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Research Article

Unraveling the changes in important molecular mechanisms of *Arabidopsis thaliana* infected by *Botrytis cinerea*: insights from *in silico* analysis

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Abstract: *Botrytis cinerea* is one of the most important harmful fungi affecting agricultural products. This study focused on the expression changes of *Arabidopsis thaliana* infected with this fungus. The expression dataset of a microarray and two RNA-sequencing were integrated using the respective software. The list of differentially expressed genes was extracted, and the key genes with altered expression were identified through Cytoscape software. These key genes co-expression patterns and functional enrichment were analyzed. Subsequently, microRNAs and transcription factors associated with these genes were predicted. Ten genes, including GAPA-2, SBPASE, CRB, HCEF1, CaS, ATPD, LIL3:1, PSAH2, PRK, and PMDH2, were identified as crucial down-regulated genes. Additionally, ten genes, namely WRKY33, CZF1, SZF1, STZ, ERF11, RHL41, BAP1, AT1G07135, CMPG2, and TET8, were highlighted as key up-regulated genes. The key roles of the hub genes with a decreased expression included processes and pathways associated with the reductive pentose phosphate cycle, photosynthesis, cold response, fructose and sucrose metabolism, defense response against bacteria, and gluconeogenesis. The key over-expressed genes played important roles in responding to chitin, oxygen deprivation, temperature fluctuations, injuries, fungal attacks, and gene transcription functions. Key genes were associated with ath-miR850, ath-miR393a-5p, and ath-miR393b-5p. Transcription factor SPL7 was linked to the transcription of down-regulated key genes, while transcription factors SARD1, PIF5, CAMTA1, HY5, WRKY33, TOC1, CAMTA3, CAMTA2, BZR1, FAR1, and CAMTA5 were also predicted to be associated with up-regulated genes. Some of these results have not previously been reported. Therefore, they could be used to design practical experiments exploring the interaction between plants and pathogenic fungi.

Keywords: Microarray, RNA-sequencing, *Botrytis cinerea*, *Arabidopsis thaliana*

Introduction

The continuous increase in the world's population has created the challenge of providing food through the production of plant products in the future daily diet of the population (Araújo et al., 2023). Fungal pathogens destroy over 60 percent of crops in severe epidemics (Różewicz *et al.*, 2021). Protecting crops from major fungal outbreaks is traditionally done

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using broad-spectrum fungicides. Developing resistance to antifungal agents, environmental pollution issues, and economic losses in countries has forced plant science researchers to find new, innovative, sustainable, and environmentally compatible solutions to protect global food systems (Corkley *et al.*, 2022). Understanding the fundamental molecular mechanisms involved in host-pathogen interactions can lead to the understanding and design of effective strategies to reduce the costs and crop losses in the agricultural industry. A profound understanding of the biological processes underlying plant resistance or susceptibility is essential for developing new crops and implementing the next generation of pathogen control strategies (Peyraud *et al.*, 2017). While sequencing and gene expression measuring technologies have been available to the scientific community for over a decade, there is still no clear definitive relationship between the scientific results obtained from these experiments and meaningful methods for crop protection (Theissinger *et al.*, 2023). Microarray and RNA-seq gene expression analyses have been developed to identify various pathophysiological processes. Despite the advantages of the RNA-seq method, the microarray method remains widely used due to lower operational costs and more available robust statistical methods for processing its data. Numerous analyzed or not yet analyzed microarray data are accessible in different databases. These datasets may contain information that can reveal important facts through network-based analysis of gene expression patterns. RNA-seq is undoubtedly a more powerful technology, with a high concordance between RNA-seq and microarray results (Lodha and Basak, 2012; Yang and Wei, 2015). *Botrytis cinerea* is a ubiquitous fungal pathogen affecting various plant species. Contamination may cause significant damage both during plant growth and after harvesting. It is the primary cause of economic loss in the production of plants. *B. cinerea* is challenging to manage because it has a variety of attack mechanisms, a wide range of hosts, and long periods of survival in crop residues (Spada *et al.*, 2024). Recent molecular genetic studies have identified crucial fungal genes for successful infection. Such knowledge provides prospects for the design of new and rational plant protection strategies. Despite advancements in manipulating genes, proteins, and their levels from different sources, no complete genetic tolerance to biotic stresses has been achieved in any crop (Ross and Santiago-Tirado, 2024). Data integration is a technique used to increase the sample size of data and achieve more reliable and accurate results (Castillo *et al.*, 2017). *Arabidopsis thaliana* serves as a model plant for investigating plant developmental processes due to its short reproduction period and possession of a small, fully-sequenced genome (Ferjani *et al.*, 2023). Consequently, this study utilized integrative microarray and RNA-seq data analysis to identify modulated molecular events in *B. cinerea*-infected *A. thaliana*.

