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The mechanism by which exogenous GABA induces salt tolerance in *Phaseolus vulgaris* was revealed through integrated physiological, transcriptomic, and metabolomic analyses.

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Abstract

Background Soil salinization is a critical abiotic stressor that constrains crop production. By disrupting photosynthetic structures, inducing oxidative damage, and causing ion imbalance, it severely compromises the yield and quality of common bean (*Phaseolus vulgaris* L.). Gamma -Aminobutyric acid (GABA), a versatile non-protein amino acid, plays a pivotal role in regulating plant salt tolerance. However, whether GABA can effectively alleviate salt-induced damage in common bean, and the precise underlying regulatory mechanisms, remain to be fully elucidated. This study examines the effect of exogenous GABA on enhancing salt tolerance in common beans, laying the groundwork for breeding salt-tolerant varieties.

Results Using the salt-sensitive common bean cultivar ‘Kaiman Brown’ as material, this study systematically elucidated the GABA-mediated salt tolerance regulatory network through integrated physiological-biochemical, transcriptomic, pan-targeting

metabolomic, and WGCNA analyses. Results indicate that GABA significantly alleviates salt stress-induced inhibition of seedling growth by enhancing SOD, POD, and CAT activities, increasing osmotic regulator content, reducing MDA accumulation and electrolyte leakage, and improving photosynthetic systems. Additionally, GABA promotes synergistic oxygen-carboxylate metabolism via GABA shunting and 2-oxocarboxylic acid metabolism, thereby elevating citrate, aspartate, branched-chain amino acids, and proline levels. This reshapes osmotic homeostasis and carbon-nitrogen balance, laying a material foundation for subsequent stress responses. At the signal-regulation level, GABA effectively alleviates salt stress-induced inhibition of phospholipase PLIP1, thereby promoting its hydrolysis of ester bonds to generate lysophospholipids (LPL), thereby amplifying ABA signal intensity. By activating the PYR/PYL-PP2C-ABF core signaling module, it upregulates Perid genes to promote lignin synthesis, thereby fortifying cell walls and limiting Na⁺ influx. It also upregulates F3H genes to optimize flavonoid metabolism and increase rutin content, thereby enhancing the ROS scavenging capacity.

Conclusion This study clarifies the physiological and molecular mechanisms by which exogenous GABA mitigates salt stress injury in common bean, providing a theoretical basis and practical reference for the genetic improvement and cultivation of salt-tolerant common bean varieties.

Keywords: Common Bean; Salt stress; GABA; Integrated analysis of transcriptional and metabolic profiles

Introduction

Soil salinization poses a severe challenge to global agriculture. In China, 9.3×10^6 hectares of saline-alkali land lie within the cultivated land red line [1, 2], severely limiting agricultural development. Common beans (*Phaseolus vulgaris* L.), a globally significant food legume, are highly sensitive to salt stress. Under adverse conditions, they readily develop physiological disorders, including damage to the photosynthetic

system, disruption of antioxidant defense balance, and disturbance of metabolic homeostasis [3]. Salt stress significantly inhibits seed germination, as evidenced by concurrent reductions in initial germination vigor, germination rate, and germination index. During the seedling stage, it restricts primary root elongation, slows cotyledon growth, and impairs secondary root development [4,5]. Salt ion stress also disrupts plant ion homeostasis, inducing excessive sodium accumulation and impeding the transport and uptake of essential elements such as potassium, calcium, and magnesium, thereby triggering cellular metabolic disorders [6]. At the photosynthetic metabolic level, salt stress not only reduces total leaf chlorophyll content but also inhibits the activity of key enzymes such as RuBP carboxylase. This disrupts electron transport chains in the light reactions and reduces carbon assimilation efficiency in the dark reactions, ultimately affecting the accumulation and distribution of assimilates [7].

Researchers have widely validated the significant effects of exogenous regulatory substances in enhancing salt tolerance across multiple crops. Research indicates that these substances mitigate salt damage through multiple physiological pathways. In terms of stress signal transduction, exogenous Ga^{2+} is crucial for maintaining K^+/Na^+ ion homeostasis in honeysuckle under salt stress [8]; Regarding osmoregulation and redox balance, exogenous melatonin (MEL) significantly alleviates osmotic stress and oxidative damage in Ginkgo biloba and cotton seedlings [9, 10] exogenous abscisic acid (ABA), as a key stress-resistant hormone, plays a central regulatory role in mediating synergistic stress responses in maize and Siberian whitebark pine [11, 12]. Among numerous exogenous regulators, GABA has attracted significant attention for its dual roles in signal transduction and metabolic regulation. As a non-protein amino acid, GABA exhibits multidimensional regulatory characteristics in repairing plant salt damage [13, 14]. On one hand, GABA contributes to enhancing plant height [15], regulating leaf senescence [16], promoting vegetative growth [17], and modulating cytoplasmic pH changes [18]. Furthermore, GABA demonstrates significant efficacy in combating abiotic stress. Exogenous GABA application effectively mitigates salt damage in to-

