Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Transcriptomic and Metabolomic Analyses Provide Insights into Foliar Vitamin C Application Compromises Resistance to Blast in Foxtail Millet

Yaofei Zhao

zhao_yf@163.com

Shanxi Agricultural University

Wenqin Cai

Shanxi Agricultural University

Jiajie Liu

Shanxi Agricultural University

Aonan Yang

Shanxi Agricultural University

Yaqiong Li

Shanxi Agricultural University

Short Report

Keywords: foxtail millet, blast, vitamin C, multi-omics, disease resistance

Posted Date: October 13th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-10912242/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Abstract

Blast disease is a major fungal disease constraining foxtail millet production. Exogenous vitamin C is widely applied to alleviate abiotic stresses in crops, yet its regulatory function in foxtail millet resistance against fungal pathogens remains unclear. After foliar spraying of 8 mM vitamin C on foxtail millet cultivar Jingu 21, the plant resistance to blast disease was markedly reduced. Transcriptomic screening identified a total of 734 differentially expressed genes (DEGs). Enrichment analyses revealed significant perturbations in the jasmonic acid (JA) signaling pathway and the plant-pathogen interaction pathway, with prominent downregulation of core JA pathway genes and chitin recognition receptor LysM receptor-like kinases (LYK) genes. A total of 137 differential metabolites were characterized via metabolomic profiling, among which disease-resistant secondary metabolites including jasmonic acid, indole-4-carboxaldehyde and tremulacin exhibited distinctly reduced abundances. From a multi-omics perspective, this study uncovers the molecular basis underlying the negative regulation of foxtail millet fungal resistance by vitamin C, and provides theoretical support for rational field application of vitamin C and eco-friendly management of foxtail millet blast disease.

Full Text

Foxtail millet (*Setaria italica*) has a long cultivation history, outstanding drought tolerance, and strong ecological adaptability, and it remains a widely cultivated dual-purpose (grain and forage) crop in Asia (Yang et al., 2012). In addition, foxtail millet possesses a short growth cycle, self-pollination, rich genetic diversity, high genetic transformation efficiency, and a relatively small genome, and it has been developed into a classic model plant for C_4 photosynthesis research (Doust et al., 2009; Peng and Zhang, 2020). Although foxtail millet shows excellent environmental adaptability, it is susceptible to infection by various pathogenic fungi during its growth. Among foxtail millet diseases, blast disease caused by *Pyricularia setariae* induces spindle-shaped lesions on leaves, and under severe infection the diseased leaves gradually turn yellow and necrotic from the tip downward. Evaluating resistant germplasm resources and mining functional resistance genes are core strategies for blast disease management and for breeding resistant foxtail millet varieties. By leveraging genetic segregation populations combined with multi-omics sequencing technologies, multiple quantitative trait loci (QTL) for leaf blast resistance have been identified and candidate functional genes have been discovered, providing core targets for molecular breeding of blast-resistant foxtail millet. Specifically, using the resistant cultivar Yugu 1 as experimental material, the integration of bulked segregant analysis-sequencing and gene expression analysis identified a stable resistance locus, qLB-03.1, within a 56.8 kb fine-mapping interval on chromosome 3 (Xia et al., 2025). In another study, using a recombinant inbred line population, a high-density SNP genetic map, and multi-year multi-location disease nursery phenotyping, three novel leaf blast resistance QTL were detected. Among these, QLB-czas8 on chromosomes 8 exhibited stable resistance effects across multiple environments, and BSR-Seq analysis further corroborated the accuracy of this QTL mapping (Tian et al., 2021). These studies have enriched the genetic resources for blast resistance in foxtail millet and laid a foundation for elucidating the molecular regulatory mechanisms underlying blast resistance.

Breeding resistant varieties is the most effective strategy for crops to combat diseases, yet varietal development is often time-consuming. Consequently, alternative approaches are frequently pursued to reduce disease pressure in the short term, such as the application of immune elicitors. These immune elicitors typically trigger induced resistance in plants through distinct signaling pathways (Conrath et al., 2015). Immune elicitors encompass molecules derived from the plants themselves and from pathogens, as well as certain chemically synthesized exogenous substances. For instance, microbe-associated molecular patterns (MAMPs/PAMPs) such as bacterial flagellin, glucans, chitin, and lipopolysaccharides, along with plant-derived damage-associated molecular patterns (DAMPs), can be recognized by pattern recognition receptors (PRRs) on the plant cell membrane, thereby initiating immune signaling cascades (Boutrot and Zipfel, 2017). Chemical activators represent another valuable category of substances; some compounds not only exhibit direct antimicrobial activity but also enhance plant immunity. For example, phosphonic acid-based fungicides can directly inhibit oomycete pathogens and concurrently act as immune elicitors to induce broad-spectrum resistance in plants (Dann and McLeod, 2021).

Vitamin C (ascorbic acid) is a ubiquitous molecule in eukaryotic organisms, functioning both as an antioxidant and as an enzyme cofactor. It participates in photosynthesis, photoprotection, cell wall growth and cell expansion, and enhances plant tolerance to environmental stresses (Smirnoff and Wheeler, 2000). Vitamins, including riboflavin and thiamine, have been demonstrated to act as resistance inducers; owing to their high safety profiles and favorable cost-effectiveness, related research has attracted considerable attention (Boubakri et al., 2016). As a core component of the plant antioxidant defense system, vitamin C is involved in regulating intracellular H_2O_2 homeostasis (Asensi-Fabado and Munné-Bosch, 2010). As a core component of the plant antioxidant defense system, vitamin C participates in regulating intracellular hydrogen peroxide levels and maintaining organellar ROS homeostasis. In addition, vitamin C can boost the accumulation of secondary metabolites and enhance plant resistance to pathogens (Zhang et al., 2019; Jiang et al., 2025). However, reactive oxygen species (ROS) function as crucial immune signals, and as a highly potent antioxidant, vitamin C-mediated ROS scavenging may suppress plant disease resistance. Arabidopsis mutants *vtc1-1* and *vtc2-1*, which carry point mutations that reduce vitamin C content by 70%-80% compared with the wild type, exhibit enhanced resistance to *Pseudomonas syringae*, accompanied by upregulation of *PR* genes, elevated salicylic acid (SA) levels, and premature senescence (Pavet et al., 2005; Mukherjee et al., 2010). In summary, vitamin C exerts both positive and negative dual effects on plant immunity by modulating ROS, redox homeostasis, and hormonal networks in a context-dependent manner. Currently, the molecular mechanisms by which vitamin C regulates plant immunity have not been fully elucidated, and the relevant research conclusions remain somewhat controversial. As an important cereal crop, foxtail millet has grains and vegetative tissues rich in B vitamins, but its vitamin C accumulation level is relatively low. Whether exogenous vitamin C participates in the regulation of disease resistance in foxtail millet, and through which molecular and physiological pathways it mediates the defense response, remain to be further investigated.

