cDNA with a total of 500 ng RNA. The cDNA synthesis reaction was set as per kit protocol.

Results

The paired-end libraries of the transcripts of *Solanum melongena* L. plant treated without or with arbuscular mycorrhizal fungi, *Glomus macrocarpum* and *Funneliformis mosseae* were sequenced on the Illumina HiSeq platform.

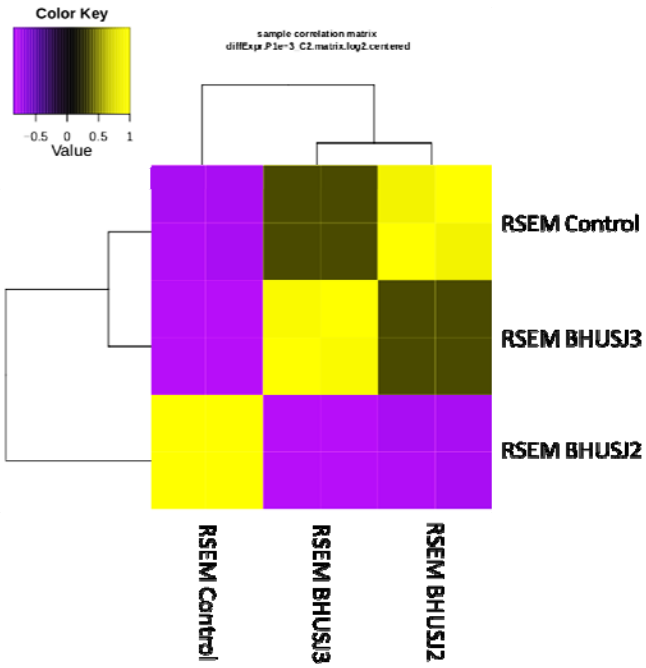

Figure 2. Sample Correlation Matrix of the transcriptome libraries

The paired-end libraries of the transcripts of *Solanum melongena* L. plant treated without or with arbuscular mycorrhizal fungi, *G. macrocarpum* and *F. mosseae* were sequenced on the Illumina HiSeq platform. The control samples i.e., *S. melongena* L. plant not treated with the AMF had mean number of 21722156.5 reads (78.81%) with 109050 fragments per kilobase per million mapped reads (FPKM) value greater than or equal to 1.0. Similarly, BHUSJ2 sample i.e., *S. melongena* L. plant treated with *Glomus macrocarpum* had mean number of 2150827 reads (80.65%) with 57733 FPKM value $\geq$ 1.0 and BHUSJ3 sample i.e., *S. melongena* plant treated with *Funneliformis mossae* had mean number of 25187514 reads (81.52%) with 95655 FPKM value $\geq$ 1.0.

The sample correlation matrix of the transcriptome libraries revealed that BHUSJ3 sample was closer to the control samples as compared to the BHUSJ2 samples (Figure 2). The observation implies that the *Glomus macrocarpum* had more pronounced effect on the transcriptomics of the *S. melongena* than that of the *Funneliformis mossae*.

The volcano plots and MA plots of the differentially expressed genes (DEGs) of the libraries are shown in the figure 3. Comparisons of the control library with BHUSJ2 i.e., genes of *S. melongena* when treated with the *Glomus macrocarpum*, revealed nearly 56897 DEGs, of which 33136 were statistically significant (Figure 3a). Out of the differentially expressed genes included 7608 upregulated transcripts and 25528 downregulated transcripts. of the upregulated transcripts 2685 genes were upregulated more than 1.5 folds while among the downregulated transcripts, 1285 genes were downregulated below -1.5 folds. Similarly, the comparisons of the control library with BHUSJ3 library i.e., genes of *S. melongena* when treated with the *Funneliformis mossae*, revealed nearly 51495 DEGs, of which 11599 were statistically significant (Figure 3b).

Out of the differentially expressed genes included 4314 upregulated transcripts and 7285 downregulated transcripts. Of the upregulated proteins, 1695 transcripts were upregulated to more than 1.5 folds while among the downregulated transcripts, 575 transcripts were downregulated below -1.5 folds. A total of 365 transcripts were upregulated more than 1.5 folds in *S. melongena* in response to both the fungi, *Glomus macrocarpum* and *Funneliformis mosseae*, while 44 transcripts were upregulated only in response to *G. macrocarpum* and 28 transcripts were upregulated only in response to *F. mosseae* (Figure 4a). Similarly, 49 transcripts were downregulated less than -1.5 folds in response to both the fungi while 78 were downregulated only in response to the *G. macrocarpum* and 36 were downregulated only in response to *F. mosseae* (Figure 4b).