mato [19], soybean seedlings [20], and wheat [15]. GABA alleviates abiotic stress-induced reactive oxygen species (ROS) accumulation by activating antioxidant defense mechanisms [21, 22], regulates chlorophyll content [23], modulates proline accumulation [24], and influences Ca^{2+} signaling pathways to promote phenolic compound accumulation [25]. On the other hand, GABA's mechanisms of action extend to more complex metabolic remodeling and molecular signaling networks. First, as a core signaling mediator, GABA induces systemic acquired resistance in plants by triggering cascade reactions of endogenous hormones. Research indicates that GABA and other compounds elevate levels of key stress hormones like salicylic acid (SA) and regulate the synthesis pathways of secondary metabolites such as phenylpropanoids and alkaloids, thereby constructing a more robust defense barrier at the molecular level [26, 27]. This cross-talk between hormones is a crucial mechanism by which plants respond to salt stress and maintain cellular homeostasis. Furthermore, as a metabolic pathway hub, GABA precisely regulates energy metabolism and carbon-nitrogen balance through feedforward regulation and GABA shunting [28]. Under abiotic stress, exogenous GABA modulates the transcriptional levels of key genes, such as GABA-T and polyamine metabolism (SSADH), alleviating the suppression of upstream transcription factors, such as LsMYB44 and LsWRKY12, induced by salt stress [29]. This remodeling of the transcription factor network not only promotes the self-compensatory accumulation of endogenous GABA but also optimizes energy output from the tricarboxylic acid (TCA) cycle [30], providing metabolic assurance for sustained plant growth under stress. Furthermore, GABA comprehensively enhances secondary metabolic defenses and basal nutrient utilization efficiency by strengthening enzyme reaction networks. Research indicates that GABA upregulates the activity of phenylalanine amide hydrolase (PAL) and tyrosine amide hydrolase (TAL), enzymes involved in phenolic biosynthesis, accelerating the accumulation of phenolic antioxidants [31]. Simultaneously, its positive regulation of nitrate reductase (NR) activity enhances nitrogen assimilation capacity in saline-alkali soils [32], thereby mitigating salt ion interference with fundamental plant nutrient metabolism. Although researchers have preliminarily demonstrated GABA's regulatory potential in model plants and certain vegetable crops, its

role in mediating the complete regulatory pathway "transcriptional response-metabolic flux-phenotype shaping" remains poorly understood in common bean, a salt-sensitive legume crop.

To elucidate the role of exogenous GABA in regulating salt tolerance in common bean, we used common bean seedlings as experimental material. Integrating multi-omics data, including transcriptomics and metabolomics, we systematically analyzed the physiological and molecular mechanisms underlying salt stress responses in common bean seedlings under exogenous GABA treatment, providing a theoretical basis for salt-tolerant breeding in leguminous crops.

Materials and Methods

Test Materials

The test material was the salt-sensitive common bean variety “Kaiman Brown”, selected earlier by the research team from 41 varieties. The Horticulture Team at Heilongjiang University provided the seeds.

Test Methods

Select plump, healthy seeds without damage and sow them into seed trays filled with vermiculite. When the seedlings develop two leaves and a heart, transfer them to hydroponic culture systems. After a 2d acclimation period, proceed with treatment. The research team previously screened a salt concentration of $100 \text{ mmol}\cdot\text{L}^{-1}$ and a GABA treatment concentration of $0.5 \text{ mmol}\cdot\text{L}^{-1}$ (Figure S1). Control and experimental groups were designated as CK (1/2 Hoagland nutrient solution), CK+GABA ($0.5 \text{ mmol}\cdot\text{L}^{-1}$ GABA + 1/2 Hoagland nutrient solution), NaCl ($100 \text{ mmol}\cdot\text{L}^{-1}$ NaCl solution + 1/2 Hoagland nutrient solution), and NaCl+GABA ($100 \text{ mmol}\cdot\text{L}^{-1}$ NaCl solution + $0.5 \text{ mmol}\cdot\text{L}^{-1}$ GABA + 1/2 Hoagland nutrient solution). We collected true leaves at 1, 3, 5, and 7 days post-salinity stress, rapidly pre-cooled in liquid nitrogen, and stored at -80°

C for systematic analysis of the following physiological and biochemical parameters: Chlorophyll content, photosynthetic parameters (including net photosynthetic rate, stomatal conductance, and intercellular CO₂ concentration), antioxidant enzyme activities (SOD, POD, CAT), osmotic regulators (soluble proteins, proline, and soluble carbohydrates), and membrane lipid peroxidation product malondialdehyde (MDA). We assessed plant growth indices at the end of the 7-day treatment period.

Measurement of Green Bean Biomass and Physiological Indicators

We determined relative moisture content and relative electrical conductivity according to the method described by Gao Junfeng [33]. Net photosynthetic rate (Pn), transpiration rate (Tr), stomatal conductance (Gs), and intercellular CO₂ concentration (Ci) were measured using the LI-6400 Portable Photosynthesis Meter (Li-Cor Inc, USA)—ultraviolet spectrophotometry for a, chlorophyll b, carotenoids, and total chlorophyll content. We measured superoxide dismutase (SOD) activity using the nitroblue tetrazolium (NBT) photochemical reduction method, determined peroxidase (POD) activity via the guaiacol method, and assessed catalase (CAT) activity using ultraviolet spectrophotometry [34]. We determined MDA using the thiobarbituric acid colorimetric method, measured soluble protein content by the Coomassie Brilliant Blue method, and assessed soluble sugar content via the anthrone colorimetric method. We analyzed proline content using the minhydrin colorimetric method [35].

RNA Extraction and Transcriptome Sequencing

Total RNA was isolated and purified from samples using TRIzol (Thermo Fisher Scientific, 15596018). We determined RNA concentration and purity using the NanoDrop ND-1000 spectrophotometer (NanoDrop, Wilmington, DE, USA). Following library preparation, we digested the dUTP-containing second strand with UDG enzyme (NEB, cat. m0280, MA, USA). PCR amplification conditions were as follows: 95°C pre-denaturation for 3 min, 98°C denaturation for 15 s, 60°C annealing for 15 s, 72°C extension for 30 s, repeated for 8 cycles. Final extension at 72°C for 5 min yielded a library with fragment sizes of 300 bp ± 50 bp (strand-specific library). After passing

quality control, the library underwent paired-end sequencing (PE150) on an Illumina Novaseq™ 6000 (LC Bio Technology Co, Ltd., Hangzhou, China) according to standard operating procedures.

Gene Annotation and Differential Gene Screening

We identified differentially expressed genes (DEGs) using DESeq2, with thresholds of $|\log_2 \text{ fold change (FC)}| > 1$ and $p\text{-value} < 0.05$. We then performed KEGG pathway enrichment analysis to detect significantly enriched pathways. Using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database, we further characterized the key biological pathways and signaling cascades associated with these DEGs.

