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Research Article

Deciphering the network of interconnected pathways of *Chaetomium globosum* antagonistic related genes against *Bipolaris sorokiniana* using RNA seq approach

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ABSTRACT: *Chaetomium* species are known as potential biocontrol agents against phytopathogens due to their multiple antagonistic mechanisms. Plant disease is controlled by *Chaetomium* exhibit complex interactions against plant pathogen under varied conditions. Previously, mycoparasitism and antibiosis have been reported as most effective mechanism exhibited by the *C. globosum* against *Bipolaris sorokiniana.* In the present study, the examination of major biosynthetic pathways underlying *Chaetomium globosum* biocontrol activity was elucidated. It was shown that the pathways related to biosynthesis of secondary metabolites, hydrolytic enzymes and other key regulator genes were involved in production of hydrolytic enzymes and antifungal metabolites. We identified various genes of biological function with significant log2 fold change such as phosphoribosyl aminoimidazole carboxylase (9.693), protease (8.18), cyanate hydratase (Cyanase) (6.7), Fe2OG dioxygenase domain-containing protein (5.9), superoxide dismutase (5.55), glycosidase (5.34), carboxylic ester hydrolase (5.27), alpha-1,2-Mannosidase (4.44), alpha-1,4 glucan phosphorylase (3.99), endochitinase (3.87), P53-like transcription factor (Fragment) (3.55), metalloprotease (3.4), polyketide synthase (3.35), Catalase-peroxidase (CP) (3.14), protein kinase domain-containing protein (3.18) and glutamate decarboxylase (2.1) which are involved in biosynthesis of secondary metabolites, polyketide synthase, antibiotic, hydrolytic enzymes and putative fungistatic metabolites. This data provides a good foundation for continued researches into *C. globosum* Cg2 biocontrol activity for facilitating widespread application under the field conditions.

KEY WORDS: Bio-control, *Bipolaris sorokiniana, Chaetomium globosum,* CNV, interconnected pathways, secondary metabolites

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INTRODUCTION

In recent years, biocontrol strategy has received tremendous attention in controlling plant diseases due to hazardous effects of pesticides and agrochemicals on ecosystem. There are about 35 genera of fungal and bacterial species, which have been used as biocontrol agents against various plant pathogens. Plant pathogens cause significant losses to agricultural products. Traditional chemical control methods are not absolutely efficient to minimize these losses. Biological control of plant pathogens can be highly effective especially with potentials of antagonists on pathogenic fungi. *Chaetomium* is one of the extremely familiar genus of Pyrenomycetes (Ascomycotina) encountered on various agricultural commodities and various species of the genus *Chaetomium* are used as biocontrol agents against several plant pathogenic fungi like *Bipolaris oryzae, Rhizoctonia solani, Fusarium fujikuroi, Fusarium graminearum*, *Bipolaris sorokiniana, Tilletia indica, Alternaria triticina* and *Alternaria alternata*. The genus contains more than 200 species all characterised by dark coloured perithecia with short neck, which are clothed with irregularly coiled or tightly spiralled hairs or with stiff, simple or branched setae (Doveri, 2013; Wang *et al.*, 2016). It is common colonizer of soil and cellulose containing substrates.

Among various species, *Chaetomium globosum* is a ubiquitous filamentous fungus having biological control properties and reported effective in reducing damage caused by seed rot and damping off, of several seed borne and soil borne plant pathogens like *Pythium ultimum*, *Alternaria raphani, A. brassicicola, Fusarium* spp. and *B. sorokiniana* (Harman *et al.*, 1978; Vannacci and Harman, 1987; Aggarwal *et al.*, 2004). Antagonistic mechanisms of *C. globosum* fungus are exerted through different studies. *Chaetomium globosum* Cg2 strain presented the highest antagonistic activity against *Bipolaris sorokiniana* with the inhibition of 75.54%. *Chaetomium globosum* mycoparasitizes and produces antifungal metabolites that helps in lysis of cell wall of the pathogen. Among them, competition for space

and nutrients (Vannaci and Harman, 1987) as well as mycoparasitism (Mandal *et al.*, 1999) are well established. At the microscopic stage, the pathogen (*Bipolaris sorokiniana*) mycelium shows malformed and deformed conidia with distorted walls, lysis and formation of holes in the conidial wall, which significantly inhibited germination of conidia and hyphal elongation (Mandal *et al.*, 1999; Biswas *et al.*, 2000; Moya *et al.*, 2016; Darshan *et al.,* 2020). Transcriptomic studies of *C. globosum* against *B. sorokiniana* reported the significant expression of genes encoding secreted proteases such as aminopeptidase (GO:0004177), metallopeptidase (GO:0008237), aspartic-type endopeptidase (GO:0004190) and serine-type carboxypeptidase (GO:0004185) which may involve in the degradation of fungal cell walls and plasma membrane proteins (Darshan *et al.*, 2020). Further, antibiosis was also reported as a most prevalent antagonistic mechanism of *C. globosum* against *B. sorokiniana* (Aggarwal *et al.,* 2004). Aggarwal *et al.* (2013) have characterized the antifungal metabolites of *C. globosum* and identified antifungal compounds like chaetoglobosin A and cochliodinol.

High-throughput sequencing is a powerful tool for identifying genes or genetic variants associated with phenotypes, including components of *C. globosum* antagonisms. Signalling networks regulate biology of cells and organisms in normal and diseased states. Transcriptomics-based networking pathways search will fundamentally change our understanding of signalling networks. Until now, only very little information is available on these interconnected pathways during Cg2-BS112 interaction. The expression of genes associated with biocontrol appears to be regulated by intracellular signal transduction pathways, which are activated by the binding of host-derived ligands to as yet unidentified receptors. The elucidation of these pathways has recently begun and has confirmed the involvement of highly conserved signalling components. Therefore, present study aims to identify the interconnected pathways of *Chaetomium globosum* antagonistic related genes against *Bipolaris sorokiniana* using RNA seq approach.

MATERIALS AND METHODS

Maintenance of biocontrol agents and plant pathogens

The *C. globosum* strain Cg2 isolated earlier from wheat leaf surface (Mandal *et al.*, 1999) available in fungal molecular biology lab, Division of Plant Pathology, Indian Agricultural Research Institute (IARI), New Delhi was used for the present investigation.

