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Transcriptomic Analysis of Pathogenicity Genes in *Sclerotinia sclerotiorum* Affecting *Brassica napus*

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ABSTRACT

Sclerotinia stem rot (SSR) caused by *Sclerotinia sclerotiorum* is one of the most destructive diseases of rapeseed (*Brassica napus* L.) globally. This study identifies key genes involved in the pathogenicity of *S. sclerotiorum* during rapeseed infection. It examines these genes' codon usage bias (CUB) to find the optimal codons effective in gene expression and the factors effective in the formation of CUB. Protein-protein interaction network was drawn and several hub genes were determined and ranked according to the CytoHubba tool among which the genes encoding nitrate reductase, beta-glucosidase, glucanase, PA14 domain-containing protein, carbohydrate-binding module family 1, and acyl-coenzyme A oxidase were found to be associated with the SSR induction. Gene ontology (GO) analysis showed that the genes related to the metabolism of organic substances, catalytic activity, and cellular anatomical entity had the highest count. Also, KEGG pathways analysis revealed 16 biological pathways modulated by *S. sclerotiorum* among which the genes associated with the metabolic pathways exhibited the highest count. CUB indices including CAI (codon adaptation index), ENC (effective number of codons), GC, GC3S (GC content in the third open position of the codon), and RSCU (relative synonymous codon usage) were determined and the results showed a significant positive correlation between GC and GC3S. Also, the possible effects of mutation pressure and natural selection in shaping CUB were determined. The mean ENC range from 42.4-56.65 indicating less orientation in codon usage. The range CAI was found to be 0.74-0.89 indicating the importance of genes in adapting to environmental stresses. The maximum RSCU was 1.76 for the CCA codon, which encodes the amino acid (aa) proline with a high preference for that codon compared to other synonymous codons of that aa. These results demonstrated that *S. sclerotiorum* modulates some genes involved in disease induction (*e.g.*, cell wall-degrading enzymes, biosynthesis of secondary metabolites & metabolic pathways) to infect its host plant.

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Introduction

Rapeseed (*Brassica napus* L.) is the third oilseed crop responsible for 13% of the world's vegetable oil production (Tanwar and Goyal, 2021). The nutritional value of the crop and its adaptability to various climates have increased its cultivation in Iran (Colagar *et al*., 2010; Zomorodian *et al*., 2011). However, this plant species is annually invaded by a variety of pathogens, significantly reducing crop yields. *Sclerotinia sclerotiorum* (Lib.) de Bary is a highly destructive pathogen of rapeseed that causes *Sclerotinia* stem rot (SSR), adversely

affecting both yield and oil quality (Saharan and Mehta, 2008). The fungus is classified in Sclerotiniaceae (Ascomycota: Helotiales) (Wong & Willetts, 1975). *S. sclerotiorum* can cause disease in more than 400 plant species such as soybean and sunflower. Symptoms of SSR include white and gray lesions on the main stem, branches, or pods; hard black sclerotia within infected stems; early flowering; and wilting at apical organs (Khangura *et al*., 2015). Sclerotia are produced in the final stages of the infection cycle and can survive in the soil for several years (Khangura *et al*., 2015), thereby reducing the effectiveness of crop rotation as a control method (Bardin & Huang, 2001). Moreover, controlling the disease through agricultural practices is challenging due to the pathogen's wide host range (Wu *et al*., 2016). Cultivation of disease-resistant rapeseed varieties is the most efficient and cost-effective way to prevent and control SSR (Bastien *et al*., 2014). However, the lack of resistant germplasm in cultivated rapeseed has limited molecular breeding efforts (Wu *et al*., 2016) and close relatives have limited its molecular breeding. The wide host range of the fungus has led to a heavy reliance on fungicides for disease management. However, due to the side effects of these chemical substances on the environment, biological control has been proposed as an alternative method for crop protection (Duke *et al*., 2017). In this way, non-pathogenic microorganisms enhance plant defense mechanisms by inducing systemic resistance, and by "inducing defense" they lead to stronger and faster plant responses to subsequent pathogen attacks (Pieterse *et al*., 2014).

Nowadays, due to the advancement of next-generation sequencing technology, transcriptomics methods are used to undertake the molecular mechanisms of plant-pathogen interactions (Xu *et al*., 2021a). For the successful implementation of biological control agents in the induction of systemic resistance against *S. sclerotiorum*, comprehensive data on the interactions of biological control systems, including their effect on host plants, as well as the understanding of the molecular mechanisms of *B. napus* and *S. sclerotiorum* interactions is necessary to develop new sources of disease-resistant rapeseed. Systems biology approach by integrating omics data, such as transcription, proteome, and metabolome data, can provide a powerful tool to better understand how plants react by predicting cell functions and modeling plant responses to various stressors (Pazhamala *et al*., 2021). Although there are several studies on the expression of *S. sclerotiorum* genes at different post-inoculation times in *B. napus*, a study on the identification of hub genes and their clustering into functional groups and their CUB pattern is not available.