Metabolite Screening and Analysis

Metabolites were extracted from samples using organic reagent precipitation, with multiple quality control (QC) samples prepared concurrently. Samples were analyzed by mass spectrometry in both positive and negative ion modes. Peak extraction and quality control were performed using XCMS software, while metabolite identification was conducted with metaX software. Identified metabolites were annotated using common functional databases, followed by quantitative analysis and sample correlation analysis. Differentially expressed metabolites were identified based on $VIP \geq 1$, fold change (FC) ≥ 2 , and $P\text{-value} < 0.05$. KEGG pathway enrichment analysis was subsequently performed to elucidate key biochemical, metabolic, and signaling pathways involved.

Construction of Weighted Gene Co-expression Networks

We performed gene co-expression network analysis using the WGCNA package in R. First, we conducted hierarchical clustering on all samples (3 biological replicates, totaling 18) based on Euclidean distances calculated from gene expression profiles. We combined core metabolite correlation analysis with outlier detection to ensure that no

outlier samples affected the results. To construct a biologically scale-free network reflecting biological properties, network topology analysis determined the soft power to be 3. The Dynamic Tree Cut algorithm was applied with a minimum module gene count of 30 and a mergeCutHeight of 0.25, ultimately identifying 23 co-expression modules (excluding gray modules). Subsequently, the eigengenes of each module were calculated, and Pearson correlation coefficients were used to assess the association between these characteristic genes and core metabolites. Genes within modules exhibiting high connectivity were defined as hub genes. We used Cytoscape (v3.9.1) to visualize co-expression topological relationships within key modules.

Data Analysis

Experimental data were organized and visualized in Excel 2019, and correlation and cluster analyses were performed in SPSS Statistics 26.0. Transcriptome and metabolome plots were generated using the LianChuan BioCloud tool (<https://www.omicstudio.cn/tool>). Final graphic layouts were compiled using Adobe Photoshop 2021.

Result

Exogenous GABA Alleviates Growth Inhibition and Enhances Photosynthetic Capacity in Common Bean Seedlings under Salt Stress

Salt stress significantly inhibited the morphological development and biomass accumulation of common bean seedlings. Compared with the control(contrCK), plant height, leaf area, fresh weight, and dry weight all decreased substantially under NaCl treatment, while root length and stem diameter also showed a reduced trend. Nevertheless, exogenous application of GABA effectively alleviated salt-induced damage. Compared with the NaCl-only treatment, the NaCl+GABA group showed significant increases in plant height, root length, leaf area, and total fresh weight, with total dry weight restored to the control level. Physiological analysis revealed that salt stress decreased relative leaf water content and induced severe electrolyte leakage. GABA addition significantly enhanced tissue water retention capacity, reduced relative electrical

conductivity, and maintained cell membrane integrity (Table 1). Stability of photosynthetic pigment content is fundamental to maintaining plant photosynthetic efficiency. Salt stress initially increased photosynthetic pigment levels, which then declined over time, significantly inhibiting carotenoid synthesis by days 5 and 7. In contrast, exogenous GABA significantly enhanced chlorophyll b and carotenoid accumulation by day 7, restoring them to normal levels (Fig. 1A). Salt stress imposes dual limits on plant photosynthetic capacity through both stomatal and non-stomatal factors. Measurements revealed that NaCl treatment significantly reduced net photosynthetic rate, transpiration rate, and stomatal conductance in common bean seedlings. In contrast, exogenous GABA increased leaf photosynthetic rate and stomatal conductance. GABA application in the early treatment phase markedly enhanced leaf transpiration rate, although this effect weakened in the later phase (Fig. 1B). In summary, exogenous GABA systematically enhances salt stress resistance in common bean seedlings by synergistically regulating water balance, protecting the cell membrane system, and optimizing photosynthetic pigment efficiency.

Table 1. Effects of Exogenous GABA on Phenotypes of Green Bean Seedlings

Treat- ment	plant height (cm)	Root length (cm)	Stem diam- ete (mm)	Leaf area (cm2)	Total fresh weight (g)	Total dry weight (g)	Relative moisture content (%)	Relative Conductiv- ity (%)
CK	61.63±1 .13a	33.12±0. 68a	2.69±0. 17a	62.91±2 .6a	9.07±0. 68a	0.85±0. 05b	92.48±1. 07a	21.73±0.22c
G	62.52±1 .82a	31.41±0. 84bc	2.6±0.0 9a	63.21±3 .14a	7.53±0. 74b	1.01±0. 05a	88.62±2. 44a	25.37±2.97c
N	33.55±1 .13c	30.2±1.0 5c	2.59±0. 13a	32.63±1 .93d	6.33±0. 08c	0.59±0. 08c	72.73±1. 67c	38.91±1.1a

NG	44.68±2	32.08±0.	2.6±0.0	44.81±2	8.65±0.	0.88±0.	78.30±2.	31.39±2.0b
	b	54ab	6a	.32c	53ab	03b	94b	