The pathogens *Bipolaris oryzae, Rhizoctonia solani*, *Fusarium fujikuroi, Fusarium graminearum*, *Bipolaris sorokiniana, Tilletia indica, Alternaria triticina* and *Alternaria alternata* previously characterized as a highly virulent isolates of the pathogens were used as target in our experiments. The strains were grown on Potato Dextrose Agar (PDA) at 25 ± 2°C. The pure cultures of the fungi were obtained by single spore isolation and the cultures were maintained on PDA slants at 25 ± 2°C with periodic sub culturing for the further studies.

Dual culture assay of *C. globosum* against rice and wheat fungal pathogens

The antimicrobial capacity of the selected strain was evaluated by dual solid culture assay in Petri plates (Dal Bello *et al.,* 1994). The mycelial disc of *C. globosum* (9 mm diameter) was placed at one side of Petri dish (1 cm from the edge of the plate) containing 10 ml PDA medium. Subsequently, mycelial disc (9 mm diameter) of seven days old culture of different above-mentioned pathogens mycelial plug was placed 1 cm away from the margin of the opposite side of the same plate. Three replicates of each pathogen-antagonist interaction were done. The control plates were also maintained without an antagonist. The inoculated plates were sealed with kiln film and kept for incubation at room temperature (25 ± 2^0C) till control plate fully covered the plate (~9-11days). Subsequently, the radial mycelial growth of the pathogen and per cent reduction over control was calculated by using the formula following:

$$\text{Percent inhibition over control} = \frac{C - T}{C} \times 100$$

Where,
C- Mycelial growth of pathogen in control
T- Mycelial growth of pathogen in dual plate

RNA isolation and illumina sequencing

The total RNA was extracted from frozen mycelial samples drawn from the interaction zone of *C. globosum* against *B. sorokiniana* using Qiagen RNeasy Plant Mini Kit. The procedure for isolation of RNA and procedures for Illumina Sequencing was described in our previous study (Darshan *et al.,* 2020). The work flow of transcriptome sequencing by Illumina platform is presented in Figure 1. The cDNA library sequencing was performed on the Illumina high-throughput sequencing platform (HiSeqTM2500) to obtain 2 × 150 bp Pair-end (PE) reads. The raw Illumina sequence reads related to *C. globosum* (Cg2 Control), *B. sorokiniana* (BS112 control) and their interactions (Cg2-BS112) with two replicates were deposited at Sequence Read Archive (NCBI/SRA) under the accession numbers, SRR11305503; SRR11305502; SRR113055501; SRR113055500; SRR11305499; SRR11305498. The transcriptome data are deposited in BioProject in GenBank via Bioproject number PRJNA612183 accessible at: https://www.ncbi.nlm.nih.gov/bioproject/ PRJNA612183/(Darshan *et al.,* 2020).