a). Control Versus BHUSJ2 b). Control Versus BHUSJ3

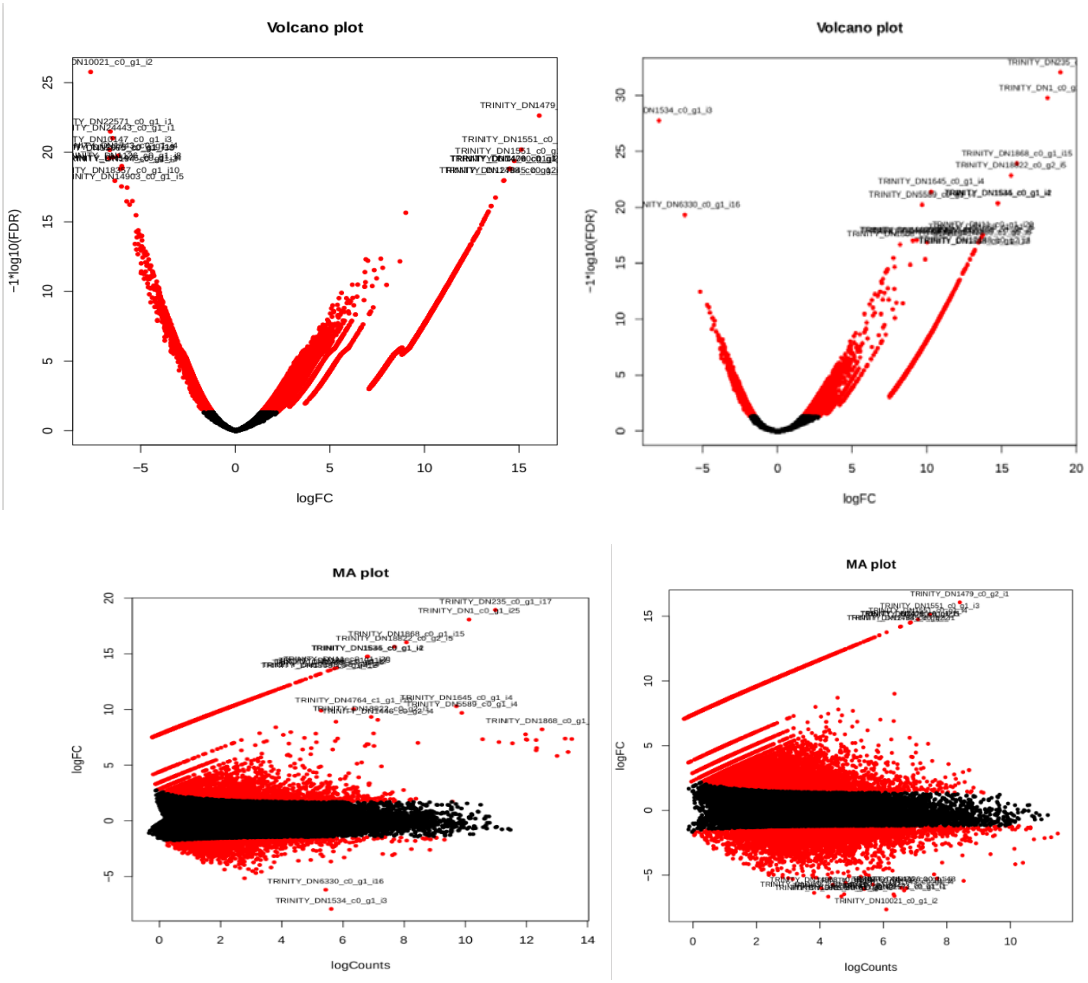

Figure 3. MA and Volcano plots of the differentially expressing libraries

a). Upregulated transcript b). Downregulated Transcript

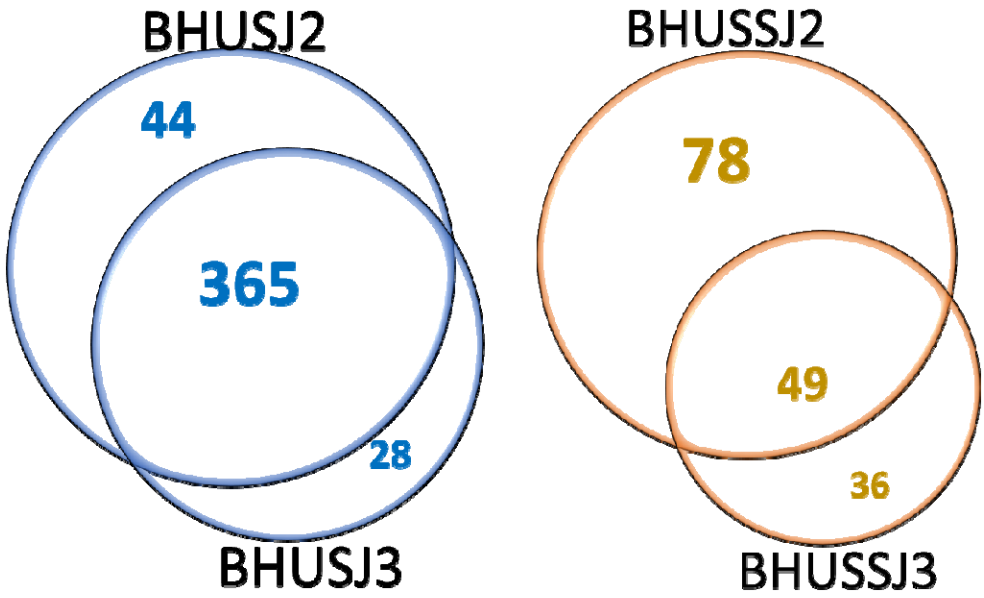

Figure 4. Upregulated and Downregulated transcripts of BHUSSJ2 and BHUSSJ3 in relation to the control samples.

Gene Ontology and KEGG Pathway analysis of the BHUSJ2 and BHUSJ3 samples

The gene ontology analysis by the DAVID software revealed identified 101 different statistically significant "molecular functions" in the differentially expressed genes with the p value less than 0.05. The analysis revealed that the genes belonging to the protein binding dominated the transcript abundance in the BHUSJ2 sample representing the effect of *Glomus macrocarpum* on *S. melongena* with 19% of the genes involved followed by the ATP binding and metal binding activity with the involvement of 11.5 % and 9.3 % of the genes. The analysis of "cellular components" in which the genes were located revealed that the transcript of the membrane, chloroplast and cytoplasm dominated the differentially expressed genes with count 966 (19.2%), 941 (18.67%) and 914 (18.13%) respectively. Similarly, the "biological process" analysis revealed that the differentially expressed transcripts were dominated by the transcript involved in the embryo development leading to breaking the seed dormancy, protein phosphorylation, and salinity responsive transcripts with transcript count 145 (2.93%), 143 (2.89%) and 114 (2.3%) respectively. Pathway KEGG analysis of the DEGs revealed that the top three pathways in which these transcripts were involved included

carbon metabolism (76 transcripts - 9.5%), biosynthesis of cofactors (71 transcripts – 8.86%), and Endocytosis (56 transcripts – 7%) (Figure 8)

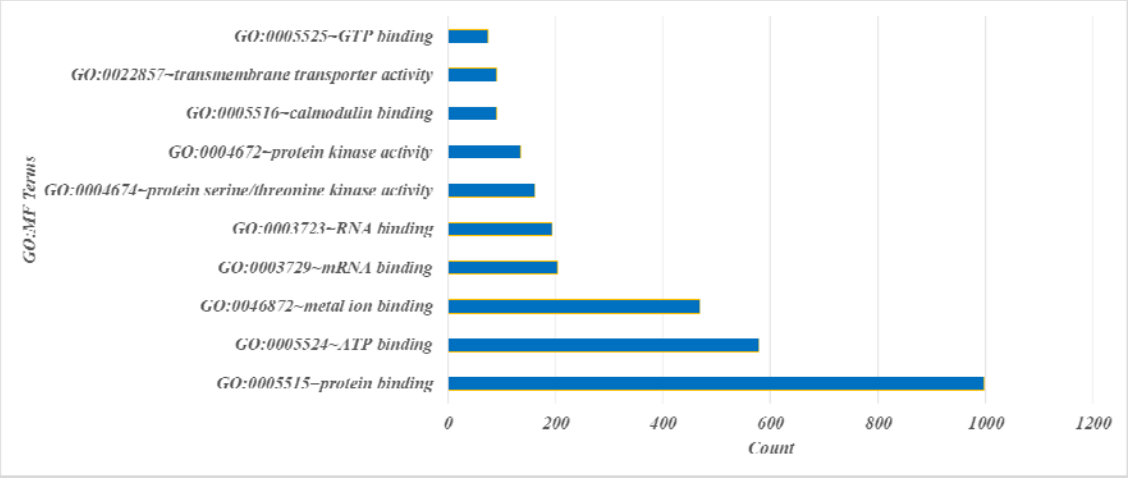

Figure 5: GO annotation representing Top 10 terms in the 'Molecular Function' category of Differential expressing transcripts of Control Versus BHUSJ2 Samples

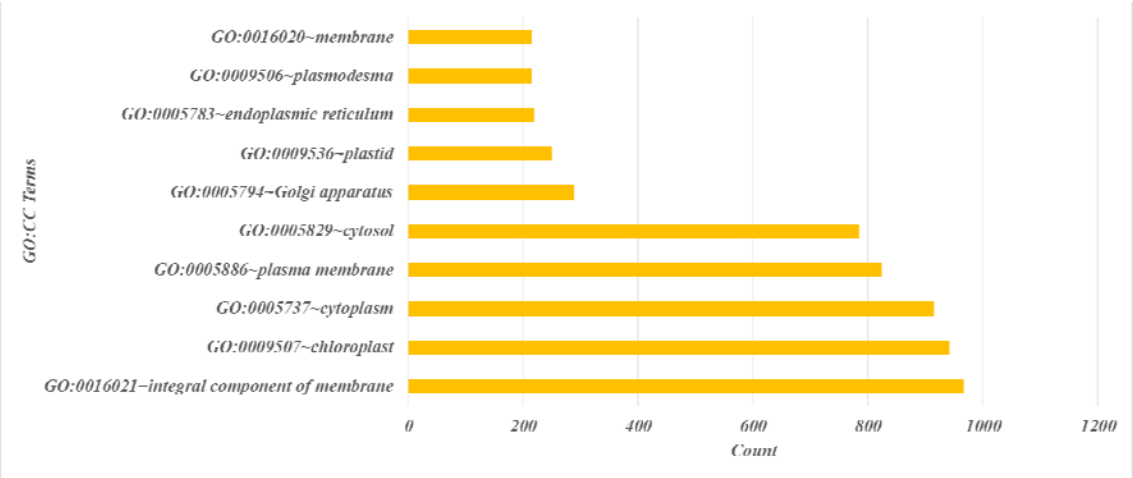

Figure 6: GO annotation representing Top 10 terms in the 'Cellular Component' category of Differential expressing transcripts of Control Versus BHUSJ2 Samples