To investigate the effect of exogenous vitamin C on blast disease resistance in foxtail millet, we used the cultivar Jingu 21 and applied foliar sprays of vitamin C solutions at different concentrations, with

sterilized water as the control. The plants were inoculated with *P. setariae* 24 h after treatment. The results showed that vitamin C application led to reduced resistance, manifested as enlarged blast lesion areas. When the spray concentration reached 8 mM, the lesion area was significantly larger than that of the control group (Fig. 1). This indicates that foliar application of vitamin C may suppress the resistance of foxtail millet to blast disease, and this suppressive effect is concentration-dependent. Although exogenous vitamin C is rarely applied in the prevention and control of plant diseases, foliar application of vitamin C has been proven effective in alleviating stress-induced damage in crops. It can mitigate oxidative damage caused by abiotic stresses, such as salinity, heavy metals, and high temperature. This is achieved through multiple pathways, including elevating endogenous vitamin C levels, modulating cellular redox homeostasis, and activating antioxidant enzyme systems (Celi et al., 2023). Under salt stress, foliar spray of 400 mg/L vitamin C on lettuce significantly upregulated the activities of SOD, POD, and CAT, as well as the contents of endogenous vitamin C and phenolic compounds, and reversed the salt-induced inhibition of growth, root system, and yield traits; the alleviating effect was weakened at lower concentrations (Naz et al., 2024). In salt-stressed passion fruit, foliar spray of 0.8 mM vitamin C effectively maintained leaf relative water content, photosynthetic pigment synthesis, and total fruit yield; however, the mitigation effect declined when the concentration was increased to 1.6 mM (Caetano et al., 2024). Under cadmium stress in maize, foliar application of vitamin C at concentrations ranging from 0.0 to 0.5 mM progressively alleviated growth inhibition, enhanced photosynthetic capacity, and elevated antioxidant contents as the exogenous vitamin C dosage increased (Sabir et al., 2022). Taken together, these studies indicate that the effects of foliar vitamin C application on plants are highly concentration-specific, and the variation in effect is closely associated with crop species and stress type. In the present study, a spray concentration of 8 mM was required to significantly suppress blast resistance in foxtail millet, which further corroborates this conclusion.

Since foliar application of vitamin C suppressed blast resistance in foxtail millet, does vitamin C promote the growth of pathogen? To verify this hypothesis, *P. setariae* was cultured on PDA medium supplemented with 8 mM vitamin C, while PDA medium without exogenous vitamin C was set as the control group. The results showed that vitamin C did not obviously promote fungal growth, and no significant difference in colony area was observed (Fig. S1). Therefore, exogenous vitamin C may inhibit the activation of innate immunity responses in foxtail millet. To explore the specific pathways by which vitamin C suppresses the activation of disease resistance in foxtail millet, transcriptomic and metabolomic analyses of leaves were performed. Leaves sprayed with 8 mM vitamin C and harvested at 24 h post-treatment were set as the experimental group, while leaves sprayed with sterile water served as the control group. Principal component analysis (PCA) of the transcriptome samples revealed that biological replicates within each group clustered together, whereas the two groups were clearly separated (Fig. 2A). Comparison between the two groups identified 734 differentially expressed genes (DEGs), including 155 upregulated and 579 downregulated genes (Fig. 2B). To gain insights into the functions of these DEGs and the mechanism by which exogenous vitamin C suppresses blast resistance, we performed GO enrichment analysis. The significantly enriched terms included multiple jasmonic acid (JA)-related pathways, such as JA-mediated signaling, regulation of JA-mediated signaling, cellular

response to JA stimulus, and JA metabolic process. In addition, genes involved in wounding and insect defense responses were significantly enriched (Fig. 2C). KEGG enrichment analysis further revealed significant enrichment of genes in the plant hormone signal transduction and plant-pathogen interaction pathways (Fig. 2D). Among the DEGs enriched in plant hormone signal transduction, 57 genes were identified (8 upregulated and 49 downregulated). Of these, 14 genes were potentially associated with JA metabolism and signaling. For instance, Seita.5G230500 encodes a Jasmonic acid-amido synthetase, which catalyzes the synthesis of bioactive JA-amino acid conjugates, a key step in JA signaling (Li et al., 2026). Seita.9G310400 encoding the MYC2 transcription factor, a master regulator of JA-responsive gene expression, was also significantly downregulated (Du et al., 2017). Moreover, the JAZ subfamily of TIFY proteins plays important roles in JA signal transduction, and 10 genes encoding TIFY family proteins including JAZ were enriched in the hormone signal transduction pathway; their expression levels were downregulated after vitamin C treatment (Supplementary Table 1).

JA is a key defense hormone in plants. Although the role of JA in foxtail millet blast resistance remains poorly characterized, JA has been demonstrated to positively regulate blast resistance in rice (Ma et al., 2022). JA biosynthesis-deficient mutants are significantly more susceptible to *Magnaporthe oryzae*. Pathogen infection strongly induces the upregulation of genes related to JA biosynthesis, signaling, and phytoalexin synthesis, thereby activating both basal and race-specific resistance in rice. The OsANN1-HAN1 protein module degrades bioactive JA-Ile and suppresses the JA pathway, weakening rice blast resistance, whereas OsGLP3-7 enhances resistance by increasing endogenous JA levels and activating phytoalexin biosynthesis (Sun et al., 2023; Zhao et al., 2023). Therefore, the inhibitory effect of foliar vitamin C application on the expression of key genes involved in jasmonic acid metabolism and signalling likely attenuates foxtail millet resistance to blast.