Materials and Methods

Data collection, processing, and differentially-expressed genes (DEGs) finding

The raw data of microarray (GSE5684) and RNA-seq (SRP234685 and SRP055503) concerning to the interaction of *B. cinerea* with *A. thaliana*, were downloaded from GEO (https://www.ncbi.nlm.nih.gov/geo/), and SRA (https://www.ncbi.nlm.nih.gov/sra) respectively.

The microarray dataset was extracted using the GEOquery package using the getGEOSuppFiles function (Davis and Meltzer, 2007), and primary data analysis was conducted using the Limma package (Ritchie *et al.*, 2015). Gene expression profiles from RNA-seq datasets were extracted using the SRA tool kit (Sherry *et al.*, 2012), and data quality control was performed with FastQC software version 0.11.5 (Schmieder and Edwards, 2011). Trimming tasks are done with Trimomatic software version 0.32 (Bolger and Giorgi, 2014). The readings were aligned and counted with Hisat2 version 2.2.1 and Htseq software version 2.0.2 (Wen, 2017). Then, integration data mentioned above

was done with the linear models for microarray data (Limma) R package package for normalization, removing the batch effect (the influence of non-biological factors in changing the data produced by the test). The Grammar of Graphics plot (ggplot2) package (Wickham *et al.*, 2021) generated a volcano plot illustrating the increased and decreased expressed genes. Statistical analysis of data was conducted with DESeq2 software version 1.36.0 (Wen, 2017) to find the list of differentially expressed genes (DEGs) with logFC > 1 and logFC <-1 and adj.P.Val < 0.05 by comparing the expression data of control (no fungal treatment) and test (*B. cinerea*-inoculated) groups.

Gene network analysis

Differentially expressed gene lists were inputted to STRING (Szklarczyk *et al.*, 2019) (https://string-db.org/), and interactions with a score greater than 0.400 were extracted. The output data were downloaded in TSV file format and then were visualized using Cytoscape software version 3.9.1 (Shannon *et al.*, 2003). Furthermore, a statistical significance test was performed through the cytoHubba plugin. The Maximal Clique Centrality (MCC) method detected hub genes. The maximal clique centrality (MCC) algorithm is the most effective method to detect genes with the highest number of connections in the network (Chin *et al.*, 2014) using the maximal clique centrality method. Finally, key genes (Hubs) were extracted and visualized.

Hub genes co-expression and correlation analysis

To analyze the relationship between the key genes, the correlation coefficient was computed through the TF2Network database (Kulkarni *et al.*, 2018) (http://bioinformatics.psb.ugent.be/webtools/TF2Network/), which utilizes TFbinding site information to find co-expressed genes in *A. thaliana*.

Enrichment analysis of the hub genes

The lists of up and down-regulated hub genes were utilized in the database for annotation, visualization, and integrated discovery (DAVID) (Huang *et al.*, 2007) (www.david.ncifcrf.gov/). Subsequently, gene biological processes and pathways were analyzed with a false discovery rate (FDR) < 0.05. The PlantGSAD website (Ma *et al.*, 2022) (http://systemsbiology.cau.edu.cn/PlantGSEAv2/) was also employed to extrapolate gene ontology. This platform provides gene sets for plant species, including *A. thaliana*, and is used to elucidate gene product functions and interactions.

Identification of microRNAs associated with hub genes

In plants, the vast majority of small regulatory RNAs are microRNAs. These molecules act as gene regulators and are produced through a distinct pathway involving proteins and other regulatory mechanisms. To evaluate the role of microRNAs in key genes, the plant small RNA target analysis server (psRNATarget) database (Dai *et al.*, 2019) (www.zhaolab.org/psRNATarget/) was utilized, selecting microRNAs with an expectation score of 3 or less. Furthermore, the PlantGSAD server (Ma *et al.*, 2022) was also employed to analyze microRNAs related to hub genes.