Materials and Methods

Data collection

Initially, genes exhibiting differential expression during *S. sclerotiorum* infection were selected based on previous transcriptome profiling studies, the genes that showed differential expression in the plant and the fungus during *S. sclerotiorum* infection were selected. The details of the collected data are presented in Table 1. The data were subsequently analyzed according to the flowchart presented in Fig. 1.

Interaction networks and hub genes

To evaluate the interaction between the selected genes, an analysis was conducted using the web-based STRING program at http://string-db.org with a minimum required interaction score of 0.15 (low confidence), resulting in a list of protein-protein interactions (PPIs). The PPI list was then imported into Cytoscape (version 3.9.1). CytoHubba software (version 0.1) is a plugin to Cytoscape that is applied to determine hub genes from a network constructed by Cytoscape to determine hub proteins between all nodes. Four computational algorithms from CytoHubba, including MCC, DEGREE, DMNC, and MNC (Chin *et al*., 2014), were applied to determined and rank hub genes, and four proteins were selected as hub proteins for each algorithm, and finally hub genes and their interactions were shown in a subnet.

Measurement of codon usage bias

To assess codon usage bias (CUB) indices, we first obtained the coding sequence of hub genes effective during SSR of rapeseed was obtained from the BioMart section of the Ensemblefungi website (https://fungi.ensembl.org/) in FASTA format. The software R (ver. 4.4.1) and RStudio

(ver. 2024.04.2) with CoRdon, Stringi, and Cubar packages, were applied. Then the values of CAI (codon adaptation index), ENC (effective number of codons), GC, GC3S (GC content in the third open position of the codon), and RSCU (relative synonymous codon usage) indices for the coding sequences were calculated and evaluated in the RStudio. To evaluate the factors influencing the formation of CUB, the correlation among CUB indices, regression equation, significance level, and graph drawing were also implemented in the RStudio.

Table 1. The details of the gene expression data on *S. sclerotiorum* used in the analysis.

Accession No.	Selected Genes/ Experiment Summary	References
GSE83935	RNA-seq (Illumina MiSeq) of *Sclerotinia sclerotiorum* (isolate 1980)-infected *B. napus* leaves (4-week-old, cultivar DH12075) during 1,3,6,12,24,48 hpi	Seifbarghi *et al*. (2017)
PRJNA735329	RNA-seq (llumina HiSeq 2000) of *Sclerotinia sclerotiorum* (isolate 1980)-infected *B. napus* leaves (3-month-old; cultivar Ning RS-1) during 6, 24,48 hpi	Xu *et al*. (2021a)
PRJNA601001	RNA-seq (Illumina GA-IIx platform, Illumina HiSeq 2500) of *Sclerotinia sclerotiorum* (isolate 1980)-infected *B. napus* petioles (4-week-old, NEP32 and NEP63 as susceptible and resistant lines, respectively) during 8, 16, 24,48 hpi	Chittem *et al*. (2020)