In the KEGG enrichment analysis, a total of 35 DEGs were enriched in the plant-pathogen interaction pathway. Among them, only four genes were upregulated, and the remaining 31 were downregulated (Supplementary Table 2). Notably, three genes encoding LysM receptor-like kinases (LYKs) were downregulated. LYKs are core proteins that enable plants to recognize chitin, a canonical MAMP in fungal cell walls, and trigger innate immunity (Wan et al., 2012). In Arabidopsis, AtLYK5 exhibits high binding affinity for chitin. Upon chitin perception, AtLYK5 forms a complex with AtCERK1 to initiate downstream phosphorylation cascades. AtLYK4 acts as a co-receptor that interacts with AtLYK5 to amplify chitin-triggered signals, whereas AtLYK2 participates in elicitor-induced pre-activation of defense responses. Collectively, these four types of LYK proteins coordinate to orchestrate vital immune events, including chitin recognition, elevation of calcium signals, and activation of defense-related genes (Cao et al., 2014; Xue et al., 2019; Giovannoni et al., 2021). The immune-activating functions of LYKs are highly conserved across the Arabidopsis and numerous crops such as rice, grape, tomato, and strawberry, rendering them central molecular regulators of plant resistance against fungal pathogens. In grapevine, VvLYK5 rescues the chitin-sensing defect of the Arabidopsis *lyk4/lyk5* double mutant. It specifically recognizes chitooligosaccharides (COS) and activates immune signaling via physical interaction with VvLYK1 (Roudaire et al., 2023). Rice OsCERK1 coordinately modulates chitin-triggered immunity and mycorrhizal symbiosis signaling; tomato SlLYK1 is exclusively responsible for chitin responses. Multiple

FaLYK paralogs in the strawberry genome harbor conserved chitin-binding domains and participate in the regulation of resistance to Colletotrichum pathogens (Liao et al., 2018; Zhang et al., 2022). Even the basal land plant *Marchantia polymorpha* relies on MpLYK for chitin detection, further supporting that the LYK-mediated pathway is evolutionarily conserved among terrestrial plants (Yotsui et al., 2023). In the present study, the three DEGs enriched in the plant-pathogen interaction pathway share high sequence homology with Arabidopsis *AtLYK3*, *AtLYK4* and *AtLYK5*. Given the indispensable and evolutionarily conserved roles of LYKs in fungal perception and immune signal transduction, we hypothesize that exogenous vitamin C application suppresses the expression of *LYKs*, impairs the recognition of *P. setariae* by foxtail millet, and ultimately attenuates millet blast resistance.

To identify core genes regulating disease resistance in foxtail millet, weighted gene co-expression network analysis (WGCNA) was performed based on the transcriptome data. In total, all analyzed genes were clustered into 32 co-expression modules according to their expression patterns, and genes within the same module exhibited highly consistent expression trends (Figure 2E). Significant differences in gene expression levels were observed between the vitamin C treatment group and the sterile water control group. Specifically, genes in the greenyellow, lightcyan, and pink modules were consistently downregulated in all three biological replicates of the vitamin C-sprayed group but markedly upregulated in the control group. In contrast, genes in the red, yellow, and turquoise modules displayed an opposite expression pattern (Figure S2 and Supplementary Table 3). These results indicate that these co-expression modules represent core transcriptional units specifically responding to exogenous vitamin C, and their constituent genes are likely involved in vitamin C-mediated biological processes associated with foxtail millet disease resistance. Furthermore, hub genes within the key modules were screened. Seita.8G192200, a hub gene in the greenyellow module, encodes an NBS-LRR disease-resistant protein. Its rice homolog RGA5 is a vital component responsible for pathogen effector recognition and the activation of effector-triggered immunity (ETI) in plants (Liu et al., 2021). Another hub gene in the greenyellow module, Seita.5G444600, encodes a protein homologous to RLCK185, which has been reported to mediate chitin signal transduction and serve as a critical regulator for the activation of downstream MAPK immune signaling cascades (Wang et al., 2017). Collectively, functional characterization of these hub genes in key WGCNA modules will not only help elucidate the molecular mechanism by which exogenous vitamin C modulates the immune response of foxtail millet against pathogens, but also provide valuable candidate targets and theoretical foundations for identifying uncharacterized immune-related genes and refining the regulatory network of crop disease resistance.

According to transcriptome profiling, DEGs were significantly enriched in metabolic pathways including phenylalanine metabolism and indole alkaloid biosynthesis, implying that exogenous vitamin C likely interferes with the accumulation of defensive metabolites in foxtail millet. To verify this speculation, widely targeted metabolomic profiling was further conducted. Principal component analysis (PCA) revealed tight clustering of biological replicates within each treatment group and clear separation between samples sprayed with 8 mM vitamin C and sterile water controls (Fig. 3A). A total of 137 differentially accumulated metabolites were identified via metabolite identification and quantitative comparison. Among these DAMs, 69 metabolites exhibited elevated abundance under vitamin C