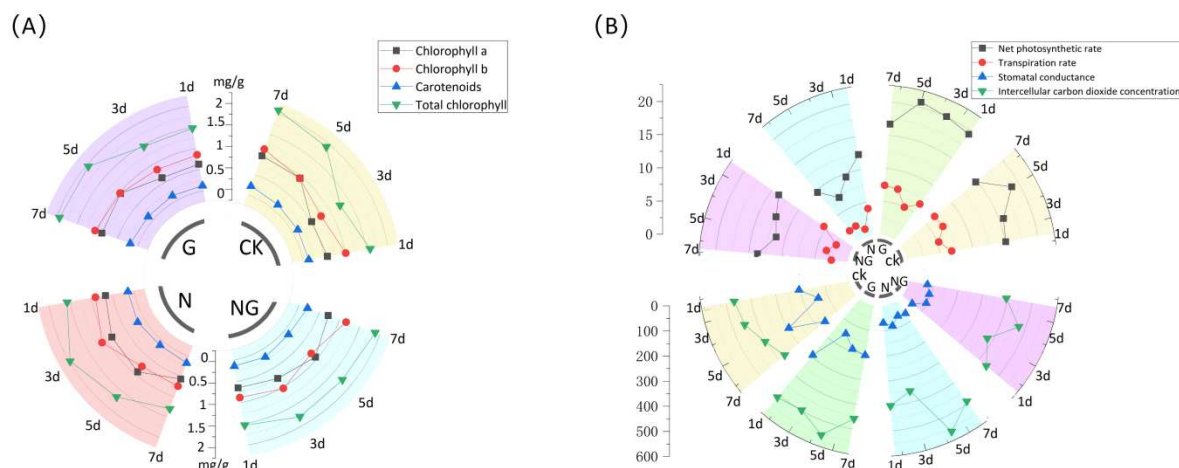


Fig.1. Effects of Exogenous GABA on Photosynthetic Parameters of Common Bean Seedlings under Salt Stress. Note: **(A)** Chlorophyll content. **(B)** Photosynthetic characteristics. The unit of net photosynthetic rate is $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, the units of transpiration rate and stomatal conductance are $\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, and the unit of intercellular CO_2 concentration is $\mu\text{mol}\cdot\text{mol}^{-1}$.

Effects of Exogenous GABA on Soluble Substances and Antioxidant Enzyme Activities in *Phaseolus vulgaris* Seedlings Under Salt Stress

Membrane peroxidation serves as a core indicator for assessing plant damage severity. Under NaCl treatment, MDA content in common bean seedling leaves showed a nonlinear pattern: an initial increase followed by a decrease, peaking on day 3. MDA levels on days 3 and 5 were significantly higher than those in the CK treatment, increasing by 61.62% and 63.77%, respectively. Under NaCl+GABA treatment, leaf MDA content on day 3 was significantly reduced by 25.59% compared to NaCl treatment. Compared to the CK treatment, GABA treatment showed no significant differences in leaf MDA content across all time points. The restoration of osmotic homeostasis is critical for plants to cope with salt stress. The study found that salt stress significantly increased the content of soluble sugars, soluble proteins, and proline in leaves, while

exogenous GABA further elevated these levels (Fig. 2A). These results suggest that exogenous GABA promotes the accumulation of soluble sugars and free amino acids in common bean seedlings, effectively mitigating the decrease in turgor pressure caused by cellular dehydration. Such osmotic adjustment enables common bean seedlings to maintain normal cellular physiological functions under salt stress. Beyond osmotic regulation, enhanced antioxidant enzyme activity represents another crucial pathway through which GABA alleviates salt damage. Results showed that NaCl treatment reduced leaf SOD, CAT, and POD activities, while NaCl + GABA treatment significantly increased these activities compared to NaCl alone (Fig. 2B). These findings indicate that exogenous GABA enhances antioxidant enzyme activity in seedlings. By regulating the antioxidant defense system of fava bean seedlings, it significantly enhances hydrogen peroxide metabolism capacity, effectively suppresses salt-stress-induced membrane lipid peroxidation, and consequently reduces the plant's salt damage index.

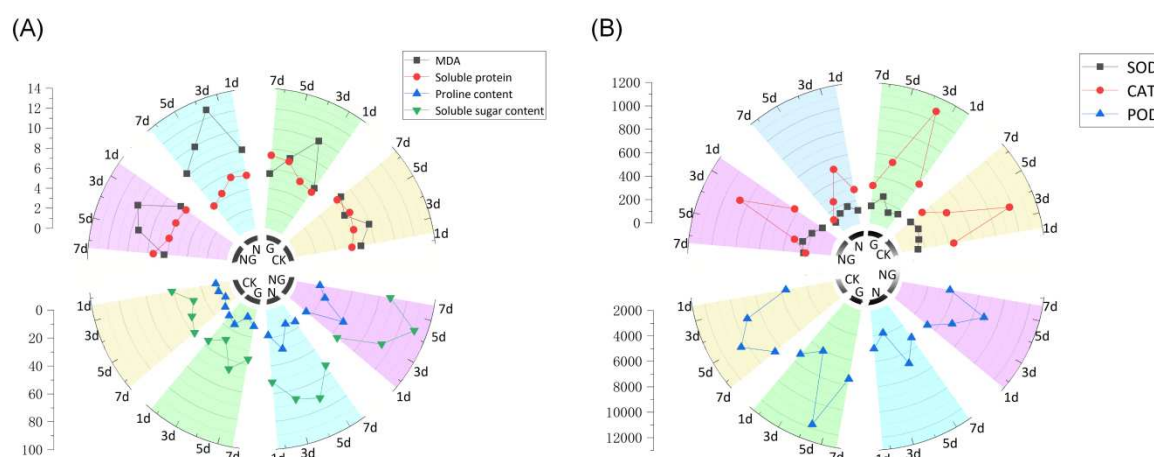


Fig. 2. Effects of Exogenous GABA on Soluble Substances and Antioxidant Enzyme Activities in Common Bean Seedlings under Salt Stress. Note: **(A)** Contents of MDA and osmo-regulatory substances. The unit of MDA content is $\mu\text{mol}\cdot\text{mol}^{-1}$, the units of soluble sugar and soluble protein contents are $\text{mg}\cdot\text{g}^{-1}$, and the unit of proline content is $\mu\text{g}\cdot\text{g}^{-1}$. **(B)** Activities of antioxidant enzymes. The units of SOD and CAT activities are $\text{U}\cdot\text{g}^{-1}\text{FW}$, and the unit of POD activity is $\text{U}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$.