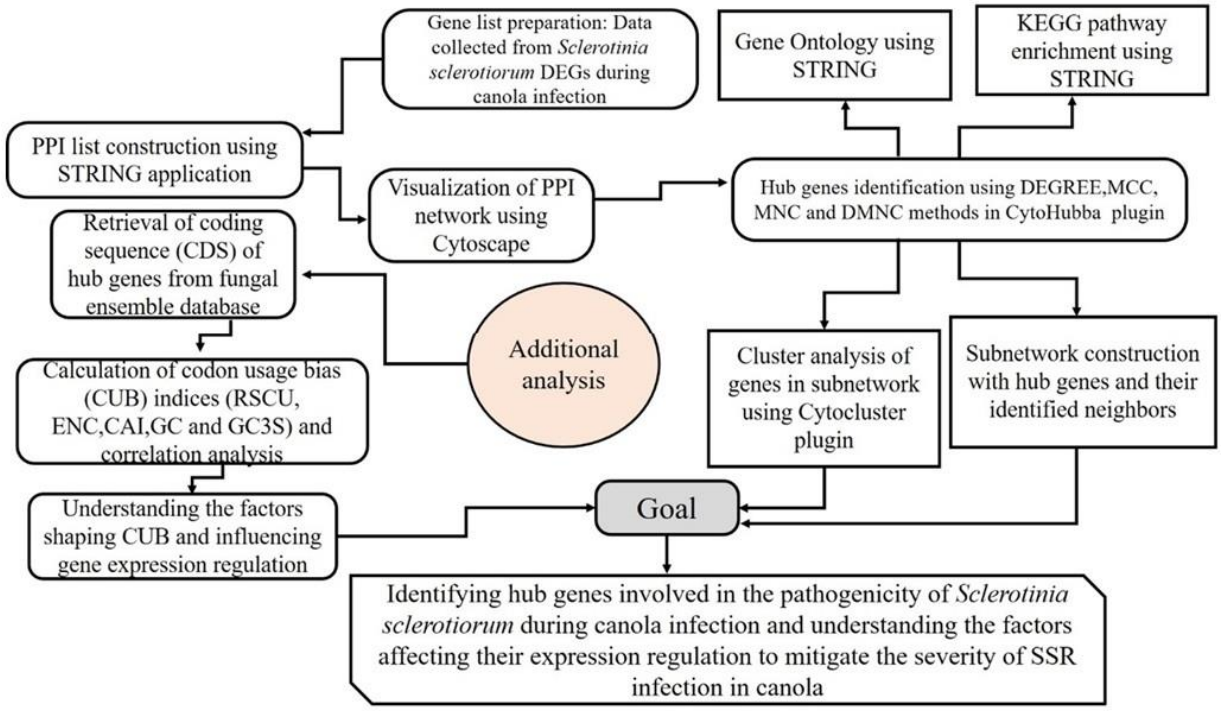

Fig. 1. Schematic overview of applied analyses for understanding the gene expression pattern of *S. sclerotiorum* during rapeseed infection.

Gene ontology and analysis

The Kyoto Encyclopedia of Genes and genomes (KEGG) database was applied to investigate the biochemical pathways of the hub network and the web-based program STRING was applied for GO, which includes molecular function (MF), cellular components (CC), and biological process (BP).

Biological network cluster analysis

CytoCluster (version 2.1.0) was applied to cluster the sub-network nodes. The IPCA algorithm (Li *et al*., 2017) was used for sub-network cluster analysis. The threshold was adjusted to 10 and in the next step, the genes of each cluster were checked in the STRING10 version to find the KEGG pathways related to these genes.

Results and Discussion

Identification of critical nodes

We identified critical nodes after entering known protein interactions into Cytoscape software using algorithms from the CytoHubba plugin. The ranking and functional properties of these genes are shown in Table 2 and the interaction network graphic is shown in Fig. 2. 21 hub genes of *S. sclerotiorum* were identified and subsequently ranked in six levels using the CytoHubba plugin (Table 2). Additionally, we identified 30 biological functions associated with these genes of *S. sclerotiorum* and then ranked in four levels using the IPCA algorithm in the CytoCluster plugin (Table 3).

Table 2. Ranking of identified hub genes of *S. sclerotiorum* using the CytoHubba plugin

Gene Description	Method	Gene ID	Rank
Nitrate reductase	Degree, MNC	SS1G_01885	1
Beta-glucosidase; glycosyl hydrolase 3 family	MCC, Degree	SS1G_01021	1, 6
Glucanase; glycosyl hydrolase 7 (cellulase C) family	DMNC	SS1G_02334	1
Glucanase; cellulase C family	DMNC	SS1G_04945	1
Glucanase; glycosyl hydrolase 6 family	DMNC	SS1G_00892	1
Glucanase; cellulase C family	DMNC	SS1G_09020	1
Elongation factor 3	Degree	SS1G_04907	2
Beta-glucosidase; glycosyl hydrolase 3 family	MCC	SS1G_05368	2
Beta-glucosidase; glycosyl hydrolase 3 family	MCC	SS1G_07146	2
Beta-glucosidase; glycosyl hydrolase 3 family	MCC	SS1G_07162	2
PA14 domain-containing protein	MCC	SS1G_06304	2
Fn3-like domain-containing protein	MCC	SS1G_09366	2
CBM1 domain-containing protein	MCC	SS1G_07847	2
Acyl-coenzyme A oxidase; acyl-CoA oxidase family	MNC, Degree	SS1G_10238	2,3
thiolase-like superfamily	MNC, Degree	SS1G_08207	2,4
thiolase-like superfamily	MNC, Degree	SS1G_02923	4,5
CBM1 domain-containing protein	DMNC	SS1G_00891	5
CBM1 domain-containing protein; cellulase A family	DMNC	SS1G_03387	5
RPOLD domain-containing protein	MNC	SS1G_02959	5
Cellulase domain-containing protein; cellulase A family	DMNC	SS1G_08837	5
enoyl-CoA hydratase/isomerase family	MNC	SS1G_00237	6

Our results indicate that gene ID SS1G_01885 encodes nitrate reductase, which ranks among the hub genes by two methods, *i.e.*, Degree & MNC. In addition to its biochemical role in protein synthesis (Unkles *et al*., 2004), nitrate reductase has been found to play a role in important biological events of *S. sclerotiorum* including sclerotium production and pathogenicity (Wei *et al*., 2023). It seems that the gene is essential for the fungal infection in rapeseed plants.

Beta-glucosidase is a cell wall-degrading enzyme (CWDE) in *S. sclerotiorum* associated with SS (Waksman, 1989). Based on our results, gene ID SS1G_01021 was identified as both the first-ranked and sixth-ranked hub gene using two methods (MCC & Degree). This suggests that the fungus modulates CWDE expression to facilitate infection.