Gene regulatory network analysis

To identify transcription factors associated with the regulation of up and down-regulated genes, TF2Network website (Kulkarni *et al.*, 2018) was used. This platform provides a tool to find potential regulatory factors in *A. thaliana*. Furthermore, PlantGSAD (Ma *et al.*, 2022) was used to analyze the potential role of key genes as regulatory factors.

Results

DEGs and hub genes

After merging related microarray and RNA-seq datasets, differentially expressed genes were identified. The list of 341 down-regulated and 504 up-regulated genes in the integrated group was presented in the volcano plot (Fig. 1). Cytoscape was used to visualize the gene interaction network of key downregulated

(GAPA-2, SBPASE, CRB, HCEF, CaS, ATPD, LIL3:1, PSAH2, PRK, and PMDH2) and up-regulated (WRKY33, CZF1, SZF1, STZ, ERF11, RHL41, BAP1, AT1G07135, CMPG2, and TET8) genes, the results can be seen in Fig. 2. Among hub downregulated genes, CRB and SBPASE possessed the highest expression correlation (0.9741), while BAP1 and ERF11 possessed the highest correlation (0.9326) among up-regulated hub genes.

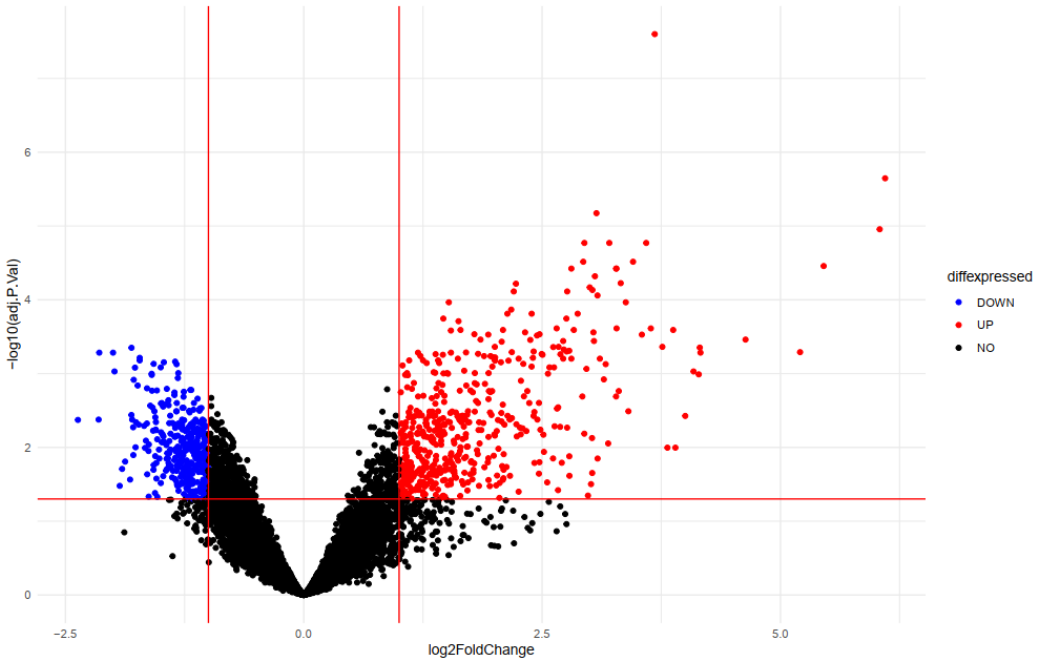

Figure 1 Volcano plot representing differentially expressed genes. The horizontal axis represents the log2 fold change, and the vertical axis represents the -log10 (adj.P.Val). Red denotes up-regulated genes with log2FC > 1 and adj.P.Val <0.05, while blue denotes down-regulated genes with log2FC < -1 and adj.P.Val <0.05. Black dots are genes that demonstrate no significant difference in their expression.

Analysis of critical biological processes and pathways related to hub genes

The results of GO analysis of differentially expressed key genes obtained from the DAVID and PlantGSAD database indicated that the biological processes related to downregulated genes include pentose phosphate reduction cycle, photosynthesis, cold stress response, fructose and sucrose metabolism, defense against bacterial infection, and gluconeogenesis. Furthermore, up-regulated genes contributed to processes such as defense response to fungus, response to chitin, hypoxia, temperature changes, and wounds. The analysis of these databases highlights that carbon fixation in photosynthetic organisms and plant-pathogen interaction are the main pathways associated with down and up-regulated hub genes, respectively.

microRNA's, transcription factors related to hub genes

Upon psRNATarget database, ath-miR850 had proper qualifications for interactions with one of the hub downregulated genes (CRB), and ath-miR393a-5p and ath-miR393b-5p passed relevant qualifications to regulate one of the up-regulated genes (WRKY33). More information about these microRNA molecules can be found in Table 1.