treatment, whereas the remaining 68 metabolites showed reduced accumulation (Fig. 3B, Supplementary Table 4). Notably, two out of the three biological replicate samples in the control group exhibited high levels of jasmonic acid (JA) and jasmonoyl-L-leucine (JA-Leu), whereas all three samples from the vitamin-C-treated experimental group had low JA and JA-Leu contents (Fig. 3C-3D). Although the differences between the two groups did not reach statistical significance ($P = 0.368$ and $P = 0.357$, respectively), considering that minor fluctuations in phytohormone abundance are sufficient to alter plant physiological responses, this trend suggests that vitamin C may modulate JA levels in foxtail millet. Meanwhile, this observation is consistent with the enrichment of JA-metabolism-related genes revealed by transcriptome analysis. JA accumulation constitutes a core defensive step for plant resistance against pathogenic fungi. Previous studies demonstrated that heat stress activates rice JA biosynthetic gene *AOC1* via the transcription factor HsfA1; knockout of *HsfA1* or *AOC1* abolishes heat-induced JA production and blast resistance, which can be rescued by exogenous methyl jasmonate (MeJA) application(Qiu et al., 2025). In soybean, GmST2 directly binds the promoters of two key JA biosynthetic genes, GmAOC3 and GmAOC4, to activate their transcription, thereby boosting endogenous JA levels and conferring dual tolerance to salt stress and gray mold disease (Li et al., 2025). In addition, JA-Leu acts as a pivotal immune-related metabolite: it functions as a ligand to facilitate the interaction between rice COI1 and JAZ proteins, triggers the degradation of OsJAZ4, and ultimately activates the transcription of JA-responsive defense genes (Fu et al., 2022). Collectively, the repressive effect of exogenous vitamin C on the accumulation of JA and its conjugated derivatives may substantially hamper the full activation of disease resistance in foxtail millet. Combined with our transcriptome data, foliar vitamin C application downregulates numerous key genes involved in JA biosynthesis and signal transduction. This reduction in JA abundance may either be a downstream consequence of suppressed gene expression, or alternatively, vitamin C may first repress JA pathway gene transcription to limit JA production. In Arabidopsis and tobacco BY-2 suspension cells, MeJA treatment upregulates genes encoding rate-limiting enzymes of the vitamin C biosynthetic pathway and stimulates de novo vitamin C synthesis (Wolucka et al., 2005). These findings raise a hypothesis that excess vitamin C may serve as a feedback repressor to restrain JA biosynthesis and signal transduction, possibly through transcriptional regulation or inhibition of core biosynthetic enzyme activity. Although direct experimental evidence supporting this regulatory feedback remains lacking, our results together with published literature collectively confirm an intimate crosstalk between vitamin C and the JA signaling cascade.

Further analysis of the metabolomic data revealed that the contents of several disease resistance-related metabolites were significantly decreased after vitamin C treatment, including indole-4-carboxaldehyde, tremulacin, and caffeoylputrescine (Fig. 3E-3G). Indole derivatives have been shown to inhibit mating and hyphal growth of *Sporisorium scitamineum*, and application of indole-3-carboxaldehyde substantially reduces the incidence of corn smut; tremulacin, a phenolic defense compound in Populus species, renders trembling aspen more susceptible to chewing insect herbivory and fungal infection when its content declines; caffeoylputrescine, a phenylpropanoid defense compound, is massively synthesized in tobacco upon lepidopteran oviposition-mediated priming and exerts insect resistance (Bandoly et al., 2016; Liu et al., 2020; Thakur et al., 2021). The accumulation

levels of these metabolites were significantly lower in the vitamin C-treated group than in the sterile water control. This may be attributed to the effects on key genes in their biosynthetic pathways, as genes involved in indole metabolism and phenylalanine metabolism were indeed significantly enriched in the transcriptome enrichment analysis of differentially expressed genes (As shown in Fig. 2D). Another possible reason is that excessive vitamin C may lead to the degradation of intracellular metabolites. Although no direct evidence currently demonstrates that vitamin C causes the degradation of indole-4-carboxaldehyde, tremulacin, or similar compounds, 0.1% vitamin C has been reported to significantly reduce the contents of various acylated and non-acylated anthocyanins in black carrot extract within 24 h, directly promoting anthocyanin degradation. Low concentrations of vitamin C can also induce the degradation of natural polysaccharides via hydroxyl radical generation (Zou et al., 2020; Guldiken et al., 2021). These findings suggest that the influence of vitamin C on the intracellular microenvironment can profoundly affect metabolite accumulation. On the other hand, indole-4-carboxaldehyde, tremulacin and caffeoylputrescine have been demonstrated to possess antimicrobial activities in other crops. Whether these metabolites can be applied to control foxtail millet blast disease remains to be explored. Subsequent studies can focus on these four defense metabolites to conduct in vitro and in vivo antifungal assays, systematically investigating the inhibitory effects of single compounds and their combinations on mycelial growth, conidial germination, and appressorium formation of *P. setariae*, and determining their optimal application concentrations for inhibiting the blast pathogen. Concurrently, pot and field experiments should be performed to compare the efficacy of different application methods, such as seed soaking, foliar spraying, and root drenching at the seedling stage on blast disease control. This will help to clarify the optimal application timing, frequency, and supporting techniques for these metabolites to exert stable disease control effects, thereby providing a theoretical basis and practical support for developing green fungicides against foxtail millet blast based on endogenous plant defense metabolites.

This study reveals that exogenous vitamin C treatment reduces foxtail millet resistance to millet blast. Such adverse effect does not arise from direct stimulation of pathogen proliferation, but is probably associated with the negative regulation of endogenous immune defense systems in foxtail millet. Transcriptomic and metabolomic analyses demonstrate that exogenous vitamin C impairs millet blast resistance by disrupting pathogen recognition, JA-mediated immune signal activation, and the biosynthesis of disease-resistant secondary metabolites. This work elucidates the molecular mechanism by which exogenous vitamin C negatively modulates fungal resistance in gramineous crops and deepens our understanding of functions in foxtail millet immunity. Practically, it suggests that the concentration and application timing of exogenous vitamin C should be strictly regulated when using it to relieve abiotic stresses, so as to avoid suppressed immune signaling pathways and the consequent elevated risk of millet blast outbreak.

Future research will conduct in vitro and in vivo functional verification on the core immune genes screened in this study that are markedly regulated by vitamin C. Furthermore, systematic in vitro, pot and field trials will be carried out to evaluate the inhibitory effects of indole-4-carboxaldehyde, tremulacin and caffeoylputrescine against blast, and define their optimal application conditions. Follow-up

investigations will not only provide genetic resources for molecular breeding of blast-resistant foxtail millet, but also identify natural bioactive metabolites with promising application prospects for the green prevention and control of foxtail millet diseases.

Declarations

Author contributions: Yaofei Zhao: Conceptualization, Investigation, Data curation, and Writing-original draft; Wenqin Cai: Investigation, and Data curation; Jiajie Liu: Investigation; Aonan Yang: Investigation; Yaqiong Li: Conceptualization, Writing-Review and Editing, and Supervision.

Data availability: All data used in this study are fully available without restriction.

Funding: Financial supports were provided by the project of Shanxi Province key lab construction (grant number Z135050009017-2-12), National Natural Science Foundation of China (grant number 32672676 and 32241041), the Distinguished and Excellent Young Scholar Cultivation Project of Shanxi Agricultural University (grant number 2022YQPYGC02), and the Scientific and Technological Innovation Enhancement Project of Shanxi Agricultural University (grant number CXGC2025051).