Transcriptomic Analysis of Salt Stress Response in *Phaseolus vulgaris* Seedlings Under

Exogenous GABA Treatment

Strict filtering and quality control were performed on the raw sequencing data from 19 samples, ensuring sufficient sequencing depth and quality to support subsequent biological analyses (Table S1). The high correlation coefficient ($R^2 > 0.81$) among biological replicates indicates reliable data. Significant expression differences were observed between groups, particularly in the NG (NaCl + GABA) treatment group, which showed strong uniqueness. These results confirm that experimental treatments are the primary drivers of gene expression changes (Fig. S2). Based on RNA-seq data, differentially expressed genes (DEGs) were identified and analyzed (Fig. 3A). Among the DEGs identified from common bean seedling leaves, a total of 57 transcription factors (TFs) were annotated (Fig. 3C). The most representative TF families included MYB, bHLH, AP2/ERF, and WRKY. Quantitative analysis revealed that these TF families exhibited distinct differential response patterns across treatment stages (Fig. 3D). KEGG enrichment analysis revealed the spatiotemporal expression patterns of common beans under salt stress and following GABA treatment (Fig. 3E-H). Analysis indicates that during the early stage of salt stress, DEGs were significantly enriched primarily in photosynthesis-antenna proteins, flavonoid biosynthesis, phenylpropanoid biosynthesis, and plant hormone signal transduction pathways. Compared to NaCl treatment alone, GABA intervention significantly modulated plant hormone signaling and glucosinolate biosynthesis pathways while inducing upregulation of fatty acid degradation, α -linolenic acid metabolism, and branched-chain amino acid (valine/leucine/isoleucine) degradation pathways. As stress duration increased, the NaCl-treated photosynthesis-antenna protein pathway was replaced by plant hormone signaling as the new most significant pathway. In contrast, cutin, suberin, and wax biosynthesis, along with the MAPK signaling pathway, exhibited heightened activity.

In contrast, the NG24-treated group exhibited positive biological changes, including enhanced secondary metabolic activity, adjusted cell wall metabolism, and recovery of the photosynthesis-antenna protein pathway. Comprehensive analysis revealed con-

sistent enrichment of the plant hormone signaling pathway (Ko04075) and the phenylpropanoid biosynthesis pathway (Ko00940) across all four comparison groups. This system, closely linked to Ca^{2+} signaling, ROS homeostasis, and WRKY-mediated transcriptional regulation, constitutes the core molecular basis for GABA-induced salt stress adaptation in *Phaseolus vulgaris*. Notably, although salt stress suppressed the expression of the plastid phospholipase A1, PLIP1 (*Phvul. 010G112600.v2.1*), to extremely low levels, GABA application significantly upregulated its expression (Table S2). This result suggests that PLIP1 may be a key regulatory target in the GABA-mediated salt stress response pathway.

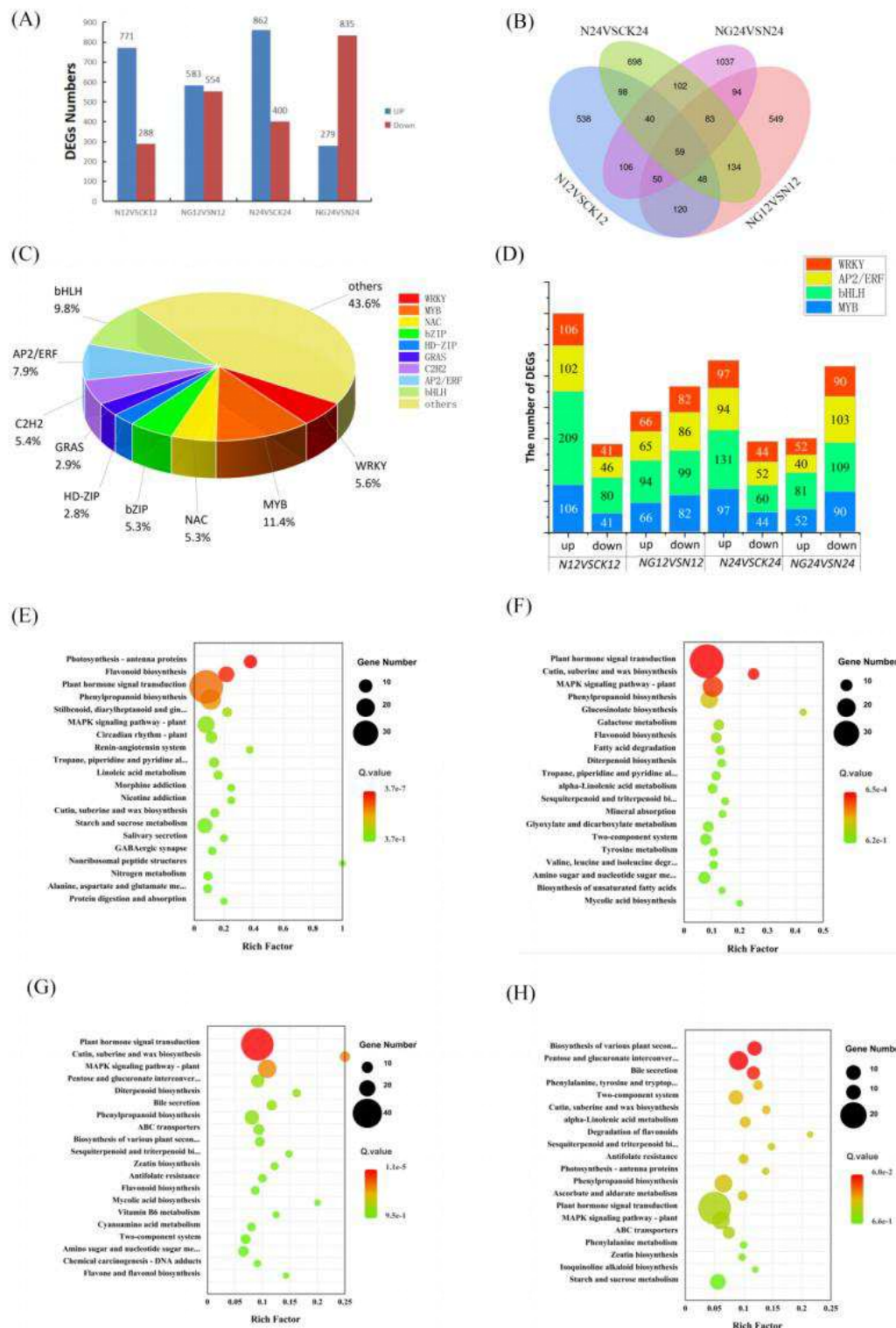


Fig. 3. Transcriptomics Data Analysis Results. Notes: **(A)** Bar chart of DEGs comparing each treatment **(B)** Venn diagram of DEGs among different treatments **(C)** Pie chart of TF family distribution among DEGs **(D)** Bar chart showing the number of up- and down-regulated DEGs in major TF families **(E)** KEGG pathways of N12VSCK12 DEGs **(F)** KEGG pathways of DEGs in NG12VSN12 **(G)** KEGG pathways of DEGs in N24VSCK24 **(H)** KEGG pathways of DEGs in NG24VSN24.