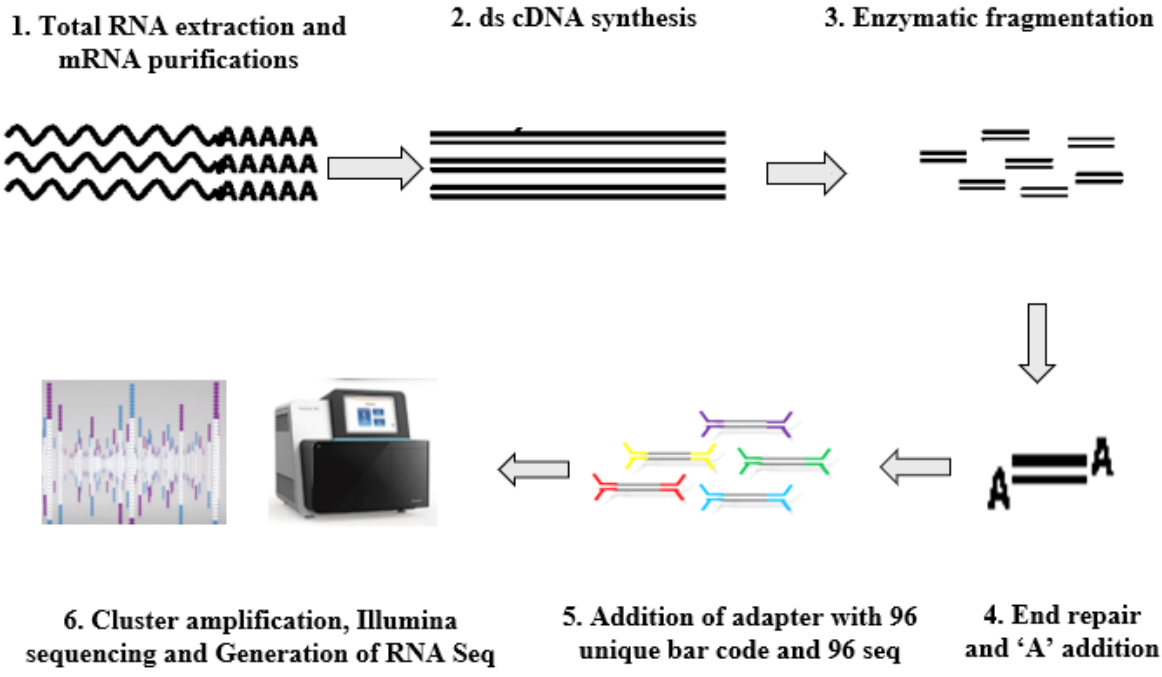

Figure 1. The work flow of transcriptome sequencing by Illumina platform

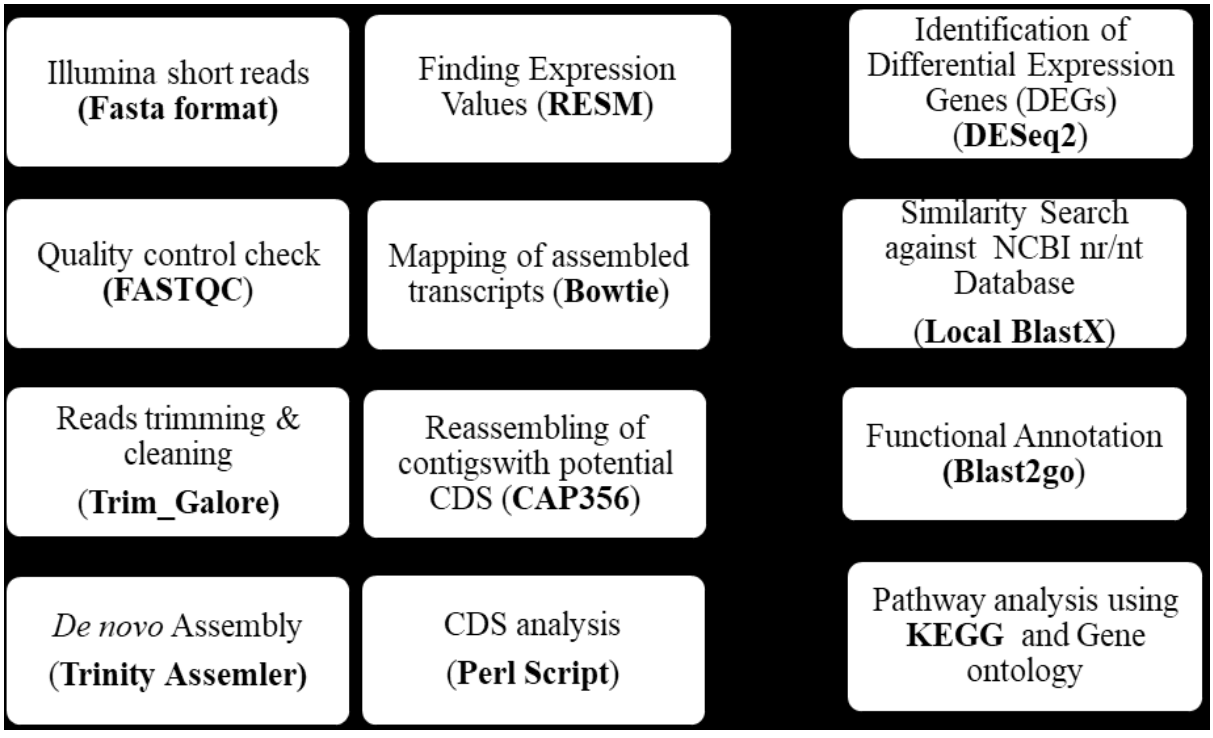

Figure 2. Flow diagram for bioinformatic analysis of transcriptomic sequencing data of *C. globosum*

Bioinformatic analysis

After visualization and checking the reads in Fast QC, the contaminants were removed using Trimmomatic tool version 0.30. The high quality filtered paired reads obtained from Cg2 and BS112 samples were used as an input for assembly of the transcriptome. *De novo* assembly of all Cg2 samples and BS112 samples total reads was performed by using trinity assembler V2.4.0. The individual assembled sequences were clustered using CD-HIT (version 4.6) to remove redundancy at 90% sequence similarity. Unigenes were extracted using the inbuilt trinity perl script. The final assembled transcripts were used for further downstream analysis. The work flow for

whole analysis of RNA seq data for identification of DEGs with its functional annotation was depicted in Figure 2.

pathways analysis

The functional annotation of individual and combined unigenes of samples was performed by aligning those unigenes to NR (non-redundant) protein database (version nr.36) of NCBI using BLASTX V 2.2.31. Further, the predicted proteins were subjected to pathway analysis using KEGG (Kyoto Encyclopaedia of Genes and Genomes) (Ogata *et al.*, 1999) database to map the proteins involved in biochemical pathways. The predicted proteins were categorized into three different functional groups such as Biological Process (BP), Molecular Function (MF) and Cellular Component (CC) with default parameters. Finally, the significant gene ontology terms having a False Discovery Rate (FDR) value ≤0.05 and P-value less than 0.01 (P<0.01) was used as the threshold to evaluate significant enrichment (Maere *et al.*, 2005). Antibiotic biosynthesis, enzymes and signaling genes were filtered from significant differential genes (log2FC and pval0.01). Copy Number Variation (CNV) analysis was done using CNVKit ToolKit to infer and visualize copy number from high-throughput sequencing data (cnvkit-import rna) (https://cnvkit.readthedocs.io/en/stable/rna.html). Scatter, and genematrics were used to generate plots. The significant enriched biological components were used for network construction and its biological relationship of genes with associated pathways was deciphered by Cytoscape R packages (version: 3.7.2) (Kohl *et al.,* 2011). The statistical charts, gene ontology graphs, pie and bar charts were generated by using Blast2GO to monitor, visualize and evaluate the biological function of the specific gene in *C. globosum* at a macro level. *MapMan was used for* visualization and profiling of data sets. To decipher protein -protein association network and its functional interaction, Protein-Protein Interaction Networks Functional Enrichment Analysis was done by using STRING v11 (STRING, http://www.string-db.org/) to achieve a comprehensive and global interacting gene network, including functional interactions (Szklarczyk *et al.,* 2019).

RESULTS AND DISCUSSION

In vitro efficacy of *C. globosum* (Cg2) against different pathogens of wheat and rice

Inhibition percentage of various seed and soil borne pathogens of wheat and rice crops in presence of *C. globosum* isolate Cg2 was performed by dual culture techniques (Dennis and Webster, 1971). Among the tested pathogens, *C. globosum* isolate Cg2 showed maximum inhibitions of 75.54 % against *B. sorokiniana* followed by *T. inidca* which showed 71.01% mycelial inhibition**.** It was also able to inhibit growth of other pathogens like *A. alternata* (67.20%), *B. oryzae* (66.66%), *A. triticina* (65.54%) *F. fujikuroi* (65.54%), *R. solani* (63.03%) and *F. graminearum* (57.72%) (Table 1, Figure 3). Inhibition percentage of the test pathogens in the presence of *C. globosum* (Cg2) by dual culture technique is shown in Figure 3. Many basic research papers have been published related to the antagonistic activity of *C. globosum* (Biswas *et al.*, 2000; Soytong *et al.*, 2001 and Aggarwal *et al.*, 2004; Aggarwal, 2015). Antibiosis was the most prevalent mechanism of action of *C. globosum* against *B. sorokiniana* (Aggarwal *et al.* 2004). *Chaetomium* spp. produces a variety of bioactive metabolites which suppress the growth of many soil and seed-borne phytopathogens. Some of these positive effects have been related to the microbial release of bioactive metabolites and elicitor proteins in the plant rhizosphere (Harman *et al.*, 2004). Present study revealed that under *in vitro* dual tests, *C. globosum* (Cg2) significantly inhibited the growth of the *B. sorokiniana* and other pathogen by producing clear inhibition zone when compared with the control. Twelve days after incubation, the percentage inhibition over control was 71.4% for *B. sorokiniana* (Figure 4).