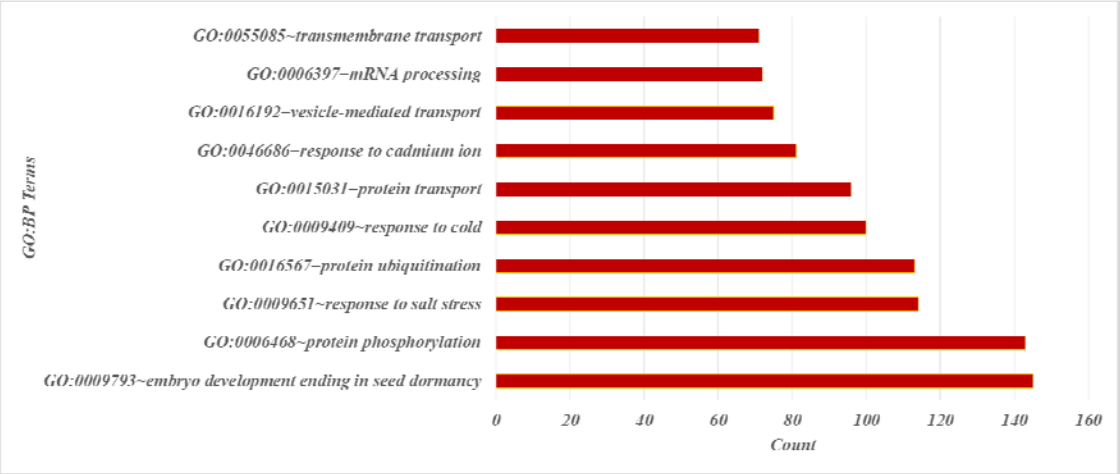

Figure 7: GO annotation representing Top 10 terms in the 'Biological Process' category of Differential expressing transcripts of Control Versus BHUSJ2 Samples

The gene ontology analysis of BHUSJ3 sample representing the effect of *Funneliformis mosseae* on *S. melongena* revealed that the genes belonging to the function of "protein binding" dominated the DEGs with count = 640 (20.50%) followed by the ATP binding and metal ion binding with count = 410 (13.14%) and 313 (10.02 %) respectively. The analysis of "cellular components" in which the genes were located revealed that the transcript of the membrane, chloroplast and cytoplasm dominated the differentially expressed genes with count 691 (22.14 %), 620 (19.87 %) and 591 (18.94 %) respectively. Similarly, the "biological process" analysis revealed that the differentially expressed transcripts were dominated by the transcript involved in the protein phosphorylation, response to abscisic acid and drought-responsive transcripts with transcript counts 119 (3.81 %), 116 (3.72 %) and 112 (3.6%) respectively. Pathway KEGG analysis of the DEGs revealed that the Glycerophospholipid and Endocytosis metabolism were the dominant metabolic pathway with 34 transcripts (9%) each (Figure 12).

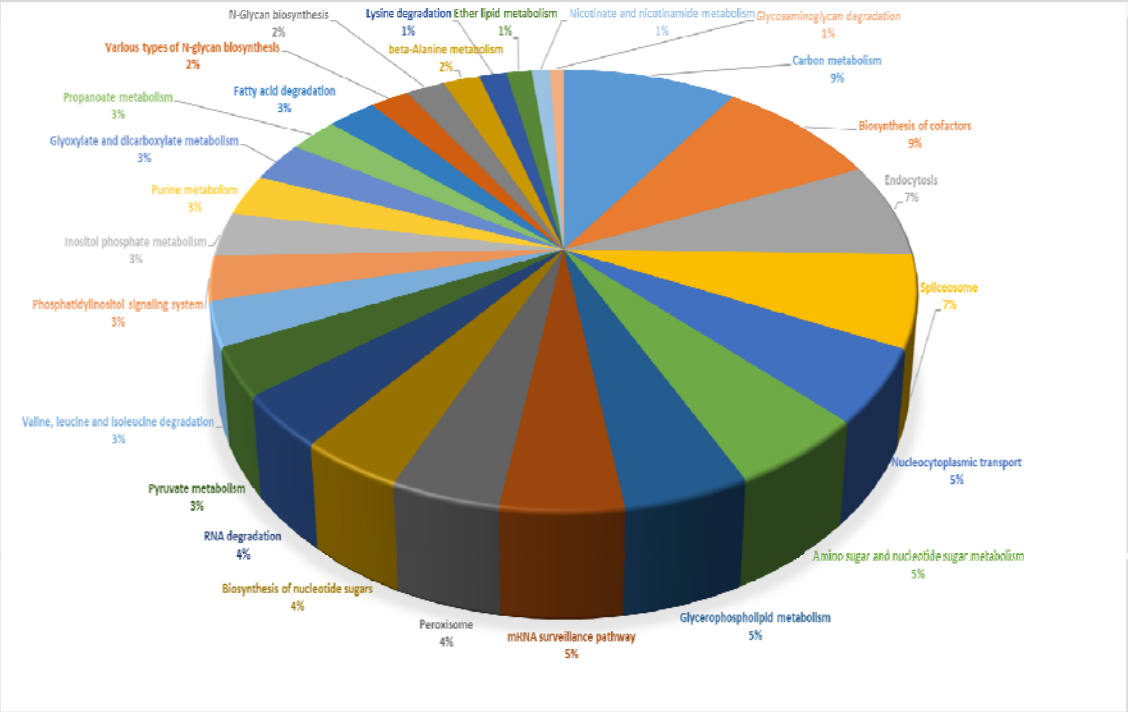

Figure 8: Figure of KEGG analysis representing pathway information related to transcript of Differential expressing transcripts of Control Versus BHUSJ2 Samples