Although the PlantGSAD database yielded no results about downregulated hub genes, it

confirmed the results of psRNATarget database for up-regulated genes. The results related to the possibility of the key genes being transcription factors analyzed by PlantGSAD suggested that STZ and RHL41 are members of C2H2 transcription factors, whereas WRKY33 belongs to the WRKY transcription factor family. Furthermore, analysis of transcription factors related to hub genes suggested that SPL7 has a direct relation with downregulated genes except for LIL3:1. Additionally, SARD1, PIF5, CAMTA1, HY5, WRKY33, TOC1, and CAMTA5 transcription factors had a relation with up-regulated genes (Fig. 3). Moreover, the analysis through TF2Network resulted to the introduction of CAMTA3, CAMTA2, BZR1, CAMTA1, and FAR1 as statistically significant transcription factors related to hub up-regulated genes (Table 2) and no transcription factors related to hub downregulated genes.

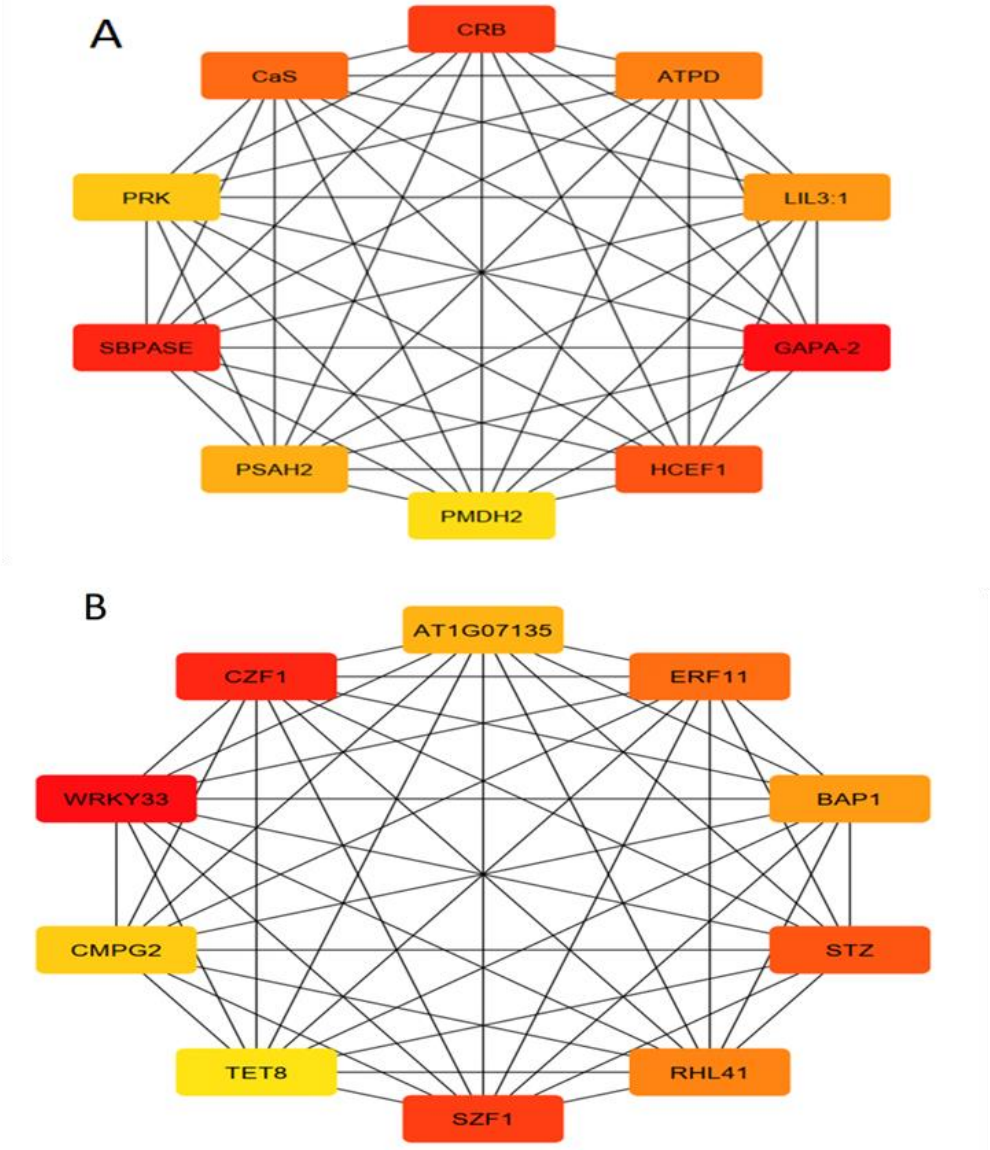

Figure 2 Network of top ten downregulated (A) and ten up-regulated hub genes from protein-protein interaction network using Cytoscape software. The color scale from yellow to red represents the top nine hub genes ranked from 1-10.

Table 1 Characterization of microRNAs associated with hub genes predicted with psRNATarget database

microRNAs	Nucleotides spanning the position of binding region in microRNAs	Nucleotides spanning the position of the binding region in the target gene	The function of microRNAs	Gene name	Expectation	Gene function