CNV analysis

Transcriptome analysis showed significant differential gene expression in *C. globosum* during challenge with *B. sorokiniana.* A total of 14366 (log2FC and pval0.01) Differentially Expressed Genes (DEGs) were detected. The top significant encoding gene with log 2-fold changes such as phosphoribosyl aminoimidazole carboxylase (9.693), protease (8.18), ABC transporter-like protein (7.8), cyanate hydratase (Cyanase) (6.7) and Fe2OG dioxygenase domain-containing protein (5.9) were overrepresented in Cg2-BS112 interaction. In addition to that some other genes like superoxide dismutase (5.55), glycosidase (5.34), carboxylic ester hydrolase (5.27), mannitol-1-phosphate 5-dehydrogenase (M1PDH) (4.92), C2H2-type domain-containing protein (4.46), alpha-1,2-Mannosidase (4.44), alpha-1,4 glucan

Table 1. Inhibition percentage of seed and soil borne fungal pathogens of wheat and rice crops by potential isolate *C. globosum* (Cg2)

SL No	Pathogen	*C. globosum* (Cg2)	
		Inhibition over control (cm)	% inhibition over control (%)
1	*Bipolaris sorokiniana*	6.82	75.54
2	*Bipolaris oryzae*	6.05	66.66
3	*Fusarium fujikuroi*	5.92	65.54
4	*Rhizoctonia solani*	5.73	63.03
5	*Alternaria triticina*	5.95	65.62
6	*Alternaria alternata*	6.01	67.20
7	*Fusarium graminearum*	5.52	57.72
8	*Tilletia indica*	6.4	71.01

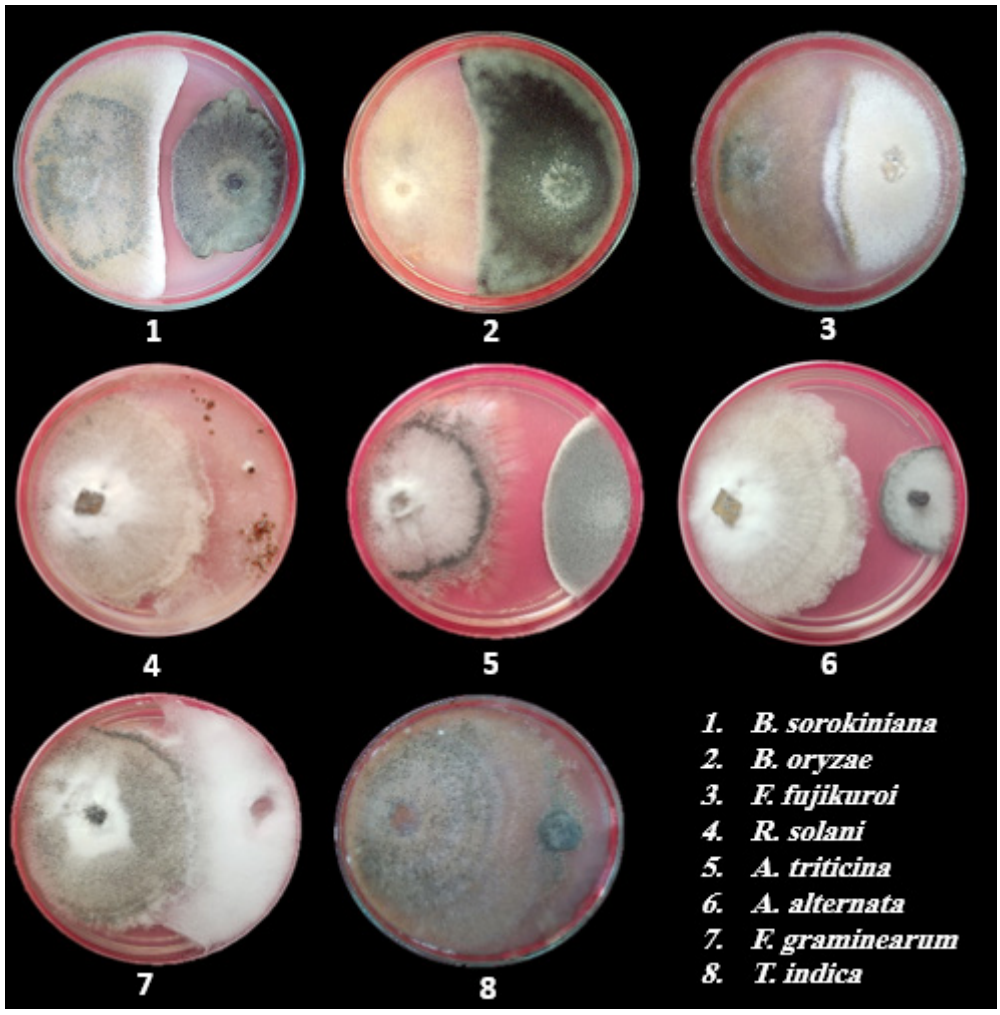

Figure 3. Dual culture plate showing antagonistic activity of *Chaetomium globosum* (Cg2) against seed and soil borne fungal pathogens of wheat and rice