Metabolomics Analysis of Salt Stress Response in *Phaseolus vulgaris* Seedlings Under Exogenous GABA Treatment

Principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) were performed on metabolic data from 19 samples, followed by confidence testing (Fig. S3). The models were stable and demonstrated significant intergroup differentiation, rendering them suitable for subsequent analysis. Non-targeted omics analysis detected 125 differentially expressed metabolites (Fig. 4A), primarily categorized as 28 lipids, 2 nucleotides and their derivatives, 1 lignan and related compound, 15 organic acids and their derivatives, among others.

KEGG pathway enrichment analysis revealed the evolutionary patterns of metabolic networks under different treatments (Fig. 4C-F). At the 12-hour treatment point, compared to the control group, salt stress alone significantly induced galactose metabolism, ascorbic acid and aldonic acid metabolism, and the biosynthesis of aromatic amino acids (phenylalanine, tyrosine, tryptophan). In contrast, exogenous GABA addition triggered more extensive and pronounced metabolic reorganization. Enrichment results indicated significant trends toward increased activity in pathways including amino acid biosynthesis, plant secondary metabolite biosynthesis, and protein digestion and absorption in the NG12-treated group. These changes suggest that GABA activates the plant's defense and protective systems early in the stress response.

By the 24-hour treatment stage, metabolic signatures under salt stress alone shifted toward cofactor biosynthesis, histidine- and purine-derived alkaloid synthesis, and the accumulation of plant secondary metabolites. Notably, exogenous GABA exhibited high regulatory specificity during this phase: pathways including arginine and proline metabolism, alkaloid synthesis (derived from ornithine, lysine, and nicotinic acid), aminoacyl-tRNA synthesis, and protein digestion and absorption pathways were highly enriched. Particularly, the heightened activity in the arginine and proline metabolic pathways indicates that GABA application enhances the transport of osmotic regulatory substances (proline), optimizes protein turnover efficiency, and activates complex anti-

oxidant secondary metabolic networks, thereby systematically improving the salt tolerance of common bean seedlings.

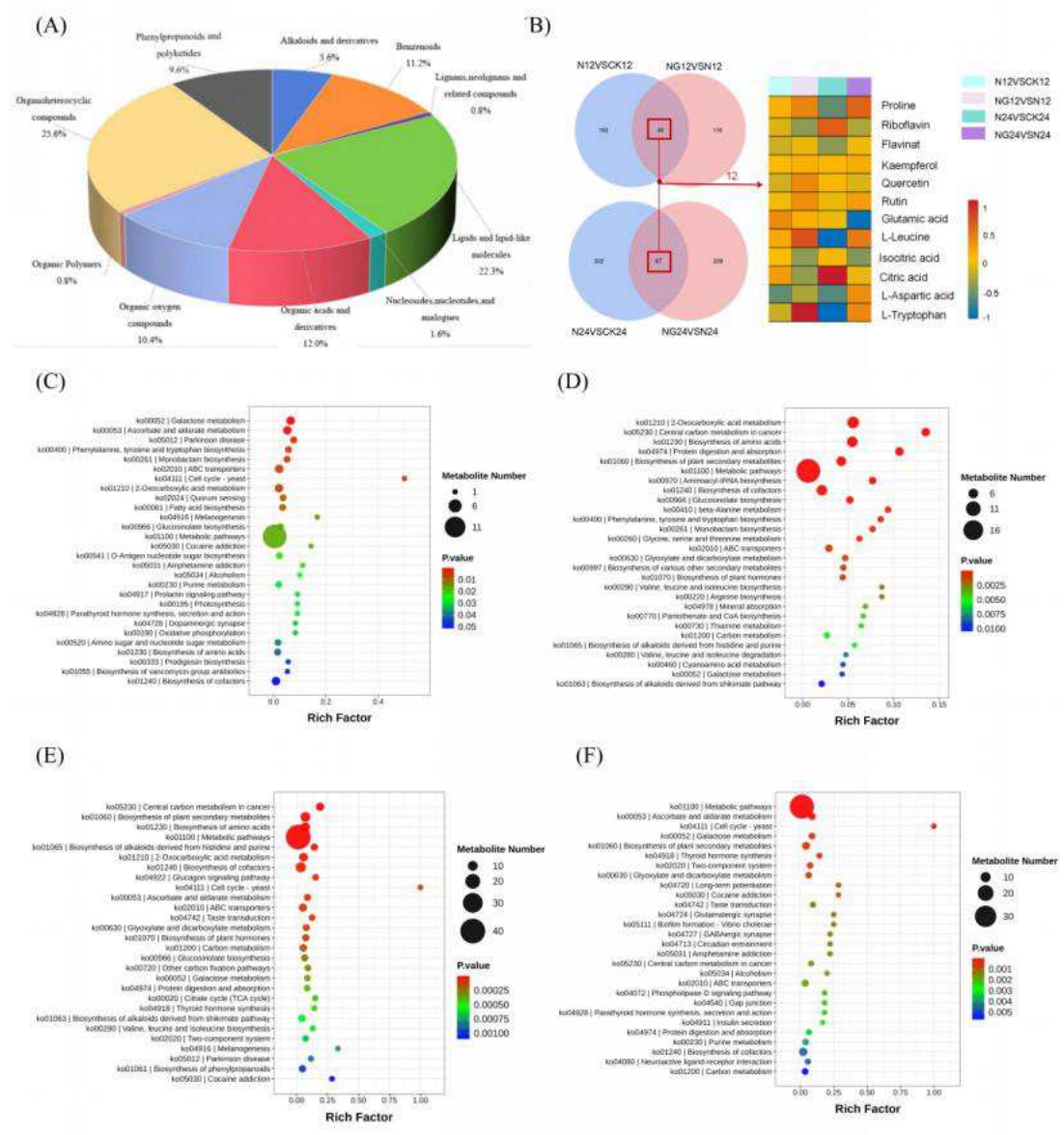


Fig. 4. Metabolomics data analysis results. Notes: **(A)** DAMs classification pie chart **(B)** Core metabolites among differentially altered metabolites under different treatments were identified using Venn diagram analysis; the heatmap displays the relative abundance of core metabolites **(C)** KEGG pathways of DAMs in N12V/SCK12 **(D)** KEGG pathways of DAMs in NG24V/SN24 **(E)** KEGG pathways of DAMs in N24V/SCK24 **(F)** KEGG pathways of DAMs in NG24V/SN24.