Declaration of competing interest

The authors declare that they have no competing financial interests.

References

1. **Asensi-Fabado, M.A., and Munné-Bosch, S.** (2010). Vitamins in plants: occurrence, biosynthesis and antioxidant function. Trends Plant Sci **15,** 582-592.
2. **Bandoly, M., Grichnik, R., Hilker, M., and Steppuhn, A.** (2016). Priming of anti-herbivore defence in Nicotiana attenuata by insect oviposition: herbivore-specific effects. Plant Cell Environ **39,** 848-859.
3. **Boubakri, H., Gargouri, M., Mliki, A., Brini, F., Chong, J., and Jbara, M.** (2016). Vitamins for enhancing plant resistance. Planta **244,** 529-543.
4. **Boutrot, F., and Zipfel, C.** (2017). Function, Discovery, and Exploitation of Plant Pattern Recognition Receptors for Broad-Spectrum Disease Resistance. Annual Review of Phytopathology **55,** 257-286.
5. **Caetano, E.J.M., Silva, A., Lima, G.S., Azevedo, C.A.V., Veloso, L., Arruda, T.F.L., Souza, A.R., Soares, L., Gheyi, H.R., Dias, M.D.S., Borborema, L.D.A., Sousa, V.D., and Fernandes, P.D.** (2024). Application Techniques and Concentrations of Ascorbic Acid to Reduce Saline Stress in Passion Fruit. Plants (Basel) **13**, 2718.
6. **Cao, Y., Liang, Y., Tanaka, K., Nguyen, C.T., Jedrzejczak, R.P., Joachimiak, A., and Stacey, G.** (2014). The kinase LYK5 is a major chitin receptor in Arabidopsis and forms a chitin-induced complex with related kinase CERK1. Elife **3**, e03766.
7. **Celi, G.E.A., Gratão, P.L., Lanza, M., and Reis, A.R.D.** (2023). Physiological and biochemical roles of ascorbic acid on mitigation of abiotic stresses in plants. Plant Physiol Biochem **202,** 107970.

8. **Conrath, U., Beckers, G.J., Langenbach, C.J., and Jaskiewicz, M.R.** (2015). Priming for enhanced defense. Annu Rev Phytopathol **53,** 97-119.
9. **Dann, E., and McLeod, A.** (2021). Phosphonic acid: a long-standing and versatile crop protectant. Pest Manag Sci **77,** 2197-2208.
10. **Doust, A.N., Kellogg, E.A., Devos, K.M., and Bennetzen, J.L.** (2009). Foxtail millet: a sequence-driven grass model system. Plant physiology **149,** 137-141.
11. **Fu, W., Jin, G., Jiménez-Alemán, G.H., Wang, X., Song, J., Li, S., Lou, Y., and Li, R.** (2022). The jasmonic acid-amino acid conjugates JA-Val and JA-Leu are involved in rice resistance to herbivores. Plant Cell Environ **45,** 262-272.
12. **Giovannoni, M., Lironi, D., Marti, L., Paparella, C., Vecchi, V., Gust, A.A., De Lorenzo, G., Nürnberger, T., and Ferrari, S.** (2021). The Arabidopsis thaliana LysM-containing Receptor-Like Kinase 2 is required for elicitor-induced resistance to pathogens. Plant Cell Environ **44,** 3545-3562.
13. **Guldiken, B., Gibis, M., Boyacioglu, D., Capanoglu, E., and Weiss, J.** (2021). Ascorbic acid-induced degradation of liposome-encapsulated acylated and non-acylated anthocyanins of black carrot extract. J Sci Food Agric **101,** 5707-5714.
14. **Jiang, X., Wang, Y., Zhao, Y., Korxeelor, X., Fan, W., Fan, X., Li, Y., Wu, X., Zhou, X., Li, F., Wu, X., Ji, W., and Cheng, X.** (2025). Cytokinesis-Defective 1 (CYT1) Positively Regulates Plant Antiviral Immunity by Promoting Callose Deposition and Ascorbic Acid Biosynthesis. Mol Plant Pathol **26,** e70126.
15. **Li, S., Wang, M., Du, X., Wang, Y., Pan, Y., Ji, D., You, J., Shan, M., Bao, G., Liu, X., Xu, J., Lu, Q., and Xiang, F.** (2025). The GmPRL1b-GmST2-GmAOC3/4 Module Confers Salt Tolerance and Botrytis cinerea Resistance by Inducing Jasmonic Acid Biosynthesis in Soybean. Plant Biotechnol J **23,** 5965-5983.
16. **Liao, D., Sun, X., Wang, N., Song, F., and Liang, Y.** (2018). Tomato LysM Receptor-Like Kinase SlLYK12 Is Involved in Arbuscular Mycorrhizal Symbiosis. Front Plant Sci **9,** 1004.
17. **Liu, S., He, F., Lin, N., Chen, Y., Liang, Z., Liao, L., Lv, M., Chen, Y., Chen, S., Zhou, J., and Zhang, L.H.** (2020). Pseudomonas sp. ST4 produces variety of active compounds to interfere fungal sexual mating and hyphal growth. Microb Biotechnol **13,** 107-117.
18. **Liu, Y., Zhang, X., Yuan, G., Wang, D., Zheng, Y., Ma, M., Guo, L., Bhadauria, V., Peng, Y.L., and Liu, J.** (2021). A designer rice NLR immune receptor confers resistance to the rice blast fungus carrying noncorresponding avirulence effectors. Proc Natl Acad Sci U S A **118**, e2110751118.
19. **Ma, J., Morel, J.B., Riemann, M., and Nick, P.** (2022). Jasmonic acid contributes to rice resistance against Magnaporthe oryzae. BMC Plant Biol **22,** 601.
20. **Mukherjee, M., Larrimore, K.E., Ahmed, N.J., Bedick, T.S., Barghouthi, N.T., Traw, M.B., and Barth, C.** (2010). Ascorbic acid deficiency in arabidopsis induces constitutive priming that is dependent on hydrogen peroxide, salicylic acid, and the NPR1 gene. Mol Plant Microbe Interact **23,** 340-351.
21. **Naz, S., Mushtaq, A., Ali, S., Muhammad, H.M.D., Saddiq, B., Ahmad, R., Zulfiqar, F., Hayat, F., Tiwari, R.K., Lal, M.K., and Altaf, M.A.** (2024). Foliar application of ascorbic acid enhances growth

and yield of lettuce (Lactuca sativa) under saline conditions by improving antioxidant defence mechanism. Funct Plant Biol **51,** Null.