According to the results, four genes (IDs: SS1G_02334, SS1G_04945, SS1G_00892 & SS1G_09) encoding glucanase have been identified as first-ranked hub genes in *S. sclerotiorum.* Glucanase is one of the CWDEs produced by *S. sclerotiorum* efficiently solubilizing the plant cellulose during the fungal pathogen (Chahed *et al*., 2014). Similarly, it has been demonstrated that CWDEs synthesized by *S. sclerotiorum* are a virulence factor in the SSR of rapeseed plants (Riou *et al*., 1991).

PA14 domain-containing protein (PA14DCP) is a member of cysteine-rich proteins (SCRPs) which are essential in disease induction of phytopathogenic fungi such as *Verticillium dahlia* (Li *et al*., 2024). Accordingly, our results showed that the PA14DCP-encoding gene (ID: SS1G_06304) is a second-ranked hub gene of *S. sclerotiorum* modulated during the rapeseed infection.

The fungi express genes whose encoded proteins can interact with plant cellulose by the existence of carbohydrate-binding module family 1 (CBM1) (Guillén *et al*., 2010). The fungal CBM1-containing proteins have been implicated

in cellulose degradation of host plants leading to cell collapse (Larroque *et al*., 2012). Consistently, three CBM1 domain-containing protein genes (IDs: SS1G_07847, SS1G_00891 & SS1G_03387) were determined as the hub genes of *S. sclerotiorum* during the rapeseed infection. These genes are therefore among the key elements in the virulence of *S. sclerotiorum* against rapeseed plants. Liu *et al*. (2018) reported that Acyl-coenzyme A oxidase (ACOX1) is involved in modulating reactive oxygen species (ROS) content during sclerotial development leading to the virulence phenotype of a plant pathogenic fungus (*Rhizoctonia solani* AG1-IA). Interestingly, a gene encoding ACOX1-binding protein (*ACBP5*) originating from rice (*Oryza sativa*) was used to induce resistance in rapeseed against *S. sclerotiorum* (Alahakoon, 2019).

Table 3. Clustering analysis of hub genes expressed in *S. sclerotiorum* using IPCA algorithm in CytoCluster plugin.

Functions	Edges	Nodes	Rank
Starch and sucrose metabolism Metabolic pathways Biosynthesis of secondary metabolites Cyanoamino acid metabolism Galactose metabolism	909	24	1
Synthesis and degradation of ketone bodies alpha-Linolenic acid metabolism Butanoate metabolism Valine, leucine and isoleucine degradation Fatty acid degradation Biosynthesis of unsaturated fatty acids, Fatty acid metabolism Propanoate metabolism Lysine degradation, Terpenoid backbone biosynthesis	409	21	2
alpha-Linolenic acid metabolism Synthesis and degradation of ketone bodies Fatty acid degradation Biosynthesis of unsaturated fatty acids Fatty acid metabolism Propanoate metabolism Valine, leucine and isoleucine degradation Lysine degradation, Butanoate metabolism beta-Alanine metabolism	408	20	3
Ribosome biogenesis in eukaryotes Assembly of large subunit precursor of preribosome Ribosomal large subunit export from nucleus Maturation of LSU-rRNA from tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA, LSU-rRNA) Maturation of LSU-rRNA Ribosomal subunit export from nucleus Ribosomal large subunit assembly	417	19	4

ACOX1 was identified as one of the second- and third-ranked hub genes of *S. sclerotiorum* infecting rapeseed plant highlighting the role of sclerotia-related genes in disease induction by the fungal pathogen.

Clustering analysis of hub genes expressed in *S. sclerotiorum* showed some functions that are associated with the pathogenicity of the fungus against rapeseed (Table 3). Based on the results, two CWDEs-associated functions including metabolic pathways and biosynthesis of secondary metabolites were identified as the first-ranked cluster of hub genes. Given their role in pathogenicity, identifying these CWDE-associated functions suggests that *S. sclerotiorum* modulates related genes to induce disease in rapeseed plants.

GO and KEGG analysis

GO analysis revealed that expression levels among hub genes from *S. sclerotiorum* were associated with several cellular components (CC), molecular functions (MF), and biological processes (BP) (Fig. 3). A total number of 11, 7, and 2 significant items were found to be associated with BP, MF, and CC, respectively.

According to the results, the genes related to the metabolism of organic substances, catalytic activity, and the cellular anatomical entity had the highest count in BP, MF, and CC. Also, KEGG analysis showed 16 biological pathways modulated in *S. sclerotiorum* (Fig. 4). Based on the results, the genes associated with the metabolic pathways exhibited the highest count number. According to the results, the hydrolase activity was found among the significant MFs in *S. sclerotiorum.* Similarly, Peng *et al*. (2017) and Seifbarghi *et al*. (2017) showed that a majority of the up-regulated DEGs of *S. sclerotiorum* were associated with hydrolase activity. This MF was considered a hallmark of CWDEs in the phytopathogenic fungus (Riou *et al*., 1991). Since the production and secretion of CWDEs is a metabolic-dependent pathway, the metabolic process was observed as a BP with the highest count number. Moreover, it has been found that CWDEs are extracellularly secreted during fungal infection (Kubicek *et al*., 2014), explaining the significance of extracellular region CC observed in our analysis.

