#### **Research Article**

## **V**

# 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 overexpressed 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

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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 new. innovative. sustainable. find 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 developed to identify various been 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 contain may 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 differentiallyexpressed 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 **GEOquery** package using the 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 STRING (Szklarczyk *et al.*, 2019) to (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 2019) (Dai et al., (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 upregulated (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 athmiR393a-5p and ath-miR393b-5p passed relevant qualifications to regulate one of the upregulated 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.

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

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



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.

#### 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 al., 2004) has been documented. et 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 microbeassociated 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 "sweet known as 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. J. Crop Prot.

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). ath-miR393a-5p Additionally, and athmiR393b-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 plant hormone signaling temperature, 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 noninducing 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.

#### References

- Abdel-Hameed, A. A., Liao, W., Prasad, K. V. and Reddy, A. S. 2024. CAMTAs, a family of calmodulin-binding transcription factors, are versatile regulators of biotic and abiotic stress responses in plants. Critical Reviews in Plant Sciences,43: 171-210.
- AbuQamar, S., Chen, X., Dhawan, R., Bluhm,
  B., Salmeron, J., Lam, S., Dietrich, R. A.
  and Mengiste, T. 2006. Expression
  profiling and mutant analysis reveals
  complex regulatory networks involved in
  Arabidopsis response to Botrytis infection.
  The Plant Journal, 48: 28-44.
- Agudelo-Romero, P., Carbonell, P., De La Iglesia, F., Carrera, J., Rodrigo, G., Jaramillo, A., Pérez-Amador, M. A. and Elena, S. F. 2008. Changes in the gene expression profile of *Arabidopsis thaliana* after infection with Tobacco etch virus. Virology journal, 5: 1-11.
- Araújo, R. G., Chavez-Santoscoy, R. A., Parra-Saldívar, R., Melchor-Martínez, E. M. and Iqbal, H. M. 2023. Agro-food systems and environment: Sustaining the unsustainable. Current Opinion in Environmental Science & Health, 31: 100413.
- Arjmand, M. P., Lahiji, H. S., Biglouei, M. H. and Golfazani, M. M. 2021. Identification of drought-responsive hub genes and their related miRNAs in *Arabidopsis thaliana*. Research Square, 1-21. DOI: https://doi.org/10.21203/rs.3.rs-773568/v1.
- Ascencio-Ibánez, J. T., Sozzani, R., Lee, T.-J., Chu, T.-M., Wolfinger, R. D., Cella, R. and Hanley-Bowdoin, L. 2008. Global analysis of Arabidopsis gene expression uncovers a complex array of changes impacting

pathogen response and cell cycle during geminivirus infection. Plant Physiology, 148: 436-454.

- Berger, S., Papadopoulos, M., Schreiber, U., Kaiser, W. and Roitsch, T. 2004. Complex regulation of gene expression, photosynthesis and sugar levels by pathogen infection in tomato. Physiologia Plantarum, 122: 419-428.
- Bolger, A. and Giorgi, F. 2014. Trimmomatic: a flexible read trimming tool for illumina NGS data. Bioinformatics, 30: 2114-2120.
- Bolouri-Moghaddam, M. R., Le Roy, K., Xiang, L., Rolland, F. and Van den Ende, W. 2010. Sugar signalling and antioxidant network connections in plant cells. The FEBS journal, 277: 2022-2037.
- Castillo, D., Gálvez, J. M., Herrera, L. J., Román, B. S., Rojas, F. and Rojas, I. 2017. Integration of RNA-Seq data with heterogeneous microarray data for breast cancer profiling. BMC Bioinformatics, 18: 1-15.
- Chen, S., Ma, T., Song, S., Li, X., Fu, P., Wu, W., Liu, J., Gao, Y., Ye, W. and Dry, I. B. 2021. Arabidopsis downy mildew effector HaRxLL470 suppresses plant immunity by attenuating the DNA-binding activity of bZIP transcription factor HY5. New Phytologist, 230: 1562-1577.
- Chen, X., Zhang, Z., Liu, D., Zhang, K., Li, A. and Mao, L. 2010. SQUAMOSA promoterbinding protein-like transcription factors: Star players for plant growth and development. Journal of Integrative Plant Biology, 52: 946-951.
- Chin, C.-H., Chen, S.-H., Wu, H.-H., Ho, C.-W., Ko, M.-T. and Lin, C.-Y. 2014. cytoHubba: identifying hub objects and sub-networks from complex interactome. BMC Systems Biology, 8: 1-7.
- Chou, H. M., Bundock, N., Rolfe, S. A. and Scholes, J. D. 2000. Infection of *Arabidopsis thaliana* leaves with Albugo candida (white blister rust) causes a reprogramming of host metabolism. Molecular plant pathology, 1: 99-113.
- Corkley, I., Fraaije, B. and Hawkins, N. 2022. Fungicide resistance management:

Maximizing the effective life of plant protection products. Plant Pathology, 71: 150-169.