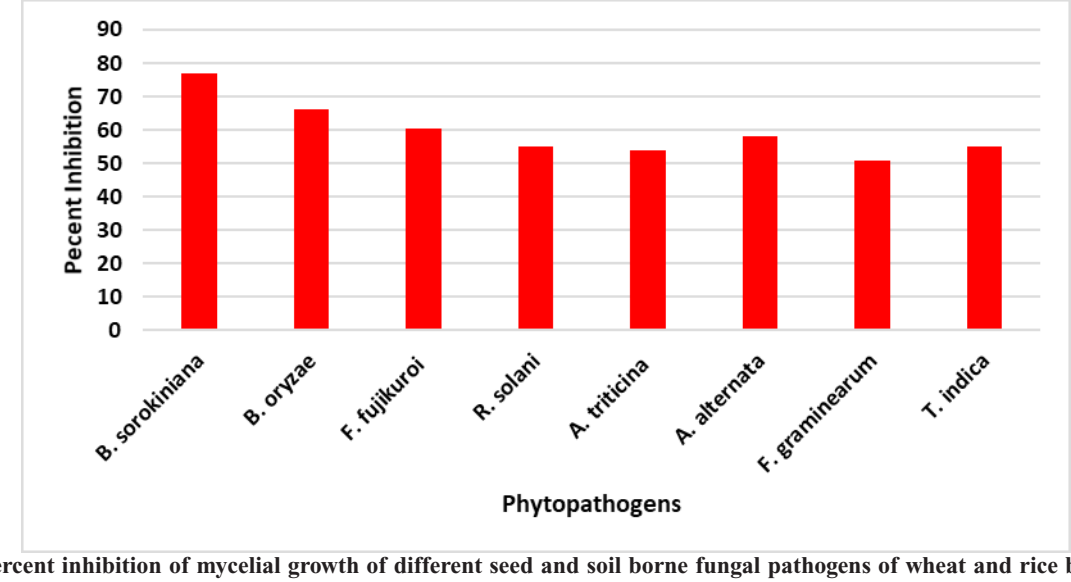

Figure 4. Percent inhibition of mycelial growth of different seed and soil borne fungal pathogens of wheat and rice by *C. globosum* (Cg2)

phosphorylase (3.99), endo-chitinase (3.87), MFS domain-containing protein (3.85), P53-like transcription factor (Fragment) (3.55), metalloprotease (3.4), polyketide synthase (3.35), catalase-peroxidase (CP)/ peroxidase/catalase) (3.14), protein kinase domain-containing protein (3.18) and glutamate decarboxylase (2.1) were expressed in Cg2-BS112 interaction when compared with Cg2 control (Table 2).

The selected potential antagonistic gene previously validated was subjected CNV analysis. It clearly depicts that the genes encoding MFS domain-containing protein (TRINITY_DN7412_c1_g1_i6), Phosphoribosyl aminoimidazole carboxylase Protein (TRINITY_DN7744_c3_g7_i2), Protein Kinase domain-containing protein (TRINITY_DN7566_c2_g2_i12), Glycosidase (TRINITY_DN7787_c5_g3_i1), Superoxide dismutase (TRINITY_DN7807_c0_g1_i1) and Mannitol-1-phosphate 5-dehydrogenase (TRINITY_DN7971_c2_g2_i1) were expressed significantly in Cg2-BS112 interaction when compared with Cg2 control. It indicated the positive correlation of differential transcriptional level and CNV in general. But the copy ratio of above-mentioned genes was significantly lower which is clearly shown in Figure 5. But in Cg2 control, the expression of the above-mentioned genes was significantly lower and it signifies that, decrease in the copy number of potential genes in control. Remarkably, for substantial number of genes, their copy numbers exert a positive correlation with the corresponding differential gene expression. Interestingly, gene-wise copy number amplification appears correlated with enhanced gene expression in Cg2-BS112 interaction when compared with Cg2 control, while copy number deletion usually led to decreased gene expression of selected potential genes in Cg2 control. Similar results were reported by Lind *et al.* (2017) where they studied extensive genetic variation in *Aspergillus fumigatus* secondary metabolic clusters and suggested that gene CNV in these regions could contribute to individual variation in secondary metabolite production.

Table 2. Categorization of top significant encoding genes with log 2-fold changes in Cg2-BS112 interaction against Cg2 control

Chromosome	Gene	Start	End	Cg2-BS112 Interaction		Cg2 Control	
				Depth	Log2	Depth	Log2
TRINITY_DN7744_c3_g7_i2	Phosphoribosyl aminoimidazole carboxylase	1	264	47395.4	9.69331	44.0682	1.65683
TRINITY_DN7674_c8_g3_i2	Protease	1863	2129	18339.4	8.18583	0	-23.8048
TRINITY_DN8092_c3_g3_i5	ABC transporter-like protein	5528	5792	38434	7.80027	0	-23.8048
TRINITY_DN7750_c11_g3_i1	Cyanate hydratase (Cyanase)	2140	2407	23115.6	6.73794	0	-23.8048
TRINITY_DN7464_c5_g1_i4	Fe2OG dioxygenase domain-containing protein	1339	1606	39883.1	5.98568	0	-23.8048
TRINITY_DN7807_c0_g1_i1	Superoxide dismutase	291	581	14255.4	5.55786	16.0224	0.53709
TRINITY_DN7787_c5_g3_i1	Glycosidase	1	270	7355.22	5.34402	0	-23.8048
TRINITY_DN7946_c4_g3_i1	Carboxylic ester hydrolase	5519	5782	6672.71	5.27128	0	-23.8048
TRINITY_DN7971_c2_g2_i1	Mannitol-1-phosphate 5-dehydrogenase (M1PDH)	1	271	3690.14	4.92671	0.237037	-5.54174
TRINITY_DN7776_c2_g8_i3	C2H2-type domain-containing protein	805	1073	2849.54	4.46275	0	-23.8048
TRINITY_DN7658_c6_g5_i2	Alpha-1,2-Mannosidase	265	530	8975.29	4.45277	0	-23.4649
TRINITY_DN7581_c4_g1_i2	Alpha-1,4 glucan phosphorylase	1074	1342	4611.26	3.99241	0	-23.4649
TRINITY_DN7969_c3_g1_i1	End chitinase	535	802	9238.81	3.87906	0.543071	-4.34571
TRINITY_DN7412_c1_g1_i6	MFS domain-containing protein	1775	2029	3848.41	3.85034	0	-23.4649
TRINITY_DN7704_c1_g5_i10	P53-like transcription factor (Fragment)	4983	5245	3882.04	3.55838	0	-23.4649
TRINITY_DN7747_c4_g1_i2	Metalloprotease	1312	1574	2675.15	3.40872	0	-23.8048
TRINITY_DN7358_c2_g1_i2	Polyketide synthase	1054	1317	2251.39	3.35696	0	-23.8048
TRINITY_DN7146_c0_g3_i2	Catalase-peroxidase (CP)/ Peroxidase/catalase)	8730	8995	3586.55	3.14476	0	-23.4649
TRINITY_DN7566_c2_g2_i12	Protein kinase domain-containing protein	1062	1327	1441.74	3.18238	65.9472	2.57831
TRINITY_DN7551_c2_g4_i5	Glutamate decarboxylase	1598	1864	2403.03	2.10551	0	-23.8048