ath-miR850	1-22	1313-1334	Cleavage	CRB	2.5	nutrient reservoir activity
ath-miR393a-5p	1-22	1084-1105	Cleavage	WRKY33	3	Transcription factor
ath-miR393b-5p	1-22	1084-1105	Cleavage	WRKY33	3	Transcription factor

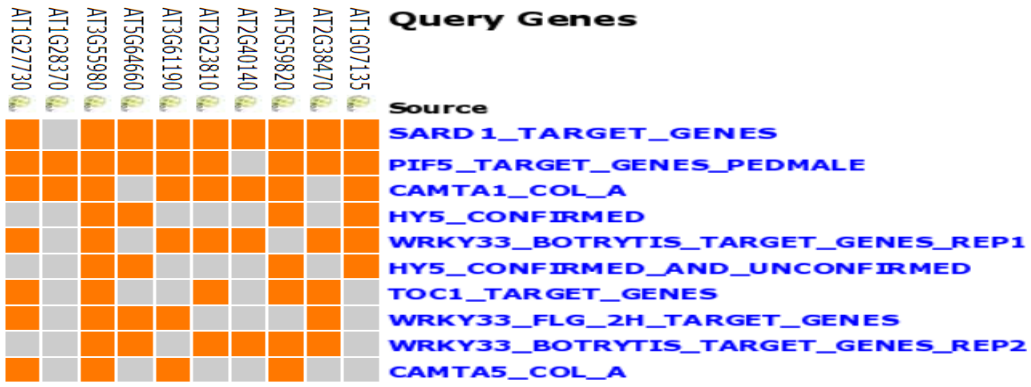

Figure 3 Prediction of the transcription factor related to the up-regulated hub genes by the PlantGSAD database.

Table 2 Detailed information about predicted transcription factors related to the up-regulated hub genes by the TF2Network database.

Transcription factor	q-value	Genes
CAMTA3	0.0001	CZF1, SZF1, STZ, ERF11, RHL41, BAP1, AT1G07135, CMPG2, TET8
CAMTA3	0.0001	CZF1, SZF1, STZ, ERF11, RHL41, BAP1, AT1G07135, CMPG2, TET8
CAMTA2	0.0001	CZF1, SZF1, STZ, ERF11, RHL41, BAP1, AT1G07135, CMPG2, TET8
CAMTA2	0.0001	CZF1, SZF1, STZ, ERF11, RHL41, BAP1, AT1G07135, CMPG2, TET8
BZR1	0.0154	WRKY33, SZF1, STZ, ERF11, RHL41, BAP1, CMPG2
CAMTA1	0.0154	CZF1, SZF1, STZ, ERF11, AT1G07135, CMPG2, TET8
FAR1	0.0154	CZF1, SZF1, STZ, ERF11, RHL41, CMPG2, TET8

Discussion

Understanding plant-microbe interactions provides crucial information about the benefits and drawbacks of infectious agents on plants and can lead to higher crop yields. The extensive and continually expanding array of "omics" and sequencing tools has significantly impacted various biological fields, offering a means to explore and comprehend intricate molecular relationships. The pros and cons of both microarray and RNA-seq methods make data merging a suitable alternative through the increase in the number of samples and the addition of the power of statistical analysis (Diwan *et al.*, 2022).