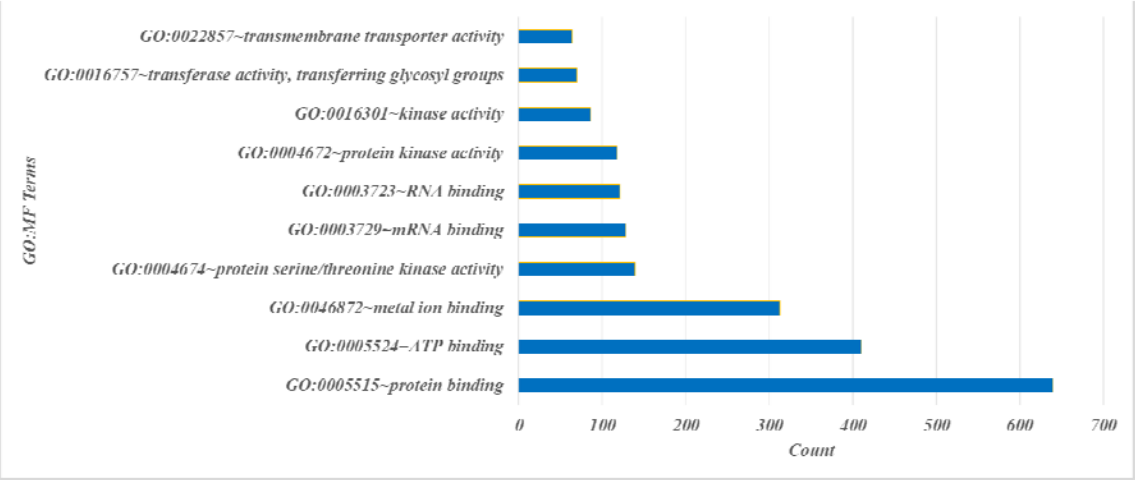

Figure 9: GO annotation representing Top 10 terms in the 'Molecular Function' category of Differential expressing transcripts of Control Versus BHUSJ3

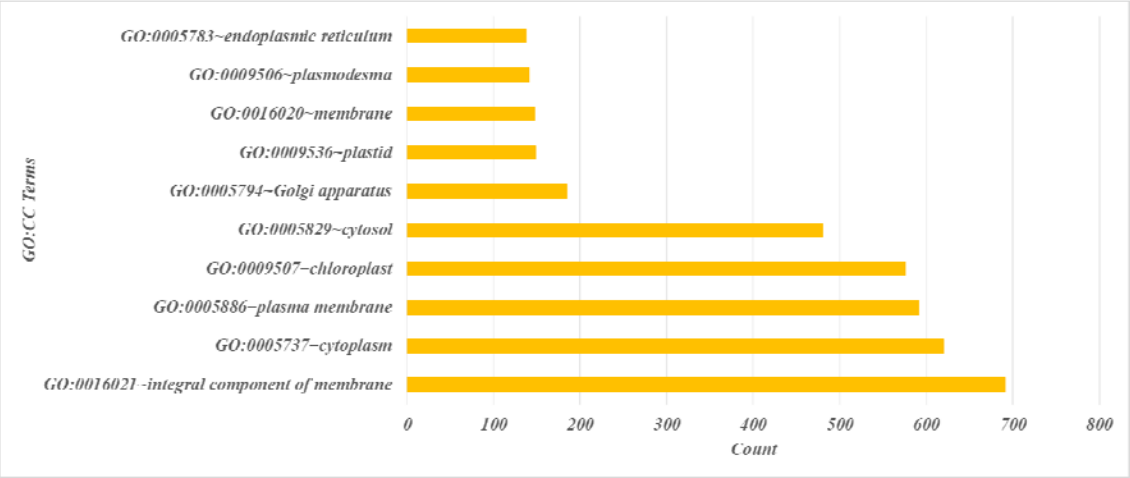

Figure 10: GO annotation representing Top 10 terms in the 'Cellular Component' category of Differential expressing transcripts of Control Versus BHUSJ3

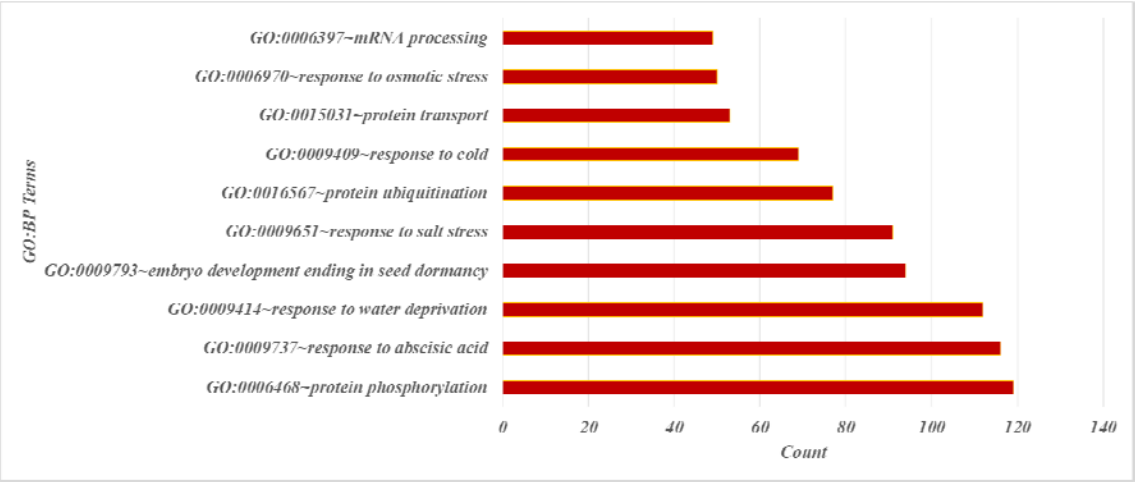

Figure 11: GO annotation representing Top 10 terms in the 'Biological Process' category of Differential expressing transcripts of Control Versus BHUSJ3

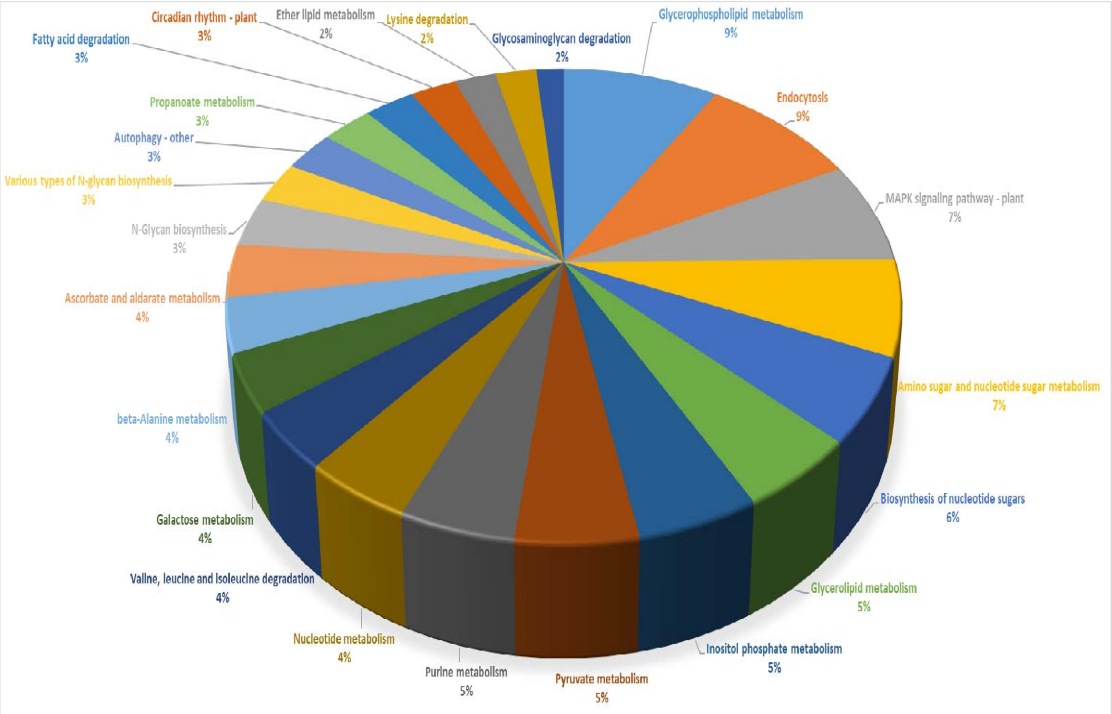

Figure 12: KEGG analysis representing pathway information related to Differential expressing transcripts of Control Versus BHUSJ3.