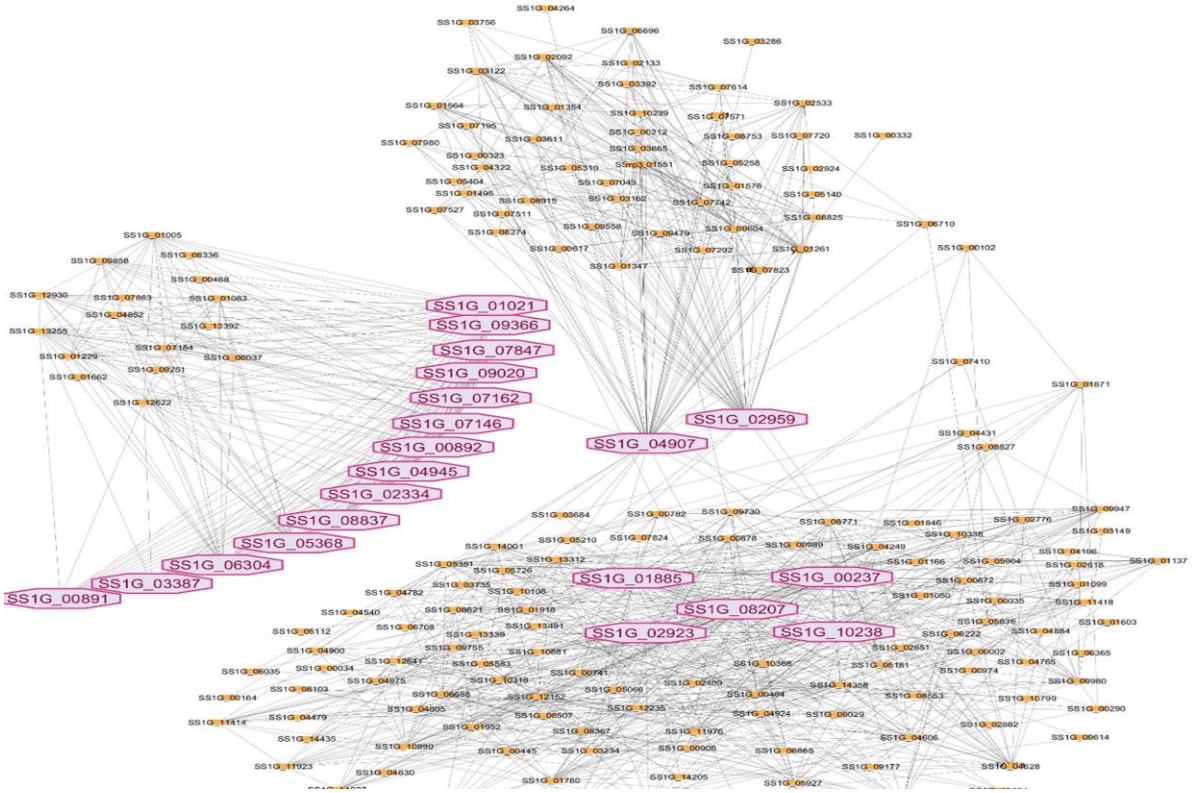

Fig. 2. A representation of expressed hub genes and their interactions with known neighbors in *S. sclerotiorum* after infection of rapeseed using the CytoHubba plugin (purple: hub genes, orange: their known neighbors). No. of Nodes: 200, No. of edges: 1045, Avg. No. of neighbors: 10.45, Characteristics path length: 3.478 Clustering coefficient: 0.551, network density: 0.053.

According to KEGG analysis results, the metabolic pathways were identified as a biological pathway in *S. sclerotiorum* with the highest gene count. Since the enzymes are included in the metabolic content of fungi (Tisch & Schmoll, 2010), fungi with CWDEs likely have relatively high levels of metabolic pathways. Also, biosynthesis of secondary metabolites was another pathway significantly modulated by *S. sclerotiorum*. Consistently, the secondary metabolism has been demonstrated to be involved in sclerotia development (Calvo and Cary, 2015) which is a pathogenicity determinant of *S. sclerotiorum* (Xu *et al*., 2018). Taken together, it seems that the DEGs related to the cell wall-destructing mission of fungal secondary metabolites are mainly modulated by *S. sclerotiorum* during rapeseed infection.