- Dai, X., Zhuang, Z. and Zhao, P. X. (2019). psRNATarget V2: a high-performance plant small RNA target analysis server. *In* "Plant and Animal Genome XXVII Conference (January 12-16, 2019)". PAG.
- Davis, S. and Meltzer, P. S. 2007. GEOquery: a bridge between the Gene Expression Omnibus (GEO) and BioConductor. Bioinformatics, 23: 1846-1847.
- Diwan, D., Rashid, M. M. and Vaishnav, A. 2022. Current understanding of plantmicrobe interaction through the lenses of multi-omics approaches and their benefits in sustainable agriculture. Microbiological Research, 265: 127180.
- Eulgem, T., Weigman, V. J., Chang, H.-S., McDowell, J. M., Holub, E. B., Glazebrook, J., Zhu, T. and Dangl, J. L. 2004. Gene expression signatures from three genetically separable resistance gene signaling pathways for downy mildew resistance. Plant Physiology, 135: 1129-1144.
- Ferjani, A., Tsukagoshi, H. and Vassileva, V. 2023. Model organisms in plant science: *Arabidopsis thaliana*. Frontiers in Plant Science, 14: 1279230.
- Ferri, M., Righetti, L. and Tassoni, A. 2011. Increasing sucrose concentrations promote phenylpropanoid biosynthesis in grapevine cell cultures. Journal of Plant Physiology, 168, 189-195.
- Fujita, M., Fujita, Y., Noutoshi, Y., Takahashi, F., Narusaka, Y., Yamaguchi-Shinozaki, K. and Shinozaki, K. 2006. Crosstalk between abiotic and biotic stress responses: a from points current view the of convergence in the stress signaling networks. Current Opinion in Plant Biology, 9: 436-442.
- Gendron, J. M., Pruneda-Paz, J. L., Doherty, C. J., Gross, A. M., Kang, S. E. and Kay, S. A. 2012. Arabidopsis circadian clock protein, TOC1, is a DNA-binding transcription factor. Proceedings of the National Academy of Sciences, 109: 3167-3172.

- Huang, D. W., Sherman, B. T., Tan, Q., Collins,
  J. R., Alvord, W. G., Roayaei, J., Stephens,
  R., Baseler, M. W., Lane, H. C. and
  Lempicki, R. A. 2007. The DAVID Gene
  Functional Classification Tool: a novel
  biological module-centric algorithm to
  functionally analyze large gene lists. Genome
  Biology, 8: 1-16.
- Koene, S., Shapulatov, U., van Dijk, A. D. and van der Krol, A. R. 2023. Transcriptional feedback in plant growth and defense by PIFs, BZR1, HY5, and MYC transcription factors. Plant Molecular Biology Reporter, 41: 59-80.
- Kulkarni, S. R., Vaneechoutte, D., Van de Velde, J. and Vandepoele, K. 2018. TF2Network: predicting transcription factor regulators and gene regulatory networks in Arabidopsis using publicly available binding site information. Nucleic Acids Research, 46: e31-e31.
- Libault, M., Wan, J., Czechowski, T., Udvardi, M. and Stacey, G. 2007. Identification of 118 Arabidopsis transcription factor and 30 ubiquitin-ligase genes responding to chitin, a plant-defense elicitor. Molecular Plant-Microbe Interactions, 20: 900-911.
- Lodha, T. and Basak, J. 2012. Plant–pathogen interactions: what microarray tells about it? Molecular Biotechnology, 50: 87-97.
- Lozano-Durán, R., Macho, A. P., Boutrot, F., Segonzac, C., Somssich, I. E. and Zipfel, C. 2013. The transcriptional regulator BZR1 mediates trade-off between plant innate immunity and growth. elife 2, e00983.
- Ma, X., Yan, H., Yang, J., Liu, Y., Li, Z., Sheng, M., Cao, Y., Yu, X., Yi, X. and Xu, W. 2022. PlantGSAD: a comprehensive gene set annotation database for plant species. Nucleic Acids Research, 50: D1456-D1467.
- Mitsuya, Y., Takahashi, Y., Berberich, T., Miyazaki, A., Matsumura, H., Takahashi, H., Terauchi, R. and Kusano, T. 2009. Spermine signaling plays a significant role in the defense response of *Arabidopsis thaliana* to cucumber mosaic virus. Journal of Plant Physiology, 166: 626-643.