Genes associated with pathogen defence

Association of plants with AMFs confer certan degree of protection ot the pathogens (Pozo et al. 1999). Though due to non-exposure to the pathogens defensive pathway did not feature in top ten physiological processes, several defensive genes were found to be modulated upon AMF association. Peptide:N-glycanase (EC 3.5.1.52) with trinity Id TRINITY_DN21710_c0_g1_i13 was induced to 9x in the case of *Funneliformis mossae* as compared with the control. Though, another N-glycanase with trinity Id TRINITY_DN50529_c0_g1_i1 was downregulated (-2x) in the case of *Glomus macrocarpum.* Similarly, chitinase (EC 3.2.1.14), Chitinase 2 (Tulip bulb chitinase-2) (TBC-2) with trinity Id TRINITY_DN45969_c0_g1_i2 was induced to 2.3 folds only in response to the symbiosis with the *F. mossae*. Though, another isoform of Chitinase 2 (TBC-2) with trinity Id TRINITY_DN38413_c0_g1_i1 was downregulated to -1.6 folds in response to the *F. mosseae*. Whereas, endochitinases, Endochitinase 2 with trinity Id TRINITY_DN2730_c0_g1_i2 , Endochitnase 3 with trinity Id TRINITY_DN2632_c2_g2_i3,

basic 30kDa endochitinase with trinity Id TRINITY_DN2730_c0_g3_i2 and acidic 27kDa endochitnase with trinity Id TRINITY_DN5280_c0_g1_i3 were induced to 5.4x, 2.4x, 3x, and 2.2x respectively in response to *F. mosseae*. Similarly, the basic 30kDa endochitinase with trinity Id TRINITY_DN2730_c0_g3_i1 and acidic endochitinase pcht28 with trinity Id TRINITY_DN2522_c0_g1_i11 and Endochitinase EP3(Chitinase class IV) (AtchitIV) with trinity Id TRINITY_DN6274_c0_g1_i4 were induced to 3x, 2.8x, and 2.3x each in response to *G. macrocarpum* . Phenylalanine ammonia-lyase (EC 4.3.1.24) with trinity Id was induced to 1.7x only in response to *F. mosseae*. Lipoxygenase 6, chloroplastic (AtLOX6) (EC 1.13.11.12) with trinity Id TRINITY_DN34921_c0_g1_i2 was upregulated to 10x in response to *G.macrocarpum*, the transcript with trinity Id TRINITY_DN34921_c0_g1_i4 was downregulated to -2.5x in the case of *F. mosseae*.

DEAD-box helicases in the transcriptomes

Among the transcripts involved in the tolerance to salinity stress, DEAD-box helicase motif are known to play significant roles. DEAD-box ATP-dependent RNA helicase 16 (TRINITY_DN6185_c0_g1_i9 and TRINITY_DN2471_c0_g1_i1), helicase 18 (TRINITY_DN15662_c0_g1_i3 and TRINITY_DN20762_c0_g1_i6), and helicase 20 (TRINITY_DN19824_c0_g1_i34) (EC 3.6.4.13) were upregulated to 7.8, 9 and 5.5 folds respectively in *S. melongena* upon association with *G. macrocarpum* and 7.7, 9.7 and 6.9 folds upon association with *F. mosseae*. Another DEAD-box ATP-dependent RNA helicase 18 protein which is also known as, BRI1-KD-interacting protein 115 (BIP115) with Trinity Id TRINITY_DN15662_c0_g1_i5 was upregulated to 2.7x in response to *G. mossae*. Helicases, DEAD-box ATP-dependent RNA helicase 5 (TRINITY_DN22150_c0_g1_i7), helicase 10 (TRINITY_DN26429_c0_g1_i4), helicase 17 (TRINITY_DN8171_c0_g1_i8), helicase 32 (TRINITY_DN48781_c0_g1_i1), helicase 35 (TRINITY_DN1888_c0_g1_i5), helicase 52C (TRINITY_DN1065_c3_g1_i5) and helicase 58, chloroplastic (TRINITY_DN37178_c0_g1_i3) were upregulated to 1.7, 9.9, 2.1, 8.4, 2.7, 3.5, and 8.4 folds respectively in the case of *S. melogena* and *G. macrocarpum* association only. On the other hand, DEAD-box ATP-dependent RNA helicase 22 (TRINITY_DN13923_c0_g1_i1), helicase 36 (TRINITY_DN17609_c4_g1_i5), and helicase 40 (TRINITY_DN14283_c1_g1_i1) were upreguated to 9, 9, and 10.6 folds respectively upon *S. melogena* and *G. macrocarpum* association only . The DEAD-box ATP-dependent RNA

helicase 50 (TRINITY_DN37563_c0_g1_i5) was upregulated upon *S. melogena* and *G. macrocarpum* association to 7.9 folds however, another variant of the helicase 50 (TRINITY_DN39418_c0_g1_i3) was decreased to 2 folds in the case of *S. melogena* and *F. mossae* association

The modulation of the DEAD box helicases during salinity stress has been correlated with the changes in the superoxide dismutase (Chen, 2016). The levels of superoxide dismutase [Cu-Zn] 2 (EC 1.15.1.1) (TRINITY_DN19272 c1 g1 i4) was upregulated to 9.7x in *S. melongena* in association with *G. macrocarpum* and 7.991x with *F. mosseae* .

Real-Time PCR analysis

Real-time PCR was carried out for Signal Peptide Peptidase, GST Zeta Class, Receptor-Like Cytoplasmic Kinase, Calcium Transporter Atpase, Citrate Synthase, FD-GOGAT, elongation factor-Tu, aminocyclopropane and DEAD-box helicases. The results of the Real-time PCR revealed significant changes in the transcript levels of GST Zeta Class (3.4 and 1.8 folds), Receptor-Like Cytoplasmic Kinase (3.7 and 4.2 folds), Citrate Synthase (2.5 and 6.7 folds), Ef-Tu (2.8 and 3.9 folds), aminocyclopropane (1.7 folds each), DEAD-box helicase 50 (2.7 and 3.3 folds), and Superoxide dismutase SOD (3.1 and 1.6 folds) in *S. melogena* upon association with either of the AMF used i.e., *F. mosseae* and *G. macrocarpum* respectively. The transcripts of the Signal Peptide Peptidase were upregulated to 1.6 folds only upon association with the *F. mossae*, while a minor downregulation of the FD-GOGAT transcripts (reduced to 0.64 folds) was noticed upon association with the *F. mosseae*. A significant downregulation in the transcripts of DEAD-box RNA helicase 16 (reduced to 0.04 and 0.71 folds) was observed upon association with either of the AMF used i.e., *F. mosseae* and *G. macrocarpum* respectively. Whereas, no significant changes in Ca-transporter ATPase transcript was observed upon association with either of the AMF used.