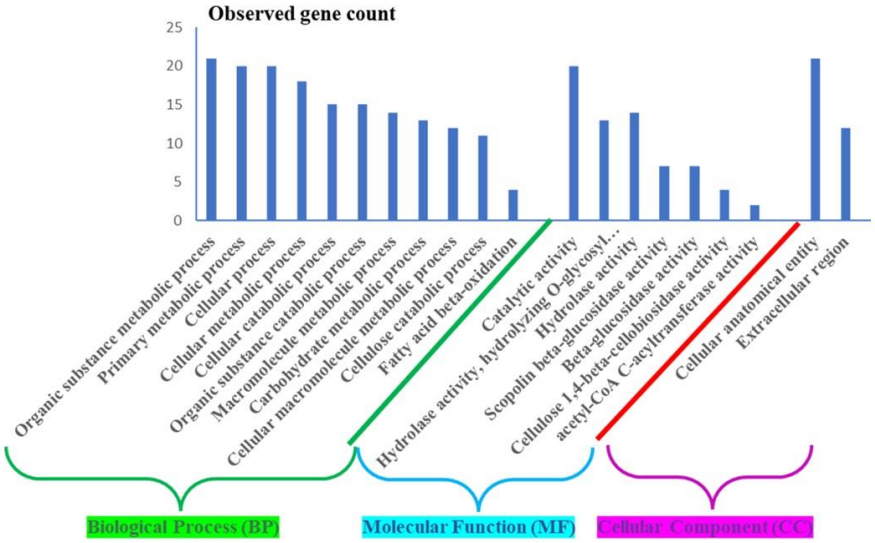

Fig. 3. Enrichment of Gene Ontology of hub genes using STRING in *S. sclerotiorum*

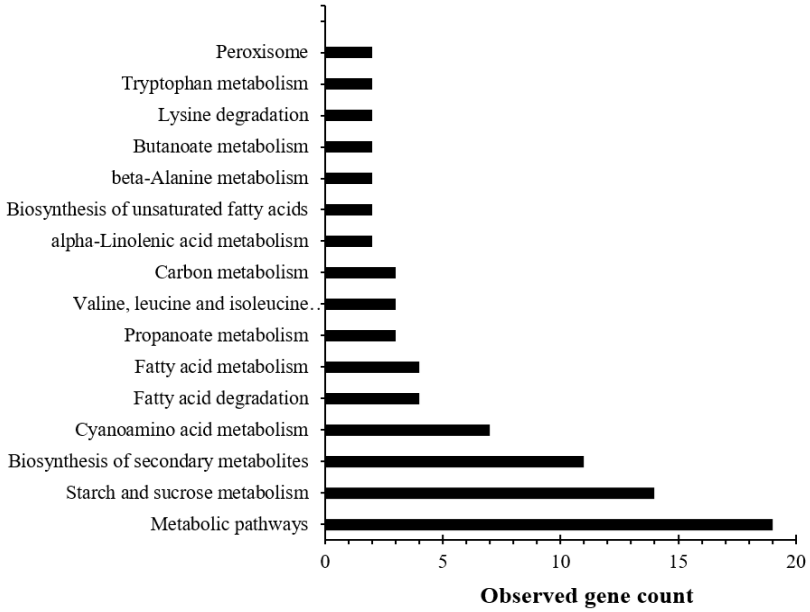

Fig. 4. The results of KEGG analysis of hub genes using STRING in *S. sclerotiorum.*

Determination of CUB

In this study, the calculation of CUB indices of SSR-associated key genes (18 genes) on coding sequences is shown in Table 4. Also, to identify the factors affecting CUB, the correlation values between the mentioned indices are shown in Table 5. GC values in the mentioned study were in the range of 0.43-0.52 and GC3S values were between 0.33-0.52 (Table 4).

It has been found that natural selection and mutation as the two main factors in the formation of CUB tendency, affect the gene expression pattern and plant growth and development processes. Therefore, to identify the factors affecting CUB, the correlation between GC (Y-axis) and GC_{3S} (X-axis) was investigated (Fig. 5A). In this diagram, each of the points represented SSR-associated key genes in the PPI interaction network (Fig. 2). If the correlation between two indices is perfect, the regression coefficient will be equal to 1 and mutation is introduced as the main factor in creating CUB, and if this coefficient is equal to 0, it means that the use of codons is affected by

selection pressure and changed non-randomly and the role of mutation in this orientation is non-significant (Fuglsang, 2008). In this study, a significant positive correlation (0.69) was observed between GC and GC_{3S} (Table 5).