- Morkunas, I. and Ratajczak, L. 2014. The role of sugar signaling in plant defense responses against fungal pathogens. Acta Physiologiae Plantarum, 36: 1607-1619.
- Peyraud, R., Dubiella, U., Barbacci, A., Genin, S., Raffaele, S. and Roby, D. 2017. Advances on plant–pathogen interactions from molecular toward systems biology perspectives. The Plant Journal, 90: 720-737.
- Qin, J., Wang, K., Sun, L., Xing, H., Wang, S., Li, L., Chen, S., Guo, H.-S. and Zhang, J. 2018. The plant-specific transcription factors CBP60g and SARD1 are targeted by a Verticillium secretory protein VdSCP41 to modulate immunity. Elife 7: e34902.
- Riseh, R. S., Fathi, F., Vatankhah, M. and Kennedy, J. F. 2024. Exploring the role of levan in plant immunity to pathogens: A review. International Journal of Biological Macromolecules, 135419.
- Ritchie, M. E., Phipson, B., Wu, D., Hu, Y., Law, C. W., Shi, W. and Smyth, G. K. 2015. limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Research, 43: e47-e47.
- Rojas, C. M., Senthil-Kumar, M., Tzin, V. and Mysore, K. S. 2014. Regulation of primary plant metabolism during plant-pathogen interactions and its contribution to plant defense. Frontiers in Plant Science, 5: 17.
- Ross, R. L. and Santiago-Tirado, F. H. 2024. Advanced genetic techniques in fungal pathogen research. Msphere, e00643-23.
- Różewicz, M., Wyzińska, M. and Grabiński, J. 2021. The most important fungal diseases of cereals—Problems and possible solutions. Agronomy, 11: 714.
- Schmieder, R. and Edwards, R. 2011. Quality control and preprocessing of metagenomic datasets. Bioinformatics, 27: 863-864.
- Scholes, J. D. and Rolfe, S. A. 1996. Photosynthesis in localised regions of oat leaves infected with crown rust (Puccinia coronata): quantitative imaging of chlorophyll fluorescence. Planta, 199: 573-582.
- Shannon, P., Markiel, A., Ozier, O., Baliga, N. S., Wang, J. T., Ramage, D., Amin, N.,

Schwikowski, B. and Ideker, T. 2003. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Research, 13: 2498-2504.

- Sherry, S., Xiao, C., Durbrow, K., Kimelman, M., Rodarmer, K., Shumway, M. and Yaschenko, E. (2012). Ncbi sra toolkit technology for next generation sequence data. In: Plant and Animal Genome XX Conference (January 14-18, 2012). Plant and Animal Genome.
- Shiade, S. R. G., Zand-Silakhoor, A., Fathi, A., Rahimi, R., Minkina, T., Rajput, V. D., Zulfiqar, U. and Chaudhary, T. 2024. Plant metabolites and signaling pathways in response to biotic and abiotic stresses: Exploring bio stimulant applications. Plant Stress, 100454.
- Singh, J. and Thakur, J. K. 2018. Photosynthesis and abiotic stress in plants. In: Vats, S. (Ed.), Biotic and abiotic stress tolerance in plants. Springer, pp. 27-46.
- Spada, M., Pugliesi, C., Fambrini, M. and Pecchia, S. 2024. Challenges and Opportunities Arising from Host–Botrytis cinerea Interactions to Outline Novel and Sustainable Control Strategies: The Key Role of RNA Interference. International Journal of Molecular Sciences, 25: 6798.
- Swarbrick, P. J., Schulez-Lefert, P. and Scholes, J. D. 2006. Metabolic consequences of susceptibility and resistance (race-specific and broad-spectrum) in barley leaves challenged with powdery mildew. Plant, Cell & Environment, 29: 1061-1076.
- Szklarczyk, D., Gable, A. L., Lyon, D., Junge, A., Wyder, S., Huerta-Cepas, J., Simonovic, M., Doncheva, N. T., Morris, J. H. and Bork, P. 2019. STRING v11: protein–protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. Nucleic Acids Research, 47: D607-D613.
- Theissinger, K., Fernandes, C., Formenti, G., Bista, I., Berg, P. R., Bleidorn, C., Bombarely, A., Crottini, A., Gallo, G. R. and Godoy, J. A. 2023. How genomics can help

biodiversity conservation. Trends in Genetics, 39: 545-559.