22. **Pavet, V., Olmos, E., Kiddle, G., Mowla, S., Kumar, S., Antoniw, J., Alvarez, M.E., and Foyer, C.H.** (2005). Ascorbic acid deficiency activates cell death and disease resistance responses in Arabidopsis. Plant Physiol **139,** 1291-1303.
23. **Peng, R., and Zhang, B.** (2020). Foxtail Millet: A New Model for C4 Plants. Trends Plant Sci **20,** S1360-1385.
24. **Qiu, J., Cao, X., Shi, H., Chen, Z., Zhang, X., Cao, Z., Huang, D., Wen, H., Chen, Y., and Kou, Y.** (2025). miR444b.2-HsfA1-AOC1 module mediates heat priming-enhanced blast resistance in rice. Proc Natl Acad Sci U S A **122,** e2505764122.
25. **Roudaire, T., Marzari, T., Landry, D., Löffelhardt, B., Gust, A.A., Jermakow, A., Dry, I., Winckler, P., Héloir, M.C., and Poinssot, B.** (2023). The grapevine LysM receptor-like kinase VvLYK5-1 recognizes chitin oligomers through its association with VvLYK1-1. Front Plant Sci **14,** 1130782.
26. **Sabir, M., Naseem, Z., Ahmad, W., Usman, M., Nadeem, F., Saifullah, and Ahmad, H.R.** (2022). Alleviation of adverse effects of nickel on growth and concentration of copper and manganese in wheat through foliar application of ascorbic acid. Int J Phytoremediation **24,** 695-703.
27. **Smirnoff, N., and Wheeler, G.L.** (2000). Ascorbic acid in plants: biosynthesis and function. Crit Rev Biochem Mol Biol **35,** 291-314.
28. **Sun, B., Li, W., Ma, Y., Yu, T., Huang, W., Ding, J., Yu, H., Jiang, L., Zhang, J., Lv, S., Yang, J., Yan, S., Liu, B., and Liu, Q.** (2023). OsGLP3-7 positively regulates rice immune response by activating hydrogen peroxide, jasmonic acid, and phytoalexin metabolic pathways. Mol Plant Pathol **24,** 248-261.
29. **Thakur, M.P., Künne, T., Unsicker, S.B., Biere, A., Ferlian, O., Pruschitzki, U., Thouvenot, L., Türke, M., and Eisenhauer, N.** (2021). Invasive earthworms reduce chemical defense and increase herbivory and pathogen infection in native trees. J Ecol **109,** 763-775.
30. **Tian, B., Zhang, L., Liu, Y., Wu, P., Wang, W., Zhang, Y., and Li, H.** (2021). Identification of QTL for resistance to leaf blast in foxtail millet by genome re-sequencing analysis. Theor Appl Genet **134,** 743-754.
31. **Wan, J., Tanaka, K., Zhang, X.C., Son, G.H., Brechenmacher, L., Nguyen, T.H., and Stacey, G.** (2012). LYK4, a lysin motif receptor-like kinase, is important for chitin signaling and plant innate immunity in Arabidopsis. Plant Physiol **160,** 396-406.
32. **Wang, C., Wang, G., Zhang, C., Zhu, P., Dai, H., Yu, N., He, Z., Xu, L., and Wang, E.** (2017). OsCERK1-Mediated Chitin Perception and Immune Signaling Requires Receptor-like Cytoplasmic Kinase 185 to Activate an MAPK Cascade in Rice. Mol Plant **10,** 619-633.
33. **Wolucka, B.A., Goossens, A., and Inzé, D.** (2005). Methyl jasmonate stimulates the de novo biosynthesis of vitamin C in plant cell suspensions. J Exp Bot **56,** 2527-2538.
34. **Xia, X., Shafique, M.S., Huang, W., Liu, Y., Zhi, H., Wang, C., Zhou, Y., and Ji, Z.** (2025). Identification and fine mapping of blast resistance gene Rpg3(t) in foxtail millet (Setaria italica L.).

Phytopathology Research **7,** 83.

35. **Xue, D.X., Li, C.L., Xie, Z.P., and Staehelin, C.** (2019). LYK4 is a component of a tripartite chitin receptor complex in Arabidopsis thaliana. J Exp Bot **70,** 5507-5516.
36. **Yang, X., Wan, Z., Perry, L., Lu, H., Wang, Q., Zhao, C., Li, J., Xie, F., Yu, J., Cui, T., Wang, T., Li, M., and Ge, Q.** (2012). Early millet use in northern China. Proc Natl Acad Sci U S A **109,** 3726-3730.
37. **Zhang, K., Wang, G., Bao, M., Wang, L., and Xie, X.** (2019). Exogenous application of ascorbic acid mitigates cadmium toxicity and uptake in Maize (Zea mays L.). Environ Sci Pollut Res Int **26,** 19261-19271.
38. **Zhang, L., Li, S., Fang, X., An, H., and Zhang, X.** (2022). Genome-wide analysis of LysM gene family members and their expression in response to Colletotrichum fructicola infection in Octoploid strawberry(Fragaria × ananassa). Front Plant Sci **13,** 1105591.
39. **Zhao, Q., Liu, R., Zhou, Q., Ye, J., Meng, F., Liu, J., and Yang, C.** (2023). Calcium-binding protein OsANN1 regulates rice blast disease resistance by inactivating jasmonic acid signaling. Plant Physiol **192,** 1621-1637.
40. **Zou, M.Y., Nie, S.P., Yin, J.Y., and Xie, M.Y.** (2020). Ascorbic acid induced degradation of polysaccharide from natural products: a review. Int J Biol Macromol **151,** 483-491.