WGCNA identifies hub genes associated with core metabolites

Based on RNA-Seq data, we employed Weighted Gene Co-expression Network Analysis (WGCNA) to identify hub genes associated with core metabolites. Using 12

core metabolites common to all four treatment groups (Proline, Riboflavin, Flavinate, Kaempferol, Quercetin, Rutin, Glutamic acid, L-Leucine, Isocitric acid, Citric acid, L-Aspartic acid, L-Tryptophan) as trait files. WGCNA analysis was performed with a soft threshold of $\beta = 3$, and the unscaled network met the goodness-of-fit criteria (Fig. 5A). Hierarchical clustering identified 24 gene modules, with strong co-expression relationships among genes within each module (Fig. 5B). The MEblue module showed significant correlation with core metabolite levels (Fig. 5C). Focusing on hub genes within this module and considering node connectivity (degree values), gene interaction networks were visualized using Cytoscape (Fig. 5D). The top 14 hub genes with the highest degree values belonged to GS(Phvul.001G229500.v2.1, Phvul.007G152500.v2.1, Phvul.008G237400.v2.1), CHS (Phvul.002G184300.v2.1, Phvul.002G039166.v2.1), CCoAOMT (Phvul.002G305200.v2.1), CAD (Phvul.003G287500.v2.1), GDH (Phvul.004G080200.v2.1), CCR (Phvul.005G034800.v2.1), Perid (Phvul.005G154900.v2.1, Phvul.006G129800.v2.1), BCAT (Phvul.009G075100.v2.1), and PRODH(Phvul.008G022000.v2.1).

exhibits a robust response to GABA treatment. Results indicate that exogenous GABA induces upregulation of ABA receptors PYR/PYL, phosphatase PP2C, and downstream transcription factor ABF-related differentially expressed genes (DEGs). ABA significantly induced riboflavin accumulation. Under salt stress, common beans exhibited some riboflavin accumulation, but flavin adenine dinucleotide (FAD) content was severely impaired. However, GABA application significantly reversed this trend by promoting the conversion of riboflavin to FMN and FAD (Fig. 6A). This enhancement of the entire "receptor-transduction-response" pathway suggests that GABA increases common bean sensitivity to ABA signaling and its capacity to mediate osmotic regulation. Although salt stress induced chalcone synthase (CHS) expression and quercetin accumulation, its downstream antioxidant, rutin, remained at low levels. GABA treatment precisely regulated CHS expression, redirecting metabolic flux toward rutin conversion (Fig. 6B). This shift from a simple stress response to efficient antioxidant accumulation represents a key mechanism by which GABA mitigates salt damage. The phenylpropanoid biosynthesis pathway played a vital role in GABA-mediated defense responses (Fig. 6C). Under salt stress, key genes for lignin synthesis—such as cinnamoyl-CoA reductase (CCR), cinnamaldehyde dehydrogenase (CAD), and peroxidase (Perid)—were significantly suppressed. GABA treatment effectively activated transcription of these rate-limiting enzymes, promoting lignin deposition by enhancing the phenylpropanoid metabolic pathway. These results suggest that GABA enhances fava bean tolerance to osmotic stress by remodeling the cell wall's physical barrier. Exogenous GABA significantly enhances the osmotic regulation capacity of *Phaseolus vulgaris* by rebalancing 2-oxoglutarate metabolism and amino acid biosynthesis. Salt stress suppressed glutamate (Glu) accumulation and disrupted organic acid metabolism associated with the tricarboxylic acid (TCA) cycle, but GABA application effectively reversed this trend. At the transcriptional level, GABA induced synergistic upregulation of proline dehydrogenase (PRODH) and glutamate dehydrogenase (GDH), directly driving proline and glutamate accumulation (Fig. 6D). Furthermore, GABA treatment significantly increased citrate and L-aspartate accumulation, with upregulation of branched-chain amino acid transaminase (BCAT) expression, consistent with increased

Salinity is a widespread environmental stress factor that severely impedes sustainable land use and agricultural productivity. Salt stress significantly suppressed plant height, leaf area, and fresh weight in common bean seedlings, while inducing electrolyte leakage and elevated malondialdehyde (MDA) content—indicating damaged cell membranes. Exogenous application of GABA markedly improved these growth parameters, reduced electrolyte leakage and MDA levels, and restored membrane integrity, consistent with previous studies [36]. Maintaining proline homeostasis is crucial for sustaining cell division and enabling plants to achieve sustained growth under prolonged stress [37]. In this study, exogenous GABA application significantly increased proline, soluble sugar, and soluble protein content in common bean seedlings, thereby enhancing plant stress response and defense capabilities [38,39]. Reactive oxygen species (ROS) in plants are toxic byproducts of aerobic metabolism. Their removal primarily relies on antioxidant enzyme systems, including SOD, CAT, and POD, which are crucial for plant stress resistance. Research indicates that exogenous GABA application significantly increases POD, SOD, and CAT activity, enabling plants to exhibit greater tolerance to salt stress [40,41]. Salt stress causes a significant reduction or partial closure of leaf stomatal aperture in various crops [42]. This physiological change directly affects photosynthetic efficiency, resulting in synchronous decreases in intercellular CO₂ concentration and net photosynthetic rate. In this study, both chlorophyll content and photosynthetic parameters declined in salt-stressed common bean seedlings. GABA treatment significantly enhanced Pn, Gs, Tr, and chlorophyll content in the seedlings. This effect likely arises because exogenous GABA effectively alleviates the inhibition of chlorophyll-binding protein activity by salt stress, suppresses excessive chlorophyll degradation, promotes chlorophyll biosynthesis, mitigates reactive oxygen species damage to leaf structure, protects the integrity of photosynthetic pigments, and safeguards the photosynthetic electron transport process, thereby improving plant photosynthetic efficiency.