Table 4. The average of CUB indices for SSR-associated hub genes

Hub Genes ID	GC	CAI	GC3S	ENC
SS1G_05368|EDO02891	0.449005	0.85473	0.337679	48.67732
SS1G_00891|EDN91488	0.47504	0.758596	0.408978	55.08428
SS1G_08207|EDN92344	0.467305	0.851402	0.403465	48.54449
SS1G_04945|EDO02469	0.516949	0.89074	0.467487	42.47502
SS1G_04907|EDO02431	0.462946	0.866243	0.42444	46.04603
SS1G_02334|EDN99480	0.522772	0.825703	0.526749	47.68533
SS1G_02923|EDO00063	0.455717	0.769181	0.339109	51.87725
SS1G_03387|EDO00913	0.479025	0.80652	0.42246	51.7551
SS1G_06304|EDO03823	0.474289	0.753467	0.418079	54.67396
SS1G_00237|EDN90837	0.471742	0.753054	0.405018	56.1012
SS1G_02959|EDO00099	0.431271	0.809197	0.392573	51.65693
SS1G_01885|EDN96957	0.463959	0.740926	0.406504	56.11587
SS1G_01021|EDN91618	0.450699	0.762933	0.38404	55.1891
SS1G_00892|EDN91489	0.481833	0.849722	0.341523	49.59352
SS1G_10238|EDN94365	0.461504	0.794787	0.435644	53.88262
SS1G_09366|EDN93499	0.467866	0.796971	0.402667	53.22015
SS1G_07146|EDO04663	0.463378	0.863041	0.359813	46.75574
SS1G_07162|EDO04679	0.478815	0.745409	0.384884	55.2969

CAI= codon adaptation index; ENC= effective number of codons; GC and GC3S= GC content in the third open position of the codon; RSCU= relative synonymous codon usage.

Table 5. Correlation between CUB indices in SSR-associated hub genes

Parameters	GC	GC3S	CAI	ENC
GC3S	0.69**	_	_	_
CAI	0.23^{ns}	0.08^{ns}	_	_
ENC	-0.38^{ns}	-0.20^{ns}	0.94^{**}-	_

CAI= codon adaptation index; ENC= effective number of codons; GC and GC3S= GC content in the third open position of the codon; RSCU= relative synonymous codon usage.

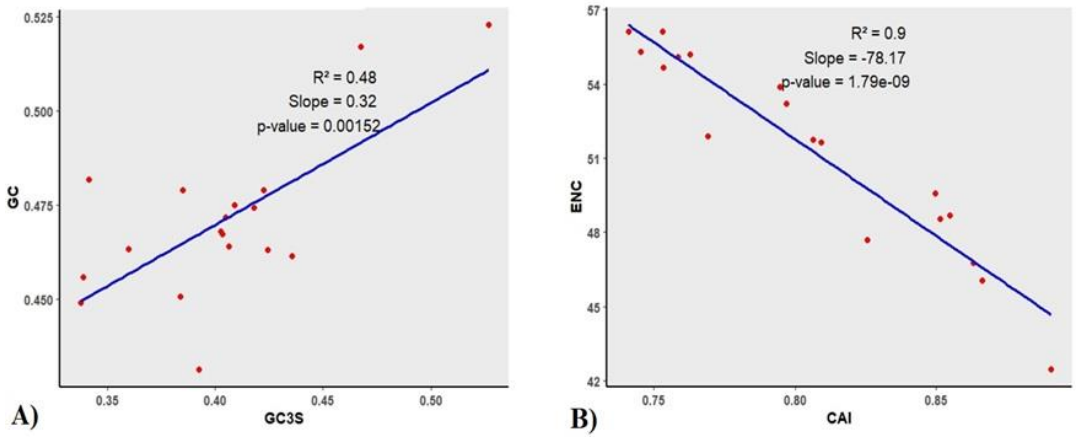

Fig. 5. The relationship between the studied CUB indices.

However, the correlation coefficient R^2 was equal to 0.48 (Fig. 5A) showing that mutation pressure alone is not enough to create this pattern and the effects of both mutation pressure and natural selection can be effective in creating CUB.

ENC, which is the codon number effectively applied in a gene, refers to the directionality of the use of amino acid codons, and its range is defined between 20 and 61. For example, if this index is 20, it means that only one specific codon has been used for the amino acids of that gene (strong orientation), and if this number is 61, it means that a variety of synonymous codons have been used (randomly) and there is no orientation in the use and no codon specification is found (Wright, 1990). In the current study, the average range of this index for the studied genes was between 42.4 and 56.65. ENC values higher than 40 indicate less orientation in codon usage. The most direction in codon usage in the studied genes was related to SS1G_02959 (RNA polymerase Rpb3 insert domain-containing protein).

CAI, under the title of codon adaptation index, measures the codon sequence of the target gene with the codon sequence of a set of high-expressed reference genes that generally use optimal codons in their sequence (such as ribosomal genes). The range of this index is variable between 0 and 1, and the closer this index is to 1, it means that more optimal codons have been used in the gene sequence. It has been found that genes with a higher CAI index are more adaptable to adverse environmental conditions (Nath Choudhury *et al*., 2017). It was also found that genes with high expression had a higher CAI index than genes with lower expression under abiotic stresses (Tyagi *et al*., 2023). Therefore, the amount of this index is considered a criterion for evaluating the level of gene expression and adaptation to environmental stresses. The range of this index in our studied genes was between 0.74-0.89, which indicates the importance of SSR-associated genes in adapting to environmental stresses. In other words, the fungi, as an infection response, by optimizing codons, has increased the possibility of using codons that are decoded by more abundant tRNAs, which leads to an increase in translation speed and adaptation to adverse environmental conditions. Conversely, rare codons have accumulated in genes with low expression (Xu *et al*., 2021b). The degree of correlation between ENC and CAI is used to detect the extent of natural selection or mutation in codon preference. On the other hand, when the amount of this correlation is high (toward -1), it means that the role of natural selection in codon preference is greater, and if the effect of mutation is stronger, the amount of this correlation is closer to 0 (Chen *et al*., 2014). In our study, a negative and significant correlation between these two indices was reported (Table 5, Fig. 5B), which shows that natural selection is also effective in the formation of CUB of SSR-associated genes.