Figures

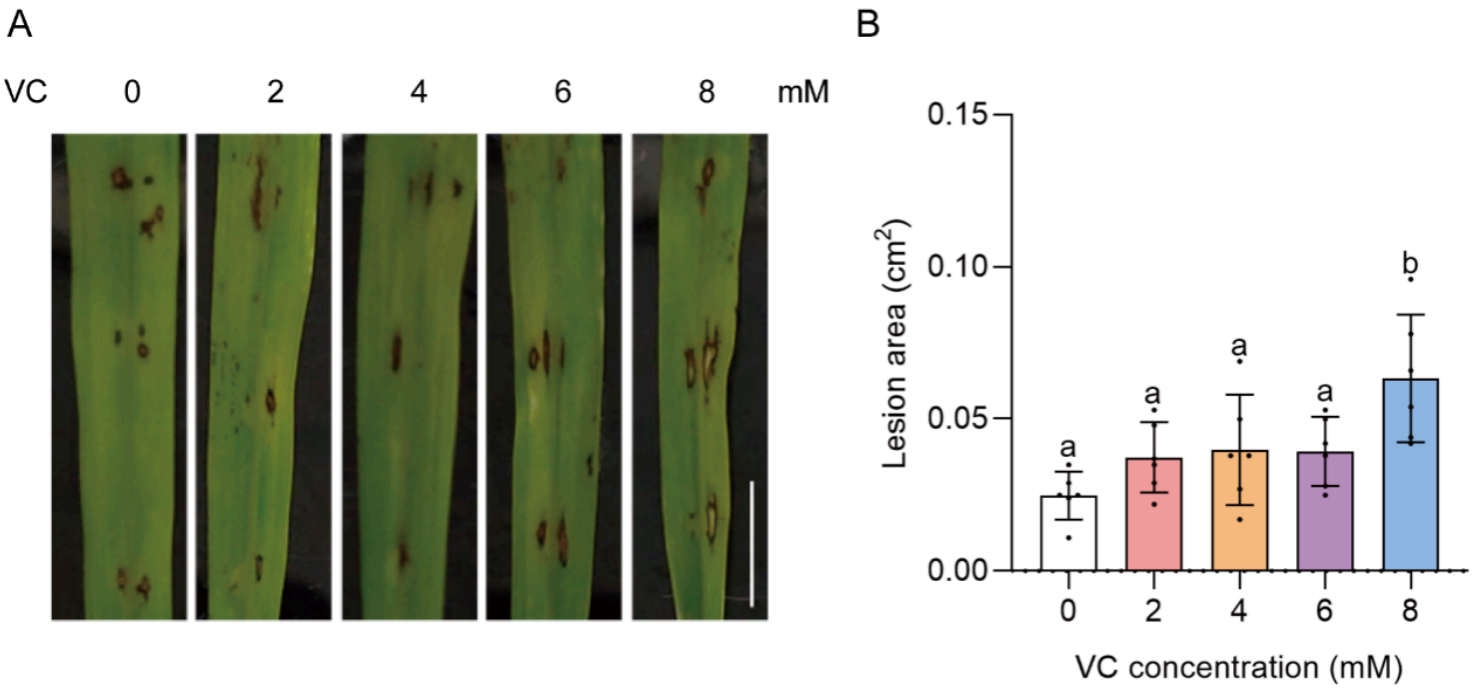


Figure 1

Effects of foliar spraying of vitamin C on resistance to blast in foxtail millet

A: Jingu 21 seedlings at the three-leaf stage were sprayed with vitamin C (VC) solutions at concentrations of 2, 4, 6 and 8 mM, with sterile water serving as the blank control (0 mM). Inoculation with *P. setariae* was carried out 24 h after foliar VC treatment. The prepared spore suspension (10^5 spores/mL) containing 0.02% Tween-20 in sterile distilled water was evenly sprayed onto leaf surfaces for inoculation, and photographs were captured at 3 days post-inoculation. Scale bar = 2 cm.

B: Statistical analysis of average lesion area. Columns represent the mean ± standard deviation of measured lesion areas. Different lowercase letters indicate statistically significant differences at $P < 0.05$.