Significantly downregulated key genes are depicted in Figure 2. A similar reduction in expression (logFC<-1) of GAPA-2, SBPASE, CRB, CaS, ATPD, and PSAH2 has been documented in *A. thaliana* infected with cabbage leaf curl virus (CaLCuV) virus (Ascencio-Ibánez *et al.*, 2008). A significant reduction in SBPASE has been observed in *A. thaliana* infected by the Tobacco etch virus (TEV) (Agudelo-Romero *et al.*, 2008). Total carbohydrate levels in plants infected by a fungal infection can change due to plant regulatory mechanisms or pathological interactions. In the latter scenario, fungal infections consistently alter carbohydrate metabolism, affecting the quality and quantity of sugars based on host-infection systems. Decreases in sugar levels commonly occur due to their utilization for energy production or structural components, as well as the inhibition of photosynthesis. Low sugar levels in infected leaf tissues usually lead to less photosynthesis (Rojas *et al.*, 2014). There are numerous reports on photosynthesis reduction in higher plants after infection by pathogenic agents. Reduction in photosynthesis relevant to interactions of plants with biotroph fungi such as *Albugo candida* (Chou *et al.*, 2000), *Puccinia coronate* (Scholes and Rolfe, 1996), *Blumeria graminis* (Swarbrick *et al.*, 2006), and *B. cinerea* (Berger *et al.*, 2004) has been documented. Dysregulation of photosynthetic genes and concentration of soluble sugar in the presence of stress is not limited to pathogenic stress responses. It has been observed in response to environmental stresses such as drought, high salt concentration, and low temperatures (Singh and Thakur, 2018). Therefore, plants have developed resistance mechanisms to confront stressful changes and stimulate growth. These resistance mechanisms are led by metabolic reprogramming and gene expression changes to achieve a new equilibrium between development and defense (Morkunas and Ratajczak, 2014). Molecular families capable of provoking immune response are pathogen-associated molecular patterns (PAMPs) and microbe-associated molecular patterns (MAMPs). PAMPs are a growing list of molecules of microbial origin, such as gram-negative bacterial lipo-oligosaccharides, flagellin, fungal chitin from the cell wall, and other compounds. These molecules are detected by transmembrane pattern recognition receptors (PRRs) and initiate PAMP-triggered Immunity (PTI). Although the role of sugars has not been highlighted in this process, some scientists believe sugars act as signaling molecules to activate the immune response (Riseh *et al.*, 2024). Furthermore, these sugars may act as beginning molecules that lead to the production of PAMP. This phenomenon is known as "sweet priming" (Bolouri-Moghaddam *et al.*, 2010). Additionally, there have been numerous attempts to understand why some plants with higher sugar levels show higher resistance toward fungal infections (Ferri *et al.*, 2011). Results in the analysis of microRNAs related to downregulated genes showed that only one mRNA, ath-miR850, had interactions with the CRB gene. There have been no reports about changes in ath-miR850 level caused by *B. cinerea* infections.

SPL7B2 was a transcription factor related to all downregulated hub genes except LIL3:1. SQUAMOSA promoter binding protein-like (SPL) genes play numerous vital roles in the growth and development of plants. SPLs are members of a small gene family with 17 genes in *A. thaliana* and 19 in rice. Primary analysis of the interaction network shows that SPLs function by regulating transcription factors. These genes may also be involved in base glucose metabolism, mineral salts and production of ATP molecules. SPLs are parts of several significant biological interactions such as leaf growth, phase shift, flower and fruit growth, spore generation, GA signaling, and response to copper and fungal toxins (Chen *et al.*, 2010). This article suggests the future analysis of differential expression levels of plant SPL7 in response to fungal toxins.