RSCU is an index based on the ratio of the determined prevalence of a codon to the expected prevalence of the same codon among the family of synonymous codons. In other words, this index evaluates the relative degree of tendency to use a codon for a specific amino acid (Wang *et al*., 2016). Accordingly, if this ratio is equal to 1 for a specific codon of an amino acid, there is no preference for using that specific codon. If this ratio is below one, it means that the use of that codon is less than expected, and if the value of this index is above one, it means that the said codon has appeared more than the expected synonymous codons.

Values higher than 1.6 also mean a high preference for that codon compared to other synonymous codons of that amino acid (Sharp and Li, 1986). Regarding the SSR-associated genes we studied, the maximum RSCU was equal to 1.76 for the CCA codon, which encodes the amino acid proline (Table 6). Figure 6 shows the column chart of CUB pattern index values measured for the studied genes in *S. sclerotiorum* infecting rapeseed plant.

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Conflict of Interest

The authors declare no conflict of interest.

Table 6. RSCU values of synonymous codons were identified in the ORF region genes.

Amino acid	Codon	RSCU	Amino acid	Codon	RSCU	Amino acid	Codon	RSCU
Phe	TTT	0.69	His	CAT	1	Ser	AGC	0.87
Phe	TTC	1.3	His	CAC	0.91	Arg	AGA	1.42
Leu	TTA	0.65	Gln	CAA	1.54	Arg	AGG	0.57
Leu	TTG	1.35	Gln	CAG	0.45	Val	GTT	1.57
Ser	TCT	1.21	Arg	CGT	1.41	Val	GTC	1.2
Ser	TCC	1.24	Arg	CGC	0.90	Val	GTA	0.69
Ser	TCA	1.13	Arg	CGA	1.46	Val	GTG	0.53
Ser	TCG	0.42	Arg	CGG	0.21	Ala	GCT	1.44
Tyr	TAT	0.87	Ile	ATT	1.38	Ala	GCC	1
Tyr	TAC	1.13	Ile	ATC	1.25	Ala	GCA	1.15
Cys	TGT	1.2	Ile	ATA	0.35	Ala	GCG	0.40
Cys	TGC	0.80	Met	ATG	1	Asp	GAT	1.40
Trp	TGG	1	Thr	ACT	1.27	Asp	GAC	0.59
Leu	CTT	1.5	Thr	ACC	1.24	Glu	GAA	1.16
Leu	CTC	1.54	Thr	ACA	1.10	Glu	GAG	0.83
Leu	CTA	0.46	Thr	ACG	0.37	Gly	GGT	1.56
Leu	CTG	0.44	Asn	AAT	0.96	Gly	GGC	0.62
Pro	CCT	1.2	Asn	AAC	1.03	Gly	GGA	1.55
Pro	CCC	0.63	Lys	AAA	0.94	Gly	GGG	0.24
Pro	CCA	1.76	Lys	AAG	1.05	-	-	-
Pro	CCG	0.35	Ser	AGT	1.12	-	-	-

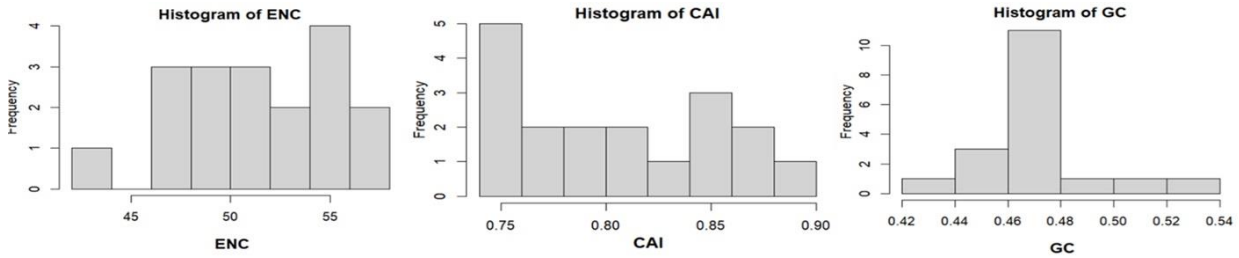

Fig. 6. Column chart of codon usage bias (CUB) pattern index values measured for the studied genes in *S. sclerotiorum*

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