- Thines, B., Parlan, E. V. and Fulton, E. C. 2019. Circadian network interactions with jasmonate signaling and defense. Plants, 8: 252.
- Wan, J., Zhang, X.-C. and Stacey, G. 2008. Chitin signaling and plant disease resistance. Plant Signaling & Behavior, 3: 831-833.
- Wang, W., Tang, W., Ma, T., Niu, D., Jin, J. B., Wang, H. and Lin, R. 2016. A pair of light signaling factors FHY3 and FAR1 regulates plant immunity by modulating chlorophyll biosynthesis. Journal of Integrative Plant Biology, 58: 91-103.
- Wen, G. 2017. A simple process of RNAsequence analyses by Hisat2, Htseq and DESeq2. In: Proceedings of the 2017 International Conference on Biomedical Engineering and Bioinformatics, pp. 11-15.
- Wickham, H., Chang, W., Henry, L., Pedersen, T., Takahashi, K., Wilke, C., Woo, K., Yutani, H. and Dunnington, D. 2021.
  Package 'ggplot2': Create Elegant Data Visualisations Using the Grammar of Graphics. R package version 3.3. 2.
- Wu, Y., Zheng, L., Bing, J., Liu, H. and Zhang, G. 2021. Deep sequencing of small RNA reveals the molecular regulatory network of AtENO2 regulating seed germination. International Journal of Molecular Sciences, 22: 5088.
- Yang, C. and Wei, H. 2015. Designing microarray and RNA-Seq experiments for greater systems biology discovery in modern plant genomics. Molecular Plant, 8: 196-206.
- Zhang, Y., Xu, S., Ding, P., Wang, D., Cheng, Y. T., He, J., Gao, M., Xu, F., Li, Y. and Zhu, Z. 2010. Control of salicylic acid synthesis and systemic acquired resistance by two members of a plant-specific family of transcription factors. Proceedings of the National Academy of Sciences, 107: 18220-18225.
- Zheng, P.-F., Wang, X., Yang, Y.-Y., You, C.-X., Zhang, Z.-L. and Hao, Y.-J. 2020. Identification of phytochrome-interacting factor family members and functional analysis of MdPIF4 in Malus domestica. International Journal of Molecular Sciences, 21: 7350.

- Zheng, X., Xing, J., Zhang, K., Pang, X., Zhao, Y., Wang, G., Zang, J., Huang, R. and Dong, J. 2019. Ethylene response factor ERF11 activates BT4 transcription to regulate immunity to Pseudomonas syringae. Plant Physiology, 180: 1132-1151.
- Zheng, Z., Qamar, S. A., Chen, Z. and Mengiste, T. 2006. Arabidopsis WRKY33 transcription

factor is required for resistance to necrotrophic fungal pathogens. The Plant Journal, 48: 592-605.

Zhu, T., Sun, Y. and Chen, X. 2022. Arabidopsis Tetraspanins Facilitate Virus Infection via Membrane-Recognition GCCK/RP Motif and Cysteine Residues. Frontiers in Plant Science, 13.

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

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**چکیدہ:** قارچ Botrytis cinerea یکی از مہمترین قارچ ہای مضر مؤثر بر محصولات کشاورزی است. این مطالعه روی تغییرات بیانی Arabidopsis thaliana آلوده به این قارچ متمرکز بود. مجموعه داده بيانی ريزآرايه (GSE5684)و دو توالييابی RNA (SRP055503, SRP234685) با استفاده از نرم افزار 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، PMDH2 و PRK ، PSAH2 ، LIL3:1 ، ATPD ، CaS ، HCEF1 ، CRB ، SBPASE بـهعنوان ژنهای کاهش بـیان یافـته شناسایی شدنـد. علاوه براين، دە ژن BAP1 ،RHL41 ،ERF11 ،STZ ،SZF1 ،CZF1 ،WRKY33 ، CMPG2 ، AT1G07135 و TET8 به عنوان 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 میکردنند. ژنهای كىليدى با microRNA هاى ath-miR393a-5p ، ath-miR850 و ath-miR393b-5p مىرتىبط بودند. فاكتور رونويسى SPL7با رونويسى ژنهاى كليدى كاهش بيان يافته مرتبط بود، درحالىكه فاكتورهاى رونويسى CAMTA2 ،CAMTA3 ،TOC1 ،WRKY33 ،HY5 ،CAMTA1 ،PIF5 ،SARD1 FAR1 ، BZR1 و CAMTA5 نيز با در ارتباط با ژنهای افزايش بيان یافته پیشبینی شدند. برخی از این نتایج قبلاً گزارش نشده است. این نـتایج را مـیتـوان بـرای طراحی آزمـایـشهای عملی برای بررسی تعامل بین گیاهان و قارچهای بیماریزا مورد استفاده قرار داد.

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