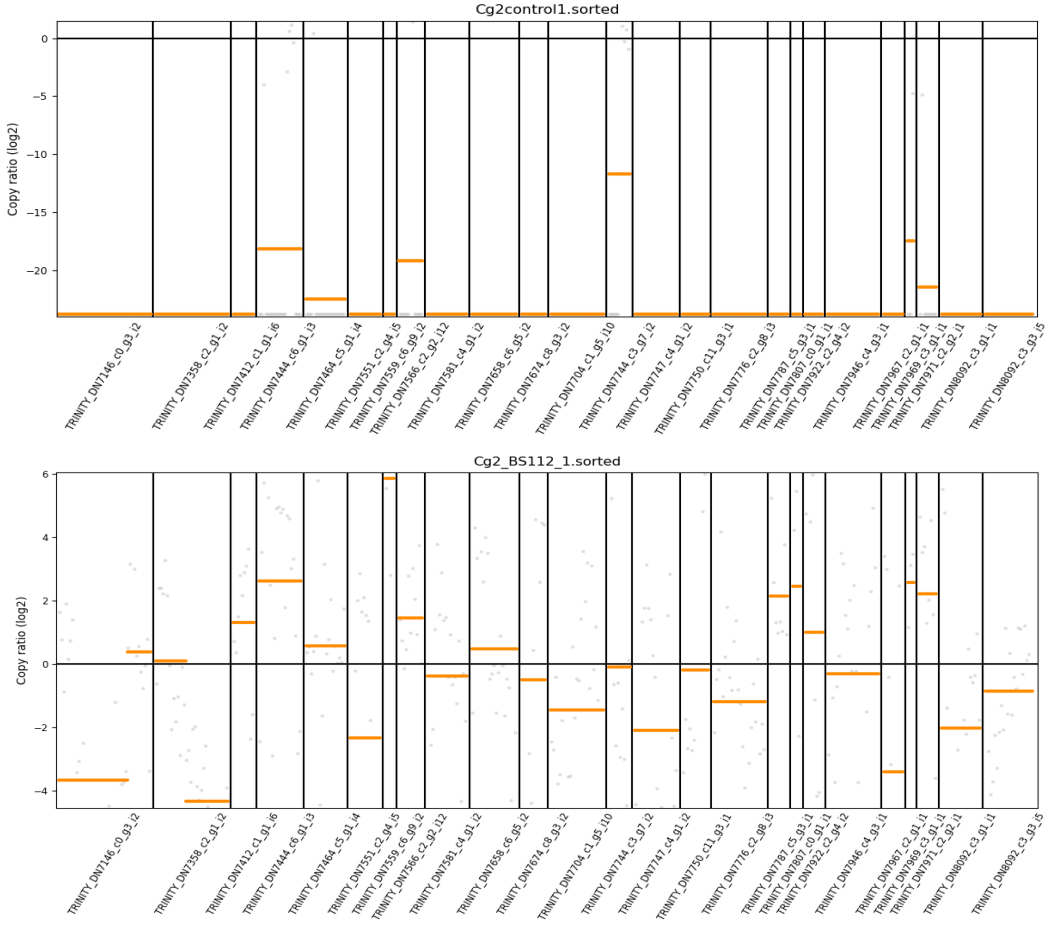

Figure 5. Copy ratio comparisons of selected potential antagonistic related genes in Cg2-BS112 interaction and Cg2 control

Exporing the network of interconnected pathways in *C. globosum*

The genes involved in cell wall degradation viz. glycoside hydrolase family consisting of GH1, GH3, GH5, GH 13, GH16, GH17, GH18, GH31, GH32, GH76, GH 78, GH 92, and GH family protein were expressed and interconnected in Cg2-BS112 interaction (Figure 6), which suggested a central role for them in *C. globosum* antagonisms, probably during host fungal cell wall degradation and mycelial inhibition. In addition, glucanase, glutamate synthase were also expressed. But in Cg2 control conditions, only glycoside hydrolase family GH 5 was associated (Figure 6). The combination of Glycoside Hydrolase family (GHs) seems to be preferentially associated with mycoparasitism-related conditions in *T. harzianum* (Monteiro *et al.*, 2010; Vieira *et al.*, 2013). Several fungi produce a variety of Carbohydrate Activity enzymes (CAZymes) for the degradation of plant polysaccharide materials to facilitate infection and/or gain nutrition. Tiwari *et al.* (2013) explored the role of βGlucosidases from the fungus *Trichoderma* as an efficient cellulase machinery for cell wall degradation of several plant pathogens. Grabowska *et al.*, (2012) reported the essential role of glutamate synthase in the metabolism of nitrogen by catalysing the condensation of glutamate and ammonia to form glutamine which acts as a source of nitrogen for its growth and development. Walsh *et al.,* (2000) reported that, the cyanate generated ammonium can serve as a source of nitrogen for growth of *C. globosum*.

Similarly, antibiosis was the most prevalent antagonistic mechanism of *C. globosum* against *B. sorokiniana* in agreement with studies by Aggarwal *et al.* (2004). In present analysis, triterpenoid (terpene cyclase, terpene synthase activity [GO:0010333]), alkaloids (aminotran_1_2 domain-containing protein), phospholipase, transketolase, amino acid transferase, phosphotransferase, S-hydroxymethyl glutathione dehydrogenase, amidophosphoribosyltransferase, isocitrate lyase and aspartate amino transferase (biosynthesis of antibiotics), phosphotransferase, succinate dehydrogenase, acetyl co-enzyme A synthase, thiolase family, catalase, serine hydroxy methyl transferase, malate dehydrogenase and methoxy-polyprenyl benzoquinol methylase (biosynthesis of secondary metabolites; ubiquinone and other terpenoid-quinone biosynthesis) were expressed significantly in *C. globosum* Cg2 when exposed to *B. sorokiniana* (Figure