Table 1: List of significantly modulated Peptide:N-glycanase, Chitinases, Endochitinases, Phenylalanine ammonia-lyase and Lipoxygenase of *Solanum melongena* in association with *F. mossae* and *G. macrocarpum*

Trinity Id	Name	*G. macrocarpum*	*F. mossae*
TRINITY_DN21710_c0_g1_i13	Peptide:N-glycanase (EC 3.5.1.52)		9.251x
TRINITY_DN50529_c0_g1_i1	N-glycanase	-1.681x	
TRINITY_DN45969_c0_g1_i2	Chitinase 2 (Tulip bulb chitinase-2) (TBC-2)		2.324x
TRINITY_DN38413_c0_g1_i1	Chitinase 2 (Tulip bulb chitinase-2) (TBC-2)		-1.622x
TRINITY_DN2730_c0_g3_i2	basic 30kDa endochitinase		2.948x
TRINITY_DN2730_c0_g3_i1	basic 30kDa endochitinase	3.054x	
TRINITY_DN2730_c0_g1_i2	Endochitinase 2		5.378x
TRINITY_DN2632_c2_g2_i3	Endochitinase 3		2.374x
TRINITY_DN5280_c0_g1_i3	acidic 27kDa endochitnase		2.171x
TRINITY_DN2522_c0_g1_i11	acidic endochitinase pcht28	2.858x	
TRINITY_DN6274_c0_g1_i4	Endochitinase EP3(Chitinase class IV) (AtchitIV)	2.274x	
TRINITY_DN3846_c1_g1_i1	Phenylalanine ammonia-lyase (EC 4.3.1.24)		1.697x
TRINITY_DN3846_c0_g1_i5	Phenylalanine ammonia-lyase (EC 4.3.1.24)	-1.572x	
TRINITY_DN2944_c0_g1_i1	Phenylalanine ammonia-lyase (EC 4.3.1.24)	-1.830x	
TRINITY_DN42824_c0_g1_i1	Phenylalanine ammonia-lyase 1 (PhPAL1) (EC 4.3.1.24)		1.812x
TRINITY_DN69444_c0_g1	Phenylalanine ammonia-lyase (EC 4.3.1.24)		1.718x
TRINITY_DN34921_c0_g1_i2	Lipoxygenase 6, chloroplastic (AtLOX6) (EC 1.13.11.12)	10.196x	
TRINITY_DN34921_c0_g1_i4	Lipoxygenase 6, chloroplastic (AtLOX6)		-2.245x

Trinity Id	Name	*G. macrocarpum*	*F. mossae*
	(EC 1.13.11.12)		

Table 2: List of significantly modulated DEAD-box ATP-dependent RNA helicases and superoxide dismutase of *Solanum melongena* in association with either *F. mossae* or *G. macrocarpum*

Trinity Id	Name	*G. macrocarpum*	*F. mossae*
RINITY_DN19824_c0_g1_i34	DEAD-box ATP-dependent RNA helicase 20	6.844	5.540
RINITY_DN6185_c0_g1_i9	DEAD-box ATP-dependent RNA helicase 16	7.786	
RINITY_DN2471_c0_g1_i1	DEAD-box ATP-dependent RNA helicase 16		7.638
RINITY_DN15662_c0_g1_i3	DEAD-box ATP-dependent RNA helicase 18	9.647	
RINITY_DN15662_c0_g1_i5	DEAD-box ATP-dependent RNA helicase 18 (BRI1-KD-interacting protein 115) (BIP115)	2.664	
RINITY_DN20762_c0_g1_i6	DEAD-box ATP-dependent RNA helicase 18		9.061
RINITY_DN22150_c0_g1_i7	DEAD-box ATP-dependent RNA helicase 5	1.696	
RINITY_DN26429_c0_g1_i4	DEAD-box ATP-dependent RNA helicase 10	9.932	
RINITY_DN8171_c0_g1_i8	DEAD-box ATP-dependent RNA helicase 17	2.059	
RINITY_DN48781_c0_g1_i1	DEAD-box ATP-dependent RNA helicase 32	8.363	
RINITY_DN1888_c0_g1_i5	DEAD-box ATP-dependent RNA helicase 35	2.657	
RINITY_DN1065_c3_g1_i5	DEAD-box ATP-dependent RNA helicase 52C	3.517	
RINITY_DN37178_c0_g1_i3	DEAD-box ATP-dependent RNA helicase 58 chloroplastic	8.399	
RINITY_DN13923_c0_g1_i1	DEAD-box ATP-dependent RNA helicase 22		9.121

Trinity Id	Name	*G. macrocarpum*	*F. mossae*
RINITY_DN17609_c4_g1_i5	DEAD-box ATP-dependent RNA helicase 36		8.911
RINITY_DN14283_c1_g1_i1	DEAD-box ATP-dependent RNA helicase 40		10.534
RINITY_DN37563_c0_g1_i5	DEAD-box ATP-dependent RNA helicase 50		7.964
RINITY_DN39418_c0_g1_i3	DEAD-box ATP-dependent RNA helicase 50	-2.004	
RINITY_DN36611_c0_g1_i1	DEAD-box ATP-dependent RNA helicase 3, chloroplastic (Protein EMBRYO DEFECTIVE 1138)	-2.023	
RINITY_DN5165_c0_g1_i4	DEAD-box ATP-dependent RNA helicase 6	-1.633	
RINITY_DN5165_c0_g1_i1	DEAD-box ATP-dependent RNA helicase 8	-1.605	
RINITY_DN17915_c0_g1_i2	DEAD-box ATP-dependent RNA helicase 21	-2.293	
RINITY_DN22733_c0_g1_i6	DEAD-box ATP-dependent RNA helicase 24	-1.738	
RINITY_DN1272_c0_g2_i1	DEAD-box ATP-dependent RNA helicase 37	-2.145	
RINITY_DN1065_c3_g1_i7	DEAD-box ATP-dependent RNA helicase 11		-2.008
RINITY_DN26075_c0_g1_i1	DEAD-box ATP-dependent RNA helicase 42 (RCF1) (REGULATOR OF CBF GENE EXPRESSION 1)		-1.794
RINITY_DN4309_c0_g1_i3	DEAD-box ATP-dependent RNA helicase 56 (UAP56 homolog B)		-3.430
TRINITY_DN19272_c1_g1_i4	Superoxide dismutase [Cu-Zn] 2 (EC 1.15.1.1)	9.730	7.991
TRINITY_DN2333_c0_g1_i4	Receptor-like cytoplasmic kinase 176 (OsRLCK176) (EC 2.7.11.1)	4.310	2.412
TRINITY_DN20147_c0_g1_i2	Calmodulin-binding receptor-like cytoplasmic kinase 1 (EC 2.7.11.1)	4.016	
TRINITY_DN4521_c0_g1_i13	Serine/threonine-protein kinase PBL34 (EC 2.7.11.1) (PBS1-like protein 34) (Receptor-like cytoplasmic kinase PBL34)	2.994	
TRINITY_DN4374_c0	Serine/threonine-protein kinase PBL36 (EC	1.890	