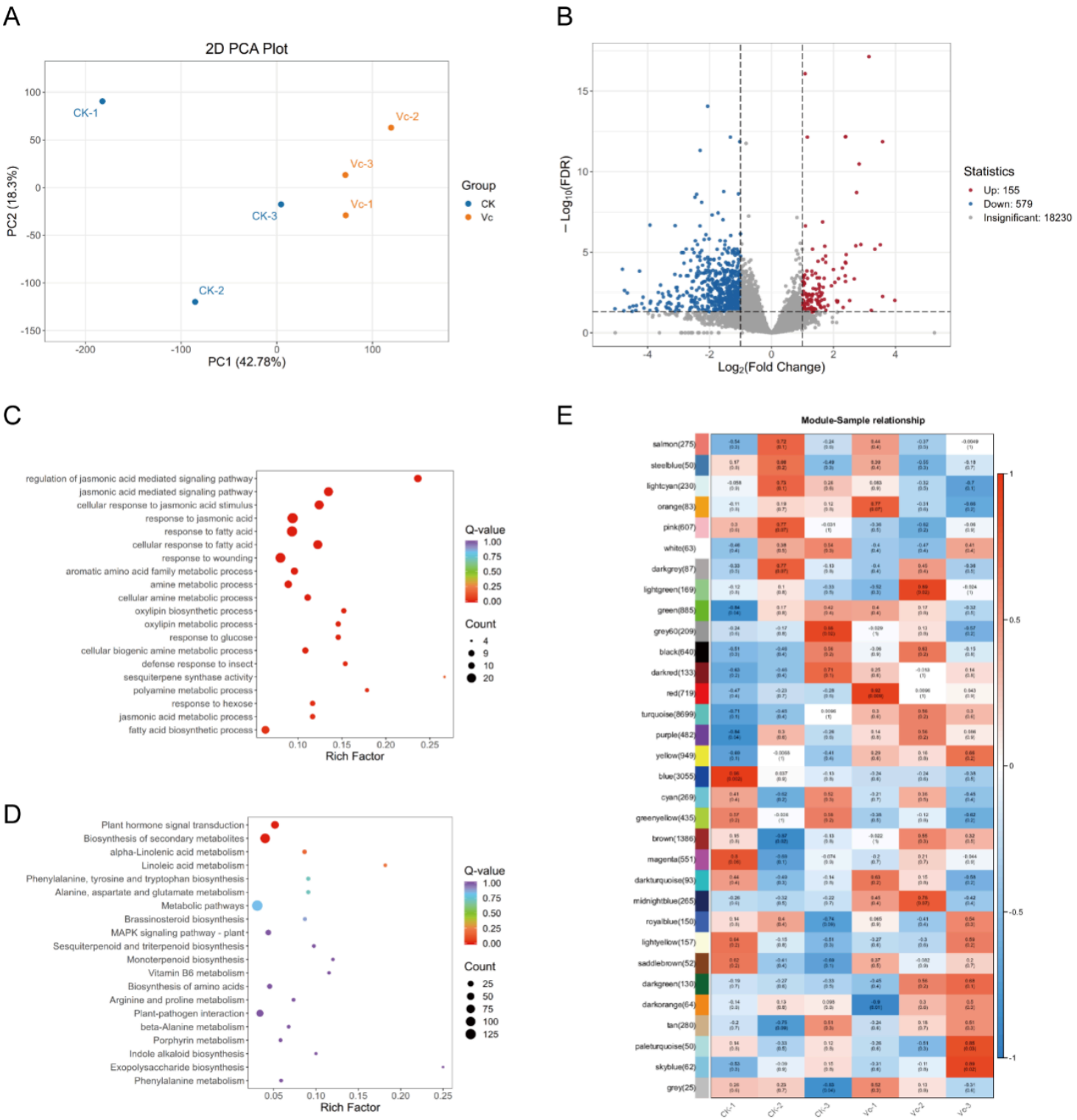


Figure 2

Transcriptomic analysis of foxtail millet after foliar vitamin C spraying

A: Principal component analysis (PCA) of transcriptome samples. B: Volcano plot illustrating differentially expressed genes (DEGs), with upregulated and downregulated DEGs separately marked. C:

GO functional enrichment of DEGs. D: KEGG pathway enrichment of DEGs. E: Module-sample association relationships of WGCNA.

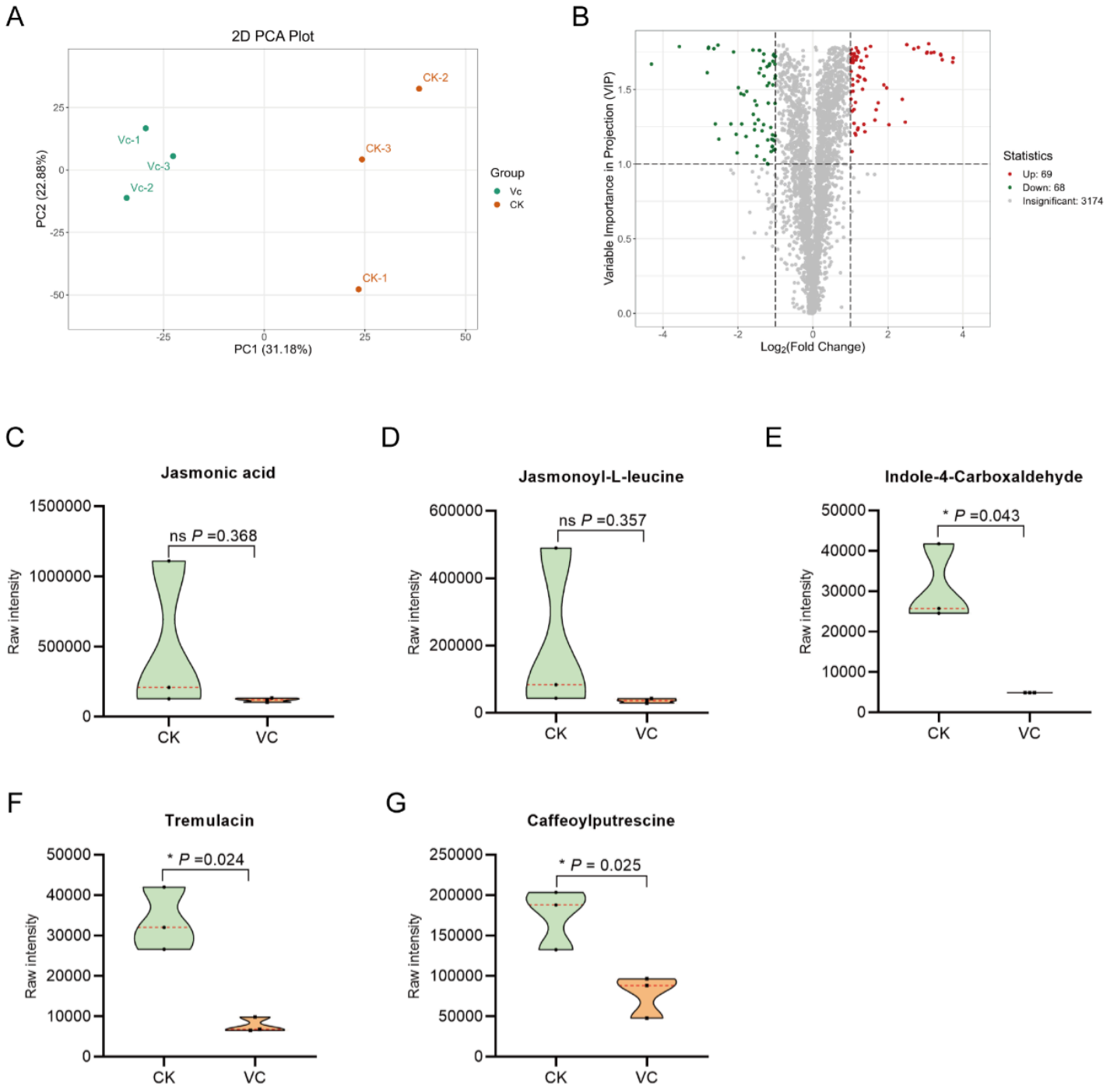


Figure 3

Metabolomic analysis of foxtail millet leaves after foliar spraying with vitamin C.

A: Principal component analysis (PCA) of metabolomic samples. B: Volcano plot showing differentially accumulated metabolites (DAMs) between the control and vitamin C treatment groups. C-H: Relative

accumulation levels of six metabolites related to plant immunity with significant differences between the two groups. The vertical axis represents the relative content of differential metabolites (original peak area). Each dot represents the value of a biological replicate. Asterisk indicate significant differences and ns means no significant difference.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementarymaterial.rar