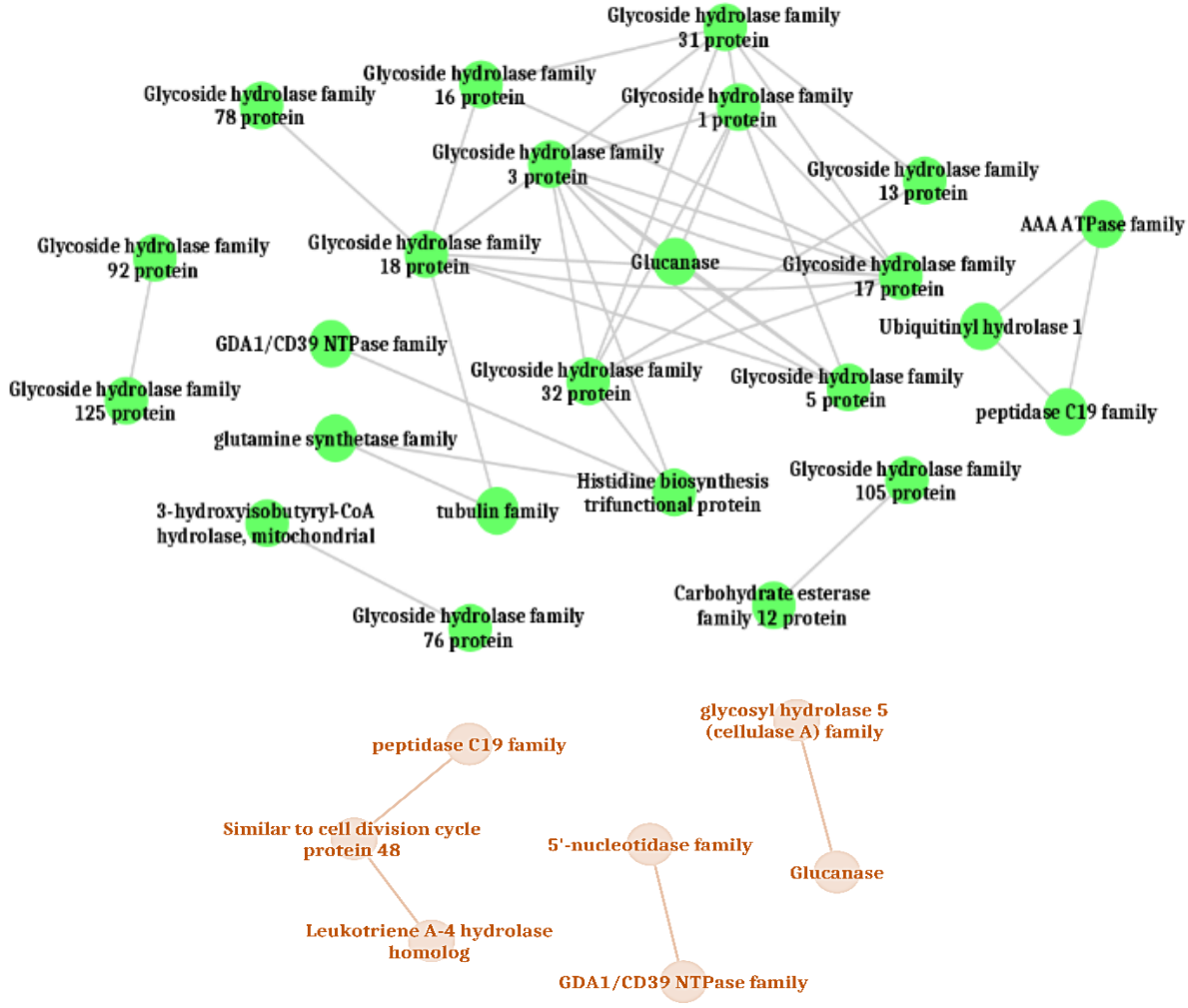

Figure 6. Hydrolase interconnected pathways involved in antagonism of *Chaetomium globosum* (Cg2) upon challenge with *B. sorokiniana* (BS112). A) Cg2-BS112 interaction B) Cg2 control

7). Similar investigation was reported earlier by Shentu *et al.* (2014) who evaluated the role of terpenoid backbone biosynthesis in *Trichoderma brevicompactum* biocontrol mechanism.

A large number of genes like TRAM domain-containing protein (CHGG_10433), RRM domain-containing protein (CHGG_10382), Utp12 domain-containing protein (CHGG_10621), Protein SDA1(CHGG_10486), CTP synthase (CHGG_11054), Chorismate synthase (CHGG_10505), Threonine dehydratase (CHGG_10834), Epimerase domain-containing protein (CHGG_10579), Pyruvate carboxyltransferase domain-containing protein (CHGG_1071), Epimerase domain-containing protein (CHGG_10579), Poly [ADP-ribose] polymerase (PARP) (CHGG_10788), RPOLD domain-containing protein (CHGG_10417), FHA domain-containing protein (CHGG_10861), Protein kinase domain-containing protein (CHGG_10629), RING-type domain-containing protein (CHGG_10844), RBR-type E3 ubiquitin transferase (CHGG_10570), E3 ubiquitin-protein ligase PEP5 (CHGG_10507), Tr-type G domain-containing protein (XP_007701615.1), MPN domain-containing protein (XP_007699947.1), Nop domain-containing protein (XP_007695297.1), J domain-containing protein (XP_007703546.1), zf-LYAR domain-containing protein (XP_007702390.1), Obg-like ATPase 1 (XP_007702860.1) were interconnected with each other in interaction (Fig. 8). But the expression of these genes in control is lower. A similar study has been conducted by Kosanovic *et al.* (2013) in the interaction of *Pseudomonas putida* and *Pseudomonas tolaasii* with *Trichoderma aggressivum.* Further studies for functional characterization of candidate genes mentioned here are necessary in order to better define the exact pathways involved in mycoparasitism and antibiosis in *C. globosum*.