Trinity Id	Name	*G. macrocarpum*	*F. mossae*
_g1_i12	2.7.11.1) (PBS1-like protein 36) (Receptor-like cytoplasmic kinase PBL36)		
TRINITY_DN7927_c0_g1_i5	Glutathione S-transferase (EC 2.5.1.18) (25 kDa auxin-binding protein) (GST class-phi)		-1.576
TRINITY_DN6516_c0_g1_i19	Glutathione S-transferase T1 (AtGSTT1) (EC 2.5.1.18) (GST class-theta member 1) (Glutathione S-transferase 10)		9.048
TRINITY_DN6516_c0_g1_i21	Glutathione S-transferase T1 (AtGSTT1) (EC 2.5.1.18) (GST class-theta member 1) (Glutathione S-transferase 10)	9.102	
TRINITY_DN19508_c0_g1_i3	Glutathione S-transferase U9 (AtGSTU9) (EC 2.5.1.18) (GST class-tau member 9) (Glutathione S-transferase 14)		2.498
TRINITY_DN4653_c0_g1_i5	Glutathione S-transferase U17 (AtGSTU17) (EC 2.5.1.18) (GST class-tau member 17) (Glutathione S-transferase 30) (Protein EARLY RESPONSIVE TO DEHYDRATION 9)	2.133	
TRINITY_DN22883_c0_g1_i1	Glutathione S-transferase Z1 (AtGSTZ1) (EC 2.5.1.18) (GST class-zeta member 1) (Glutathione S-transferase 18) (Maleylacetone isomerase) (MAI) (EC 5.2.1.-)		1.856

Discussion

Arbuscular mycorrhizal fungi (AMF) are vital endosymbionts playing an effective role in plant productivity and the functioning of the ecosystem. They are of key importance for sustainable crop improvement (Gianinazzi et al., 2010). The soil-borne AMF are known to improve plant nutrient uptake as well as improve the resistance to abiotic stress factors (Sun et al., 2018). AMF are essential endosymbionts that contribute significantly to the production of plants and the health of the ecosystem. They are crucial for improving crops in a sustainable way (Gianinazzi et al., 2010). AMF-mediated growth promotion is not only by improving water and mineral nutrient uptake from the adjoining soil but also by safeguarding the plants from fungal pathogens (Smith and Read, 2008; Jung et al., 2012).

Plants have been shown to react differently to different AMF species because of varied AMF traits (Wilson and Hartnett, 1998; Yang et al., 2014; Ramirez-Viga et al., 2018). As a result, there are a variety of plant-AMF interactions that might be favourable, neutral, or even detrimental (Pankova et al., 2018; Neuenkamp et al., 2019; Kaur et al., 2022). Our results revealed modulation of different genes of *Solanum melongena* in response to treatment with the two AMF species, *Glomus macrocarpum* and *Funneliformis mosseae*. The sample correlation matrix revealed that the *S. melongena* was found more responsive to *G. macrocarpum* than *F. mosseae*. The observation was reflected in the physiological observations where, *G. macrocarpum* was found to significantly increase the Fruit fresh weight, Root height, Root fresh and dry weight of *S. melongena* (Communicated elsewhere). However, the Shoot dry weight was increased in response to both *F. mosseae* and *G.macrocarpum* AMF. All transcripts are not translated into physiological changes owing to various post-transcriptional and post-translational changes. It seems protein profiling of the *S. melongena* in association with AMFs *G. macrocarpum* and *F. mossae* is also needed to understand the interaction at protein level and its effect on the plant physiology in a better way.

Association of plants with AMFs confer certan degree of protection from the pathogens. Peptide:N-glycanase (EC 3.5.1.52) was induced to 9x in the case of *Funneliformis mosseae* as compared with the control. Though, another N-glycanase was downregulated (-2x) in the case of *G. macrocarpum.* Induction of Glycanase in tomato (*Solanum lycopersicum*) roots treated with *F. mosseae* has been associated with conferring resistance to invading pathogen, *Phytophthora parasitica* (Pozo et al. 1999). However, the induction of the enzyme is the case of *G. mossae* treatment to *S. melongena* need further investigations upon infection.

Besides glycanase, other hydrolytic enzymes implicated in dealing with the infections includes β-1,3-glucanase, chitinase, phenylalanine ammonia-lyase (PAL) and lipoxygenase (LOX) in tomato leaves. These enzymes also confers plant development, morphogenesis, plant microbe signaling and antifungal activities (Song et al., 2015). These genes were also modulated in the *S. melongena* plant upon treatment with the fungi, *F. mosseae* and/ or *G. macrocarpum*. Phenylalanine ammonia-lyase (PAL) is known to help in countering the cold stress in eggplant, *Solanum melongena* L. (Pasbani et al., 2020).

Association with the AMFs is known to impart resistance to abiotic stress in plants particularly salinity stress. Association between *S. melongena* and *F. mosseae* modulated transcript predominantly involved in development of embryo associated with the breaking of seed dormancy, followed by protein phosphorylation and response to salinity stress. whereas, in the case of the association between *S. melongena* and *G. macrocarpum* the protein phosphorylation was the dominant biological process and seed dormancy was ranked fourth and response to salt stress was fifth dominant biological process.

Numerous studies have shown that fungal symbiosis increases a plant's ability to withstand a wide range of challenges, including as drought, salinity, herbivory, temperature, metals, and diseases (Rodriguez et al., 2008; Ahanger et al., 2014; Salam et al., 2017). By facilitating a series of intricate communication events between the plant and the fungus that result in an improved photosynthetic rate and other gas exchange-related features, as well as an increased water intake, AMF help host plants develop vigorously under adverse conditions (Birhane et al., 2012).

Association of AMF with *S. melogena* alters the expression of genes encoding various growth related factors, for example Citrate synthase, which is responsible for catalysing the initial reaction of the citric acid cycle was found upregulated upon association with both the AMFs with higher expression in the *G. macrocarpum*. Similarly, the G protein, EF-Tu that catalyses the binding of aminoacyl-tRNA to the ribosome's A-site, and aminocyclopropane which is direct precursor of ethylene hormone was also upregulated upon association of both the fungi. Signal Peptide Peptidase known to be responsible for the quality control of endoplasmic reticulum membrane proteins were upregulated only in the *F. mossae* samples, while a minor downregulation of the FD-GOGAT transcript, that code for the proteins involved in photorespiration and primary nitrogen assimilation and have some role in the leaf

senescence and the nitrogen remobilization (Zeng et al., 2017) was noticed in the *G. macrocarpum* associated egg plant samples.

Receptor-like cytoplasmic kinases (RLCKs) are essential regulators of plant immunity and development. Several DEAD-box ATP-dependent RNA helicases and salt stress associated genes were modulated in *S. melongena* in association with *F. mossae* and with *G. macrocarpum* (table 1). Real-Time PCR analysis confirmed the upregulation of the genes encoding stress proteins such as, Receptor-like cytoplasmic kinases (RLCKs), DEAD-box ATP-dependent RNA helicase 50, Class Zeta GST, and Superoxide dismutases. Resistance to salt stress has been correlated with multiple stress responsive genes that work in coordination to confer resistance to stress (Shivakumara et al., 2017). Helicases seems to be crucial in stabilizing growth in stressed plants by controlling transcription and translation during abiotic stress. Most helicases are characterized by the presence of a group of highly conserved amino acid sequences known as "helicase-motifs," which are grouped together to facilitate helicase function. (Tuteja and Tuteja, 2004a; 2004b). The largest family of RNA helicases is the DEAD-box family. DEAD motif (Asp-Glu-Ala-Asp) is a crucial motif of helicases. DEAD-box RNA helicases are thought to have a variety of important roles in plant growth and development. (Linder and Jankowsky, 2011). The motif confers salinity tolerance probably by lowering membrane ion leakage and increased activity of superoxide dismutase as was reported in cotton (Chen, 2016). The role of the motif in conferring resistance to salt stress has been demonstrated in various legumes plants (Kim et al., 2017; Neha and Kharb 2022). Besides salinity tolerance the DEAD box RNA helicase are also reported to be involved in conferring resistance to cold and other abiotic stresses (Gong et al., 2005; Kant et al., 2007; Guan et al., 2013), and pathogen and oxidative stresses (Li et al., 2008).