Up-regulated hub genes and their network exhibited ten hubs up-expressed. TET8 is involved in making exosomes during fungal infection. Furthermore, its upregulation effectively accelerates plant viral infection (Zhu *et al.*, 2022). Upregulation of CMPG2,

WRKY33, CZF1, STZ, SZF1, ERF11, and RHL41 has been shown in the chitin treatment of *A. thaliana*, as a part of the cell wall of the fungi (Libault *et al.*, 2007). CZF1 increased expression levels in *A. thaliana*, which were demonstrated after infection with *B. cinerea* (AbuQamar *et al.*, 2006). One of the central transcription regulators of hormonal and metabolic pathways against necrotrophic pathogens is WRK33 (Zheng *et al.*, 2006). Upregulation of RHL41 in *A. thaliana* treated with Polyamine spermine (an immune stimulant) has been discovered. Additionally, 90 percent of expression changes of RHL41 have been observed in plants infected with Cytomegalovirus (CMV) (Mitsuya *et al.*, 2009). ERF11 is a transcription factor that plays a role in immunity during infection with bacterial and fungal infections (Eulgem *et al.*, 2004; Zheng *et al.*, 2019).

Chitin is a polymer in fungi cell walls. Additionally, plant cells possess enzymes capable of hydrolyzing fungal cell walls and sensing released chitin fragments during fungal infection. There is insufficient information on the chitin signaling pathway. Recent studies suggest involving this molecule in the mitogen-activated protein kinase (MAPK) pathway. Plant cells recognize pathogenic agents through pathogen-specific receptors and activate the same conserved downstream pathway to show resistance. Due to fungal infection problems in agricultural systems, understanding the chitin signaling pathway may be worthy of investigation (Wan *et al.*, 2008).

Evidence indicates that hormone signaling pathways regulated by ethylene, jasmonic acid (JA), salicylic acid (SA), abscisic acid (ABA), and reactive oxygen species (ROS) play a crucial role in the crosstalk between biotic and abiotic stress signaling pathways (Shiade *et al.*, 2024). Abiotic stress can lead to higher or lower susceptibility to biotic stress and vice versa (Fujita *et al.*, 2006). Recognition of connector regulators of the biotic and abiotic stress response is vital to developing agricultural products.

In *A. thaliana*, the miR393 family is encoded by the MIR393a/b gene. It accumulates in the cells through indole acetic acid (IAA) effects. Furthermore, pathogenic response genes are regulated by miR393. miR393 overexpression provides plants with enhanced antibacterial resistance. The target genes miR393 are related to auxin receptor genes and F-box proteins, which are important in abiotic stress conditions such as drought (Arjmand *et al.*, 2021). Additionally, ath-miR393a-5p and ath-miR393b-5p are involved in plant hormone signaling and thus affect hormone synthesis (Wu *et al.*, 2021). Transcription factors that activate calmodulin binding transcription activator (CAMTA) are highly conserved among plants and other eukaryotes. In *A. thaliana* six CAMTA proteins regulate abiotic stress and defense against bacterial infection genes (Abdel-Hameed *et al.*, 2024)

SA is a plant defense hormone involved in local systemic acquired resistance (SAR). Furthermore, infectious agents induce the synthesis of SA by regulating isochorismate synthase 1 (ICS1), a key enzyme in the production of SA. It has been reported that SARD1 and CBP60g are both key regulators of ICS1 and SA synthesis. In addition, the deletion of SARD1 jeopardizes immunity and SAR, and overexpression of SARD1 activates the defense mechanisms (Zhang *et al.*, 2010). Some fungi secrete products that bind to SARD1 and inhibit immune response (Qin *et al.*, 2018).

There are debates regarding the molecular mechanisms underlying innate immunity and steroid growth. However, it seems that the activation of BZR1 suppresses immune signals by brassinosteroids (BRs). Furthermore, BZR1 induces multiple WRKY transcription factors that negatively regulate the effect on the initial immune response and interact with WRKY40 to mediate between BR and immune signaling. BZR1, combined with environmental signals, mediates growth and immunity (Lozano-Durán *et al.*, 2013).

Temperature and light with gibberellin hormones and BRs, adjust plant growth and

development through the cell wall and auxin genes. Phytochrome interacting factors (PIFs) are significant activators of the mentioned genes. Recent studies have shown that PIFs, activated through BES1 and BZR1 play a role in the synthesis and transfer of auxins (Koene *et al.*, 2023). Furthermore, in addition to negative regulators of photomorphogenesis, evidence suggests that PIFs act as a signaling center of a variety of reactions like anthocyanin synthesis, resistance against drought, high salt concentration, and low temperature, plant hormone signaling pathways, and even in plant immunity regulation (Zheng *et al.*, 2020).

HY5 transcription factor acts downstream of multiple light receptors in photomorphogenesis. HaRXLL470, being a conserved RxLR factor, is involved in plant immunity repression by binding to host HY5. Therefore, HY5 regulates light signals and positively regulates plant immunity against the mentioned fungi (Chen *et al.*, 2021).