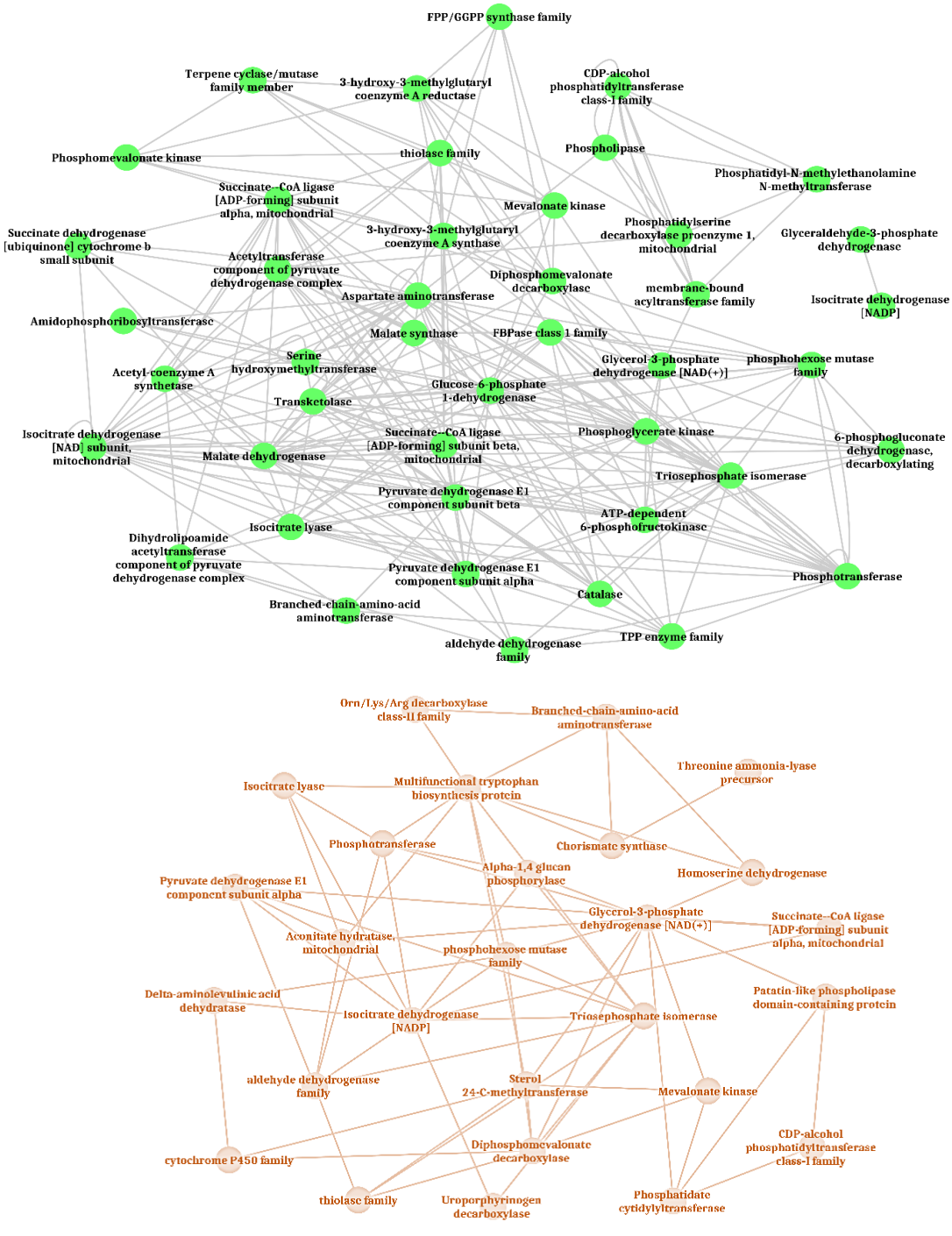

Figure 7. Secondary metabolite interconnected pathways involved in antibiosis of *C. globosum* (Cg2) upon challenge with *B. sorokiniana* (BS112). A) Cg2-BS112 interaction B) Cg2 control

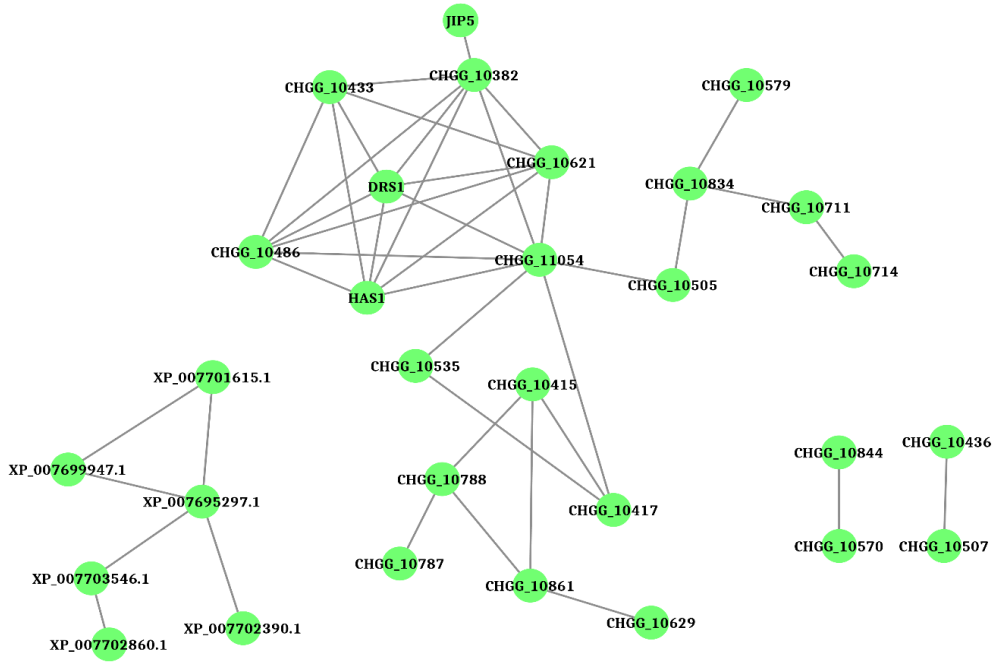

Figure 8. STRING analysis of top differentially expressed proteins present in *C. globosum* (Cg2) upon challenge with *B. sorokiniana* (BS112)

CONCLUSION

This study demonstrated the efficacy of *Chaetomium globosum* in controlling several plant pathogens. Interconnected pathways related to biosynthesis of secondary metabolites, hydrolytic enzymes and other key regulator genes were involved in the production of hydrolytic enzymes and antifungal metabolites in *C. globosum*. The extent of inhibition by *C. globosum* provides its wide usage in the management of plant pathogens in sustainable agriculture. The obtained data will greatly enrich the current *C. globosum* genetic information and provide a good foundation for better understanding of the molecular mechanism of *C. globosum* against plant pathogens.

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