Chen, (2016) reported that transgenic lines of cotton plants overexpressing *Apocynum venetum* DEAD-box helicase 1 (AvDH1) showed lower membrane ion leakage, along with increased activity of superoxide dismutase thus confering salinity tolerance. In Arabidopsis, DEAD-box protein LOS4 (low expression of osmotically responsive genes 4) and RCF1 (regulator of CBF gene expression 1) has been validated to be essential in exporting mRNA and pre-mRNA splicing by regulating the expression of CBF (C-repeat binding factor) factor under cold stress conditions (Gong et al., 2005; Guan et al., 2013). Three DEAD-box RNA helicases AtRH5, AtRH9 and AtRH25 also respond to multiple abiotic stresses in Arabidopsis (Kant et al., 2007; Kim et al., 2008). A rice DEAD-box RNA helicase OsBIRH1

(Oryza sativa BTH-induced RNA helicase 1) was shown to function in defense responses against pathogen and oxidative stresses (Li et al., 2008).

The cytosolic dimeric protein, GST (EC 2.5.1.18) catalyse the conjugation of glutathione (GSH) with a variety of endogenous and exogenous alkylating chemicals to aid in cellular detoxification. GST-Theta and GST-Tau were induced upon association with both the AMFs upto 9x and 2x respectively. The GST-Zeta class transcripts were upregulated upon association with *F. mossae* while GST-phi class was downregulated upon association with the *G. macrocarpum*.

The penultimate step in the breakdown of tyrosine and phenylalanine is catalysed by Class Zeta GSTs, also known as maleylacetoacetate (MAA) isomerases, employing GSH as a cofactor. the Class Zeta GSTs were found to be involved in the detoxification of the carcinogen dichloroacetic acid by catalysing its dechlorination to glyoxylic acid. (Pandey et al., 2015). The glutathione metabolism of the *S. melogena* was severely affected by association with the AMFs. Glutathione reductase, cytosolic (GR)(EC 1.8.1.7) was induced in *G macrocarpum* however an insignificant change was noticed in *F. mossae*. Similarly, Glutamate--cysteine ligase, chloroplastic (EC 6.3.2.2), Glutathione synthetase, chloroplastic (EC 6.3.2.3), and glutathione peroxidase 8 (EC 1.11.1.9) were induced in *G. macrocarpum*, yet the gene was not found in the *F. mossae*. On the other hand, Glutathione S-transferase DHAR1, mitochondrial (EC 2.5.1.18) and Glutathione S-transferase (EC 2.5.1.18) (25 kDa auxin-binding protein) (GST class-phi) were down regulated in the *F. mossae*, however, the genes were not found in *G. macrocarpum.* The observation clearly suggests that the genes involved in stress tolerance are largely affected in *G. macrocarpum* and not in *F. mossae*. The observation might be correlated with the improved physiology of the eggplant in association of *G. macrocarpum* than that of the *F. mossae*. The fungal species-specific ability to mitigate stress through antioxidant defense of the host plant has also been previously reported (Pasbani et al., 2020).

Table3: Comparison of GST and other enzymes involved in the Glutathione metabolism of S. melogena upon association with *G. macrocarpum* and *F. mossae*

Trinity Id	**Name**	***G. macrocarpum***	***F. mossae***
TRINITY_DN1033_c0_g2_i7	Glutathione reductase, cytosolic (GR) (GRase) (EC 1.8.1.7) (GOR2)	11.471	
TRINITY_DN1033_c0_g2_i3	Glutathione reductase, cytosolic (GR) (GRase) (EC 1.8.1.7) (GOR2)		Insignificant change
TRINITY_DN13657_c0_g1_i7	Glutamate--cysteine ligase, chloroplastic (EC 6.3.2.2) (Gamma-ECS) (GCS) (Gamma-glutamylcysteine synthetase)	9.738	
TRINITY_DN9976_c0_g1_i4	Glutamate--cysteine ligase, chloroplastic (EC 6.3.2.2) (Gamma-ECS) (GCS) (Gamma-glutamylcysteine synthetase)	Insignificant change	
TRINITY_DN18459_c0_g1_i1	Glutathione synthetase, chloroplastic (GSH synthetase) (GSH-S) (Glutathione synthase) (EC 6.3.2.3)	1.581	
TRINITY_DN11591_c0_g1_i8	Glutathione hydrolase 3 (EC 3.4.19.13) (Gamma-glutamyltransferase 3) (Gamma-glutamyltranspeptidase 3) (EC 2.3.2.2) (Gamma-glutamyltranspeptidase 4)	-3.345	-2.319
TRINITY_DN40241_c0_g1_i2	Glutathione S-transferase DHAR1, mitochondrial (EC 2.5.1.18) (Chloride intracellular channel homolog 1) (CLIC homolog 1) (Glutathione-dependent dehydroascorbate reductase 1) (AtDHAR1) (GSH-dependent dehydroascorbate reductase 1) (mtDHAR) (EC 1.8.5.1)		-1.57171
TRINITY_DN16574_c0_g1_i1	Probable phospholipid hydroperoxide glutathione peroxidase (PHGPx) (EC 1.11.1.12)	2.015	3.451
TRINITY_DN6777_c0_g1_i4	Probable glutathione peroxidase 8 (EC 1.11.1.9)		Insignificant change
TRINITY_DN6777_c0_g1_i6	Probable glutathione peroxidase 8 (EC 1.11.1.9)	2.591	

TRINITY_DN31275_c0_g1_i1	Phospholipid hydroperoxide glutathione peroxidase, chloroplastic (PHGPx) (EC 1.11.1.12)	Insignificant change	
TRINITY_DN10095_c2_g1_i8	Aminopeptidase M1 (EC 3.4.11.2) (Alpha-aminoacylpeptide hydrolase)		Insignificant change
TRINITY_DN33245_c0_g1_i3	Aminopeptidase M1 (EC 3.4.11.2) (Alpha-aminoacylpeptide hydrolase)	Insignificant change	
TRINITY_DN7927_c0_g1_i5	Glutathione S-transferase (EC 2.5.1.18) (25 kDa auxin-binding protein) (GST class-phi)		-1.576
TRINITY_DN22883_c0_g1_i1	Glutathione S-transferase Z1 (AtGSTZ1) (EC 2.5.1.18) (GST class-zeta member 1) (Glutathione S-transferase 18) (Maleylacetone isomerase) (MAI) (EC 5.2.1.-)		1.856

Conclusion

Arbuscular mycorrhizal fungi have important role in the agriculture sector. The fungi associate with the roots of plants to form vesicles, arbuscules, and hyphae in roots, and also spores and hyphae in the rhizosphere. The fungi is known to confer a lot of advantage to the host plant in return of shelter including the ability to tolerate stress. The egg plant when treated with two different species of Glomus. viz G. mossae and G. macrocarpum, the two AMF species were found to differentially contribute to the stress tolerance to Solanum melanogena. The findings suggest more studies under salinity stress to gain more insight into the process.

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