JA is a proven plant stress hormone necessary for induced defense against pests and necrotrophic pathogens and plays a vital role in growth and development. Almost all aspects of JA function, including biosynthesis, signaling, and downstream gene expression, are under a day/night cycle. Base levels of JA can trigger less powerful induction through the non-inducing agents. This base defense is considered less costly and bypasses the need for fully activating defense pathways (Thines *et al.*, 2019). TOC1 is a protein expressed at night, and part of the five-member family called Pseudo-response regulators (PRRs) expressed from day to night (Gendron *et al.*, 2012). Transposable elements play significant roles in the adaptation and evolution of the host genome. FAR1 is one of the transcription factors derived from transposons and affects plant growth and development. FAR1 regulates chlorophyll biosynthesis and seedling growth, but its role in the regulation of plant immunity through the combination of chlorophyll biosynthesis pathway and SA signaling pathway (Wang *et al.*, 2016).

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Statement of Conflicting Interests

The Authors state that there is no conflict of interest.

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کشف تغییرات سازوکارهای مولکولی مهم در *Arabidopsis thaliana* آلوده به *Botrytis cinerea*: دیدگاه‌هایی از تجزیه‌وتحلیل درون رایانه‌ای

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چکیده: قارچ *Botrytis cinerea* یکی از مهم‌ترین قارچ‌های مضر مؤثر بر محصولات کشاورزی است. این مطالعه روی تغییرات بیانی *Arabidopsis thaliana* آلوده به این قارچ متمرکز بود. مجموعه داده بیانی ریزآرایه (GSE5684) و دو توالی‌یابی RNA (SRP055503, SRP234685) با استفاده از نرم‌افزار مربوطه ادغام شدند و فهرست ژن‌های بیان شده متفاوت استخراج شد. ژن‌های کلیدی با ژن‌های بیان تغییر یافته از طریق نرم‌افزار Cytoscape شناسایی شدند. الگوهای بیان مشترک و غنی‌سازی عملکردی این ژن‌های کلیدی تجزیه‌وتحلیل شد. پس از آن، microRNAها و فاکتورهای رونویسی مرتبط با این ژن‌ها پیش‌بینی شدند. ده ژن، ازجمله GAPA-2، SBPASE، CRB، HCEF1، CaS، ATPD، LIL3:1، PSAH2، PRK و PMDH2 به‌عنوان ژن‌های کاهش بیان یافته شناسایی شدند. علاوه براین، ده ژن WRKY33، CZF1، SZF1، STZ، ERF11، RHL41، BAP1، AT1G07135، CMPG2 و TET8 به‌عنوان ژن‌های افزایش بیان یافته شناسایی شدند. نقش‌های کلیدی ژن‌های هاب کاهش بیان یافته شامل کاهش فرآیندها و مسیرهای مرتبط با چرخه پنتوز فسفات احیاکننده، فتوسنتز، پاسخ به سرما، متابولیسم فروکتوز و ساکارز، پاسخ دفاعی در برابر باکتری‌ها و گلوکونئوژنز بود. ژن‌های کلیدی افزایش بیان یافته، نقش مهمی در پاسخ به کیتین، کمبود اکسیژن، نوسانات دما، آسیب‌ها، حملات قارچی و عملکردهای رونویسی ژن ایفا می‌کردند. ژن‌های کلیدی با microRNAهای ath-miR850، ath-miR393a-5p و ath-miR393b-5p مرتبط بودند. فاکتور رونویسی SPL7 با رونویسی ژن‌های کلیدی کاهش بیان یافته مرتبط بود، درحالی‌که فاکتورهای رونویسی SARD1، PIF5، CAMTA1، HY5، WRKY33، TOC1، CAMTA3، CAMTA2، BZR1، FAR1 و CAMTA5 نیز با در ارتباط با ژن‌های افزایش بیان یافته پیش‌بینی شدند. برخی از این نتایج قبلاً گزارش نشده است. این نتایج را می‌توان برای طراحی آزمایش‌های عملی برای بررسی تعامل بین گیاهان و قارچ‌های بیماری‌زا مورد استفاده قرار داد.

واژگان کلیدی: ریزآرایه، توالی‌یابی RNA، *Botrytis cinerea*، *Arabidopsis thaliana*