This study reveals that GABA regulation of 2-oxoacid metabolism is fundamental to maintaining the carbon-nitrogen balance. Salt stress induces isocitrate accumulation, reflecting compensatory activation of the TCA cycle under adverse conditions [43]. The

GABA shunt significantly enhances citrate and L-aspartate levels. Citrate acts as both a pH buffer and a Na^+ chelator, thereby mitigating ion toxicity [44,45] while L-aspartate serves as a vital nitrogen source for the synthesis of stress-related proteins [46]. During this process, GABA synergistically upregulates GS and GDH, ensuring efficient nitrogen assimilation under salinity. This robust nitrogen supply, coupled with the differential expression of hub genes BCAT and PRODH, facilitates the accumulation of branched-chain amino acids (BCAA) and proline. The increase in BCAAs, integrated with the carbon skeletons provided by 2-oxoacid metabolism, establishes the metabolic foundation for common bean seedlings to resist osmotic pressure. PLIP1, a chloroplast-localized lipase of the α/β -hydrolase family, specifically hydrolyzes the *sn*-1 ester bond of phospholipids to generate lysophospholipids (LPL) and free fatty acids (FFA). LPL functions as a lipid messenger that amplifies ABA signaling [47], further strengthening the plant's adaptive response. This study confirms that GABA initiates stress responses by regulating the core ABA signaling module (PYR/PYL-PP2C-ABF) [48]. Once activated, ABF transcription factors induce P5CS1 expression, thereby accelerating proline accumulation. Simultaneously, these factors mediate riboflavin biosynthesis, thereby increasing FAD content. As an essential electron acceptor, FAD works synergistically with PRODH to maintain the cyclic homeostasis between proline and glutamate. This mechanism not only safeguards cellular osmotic potential but also reserves energy substrates for subsequent growth recovery [49]. At the antioxidant level, GABA finely tunes the flavonoid biosynthetic pathway. By activating the core gene *CHS*, GABA promotes the accumulation of antioxidants such as quercetin. Notably, GABA treatment shifts the metabolic flux toward rutin, which possesses higher water solubility and transport capacity. This optimized allocation of metabolic resources—potentially mediated by Ca^{2+} or MYB transcription factors—more effectively scavenges salt-induced ROS and maintains the integrity of the photosynthetic structure [50, 51]. Salt stress typically inhibits lignin synthesis, as evidenced by the downregulation of *CCR*, *CAD*, and *Perid* genes; however, exogenous GABA exerts a significant restorative effect. GABA induces the coordinated upregulation of key genes involved in lignin monomer synthesis and polymerization (*CCoAOMT*, *CCR*, *CAD*, and *Perid*), thereby promoting

lignification. This response not only enhances the mechanical strength of the cell wall but also strengthens the physical barrier, restricting non-specific transmembrane transport of Na^+ . By mitigating ion toxicity at its source [52], GABA allows common bean seedlings to maintain higher RWC and turgor pressure, effectively alleviating the physiological drought damage caused by salinity (Fig. 7).

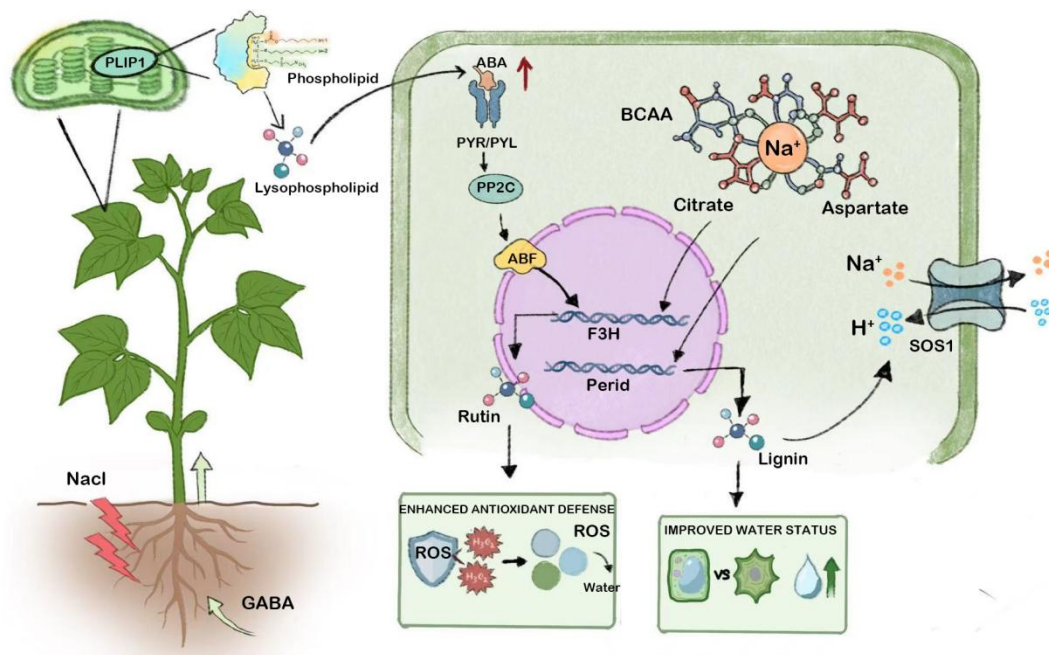


Fig. 7. The core process of enhancing plant salt tolerance through PLIP1-mediated ABA signaling after exogenous GABA treatment.

Conclusion

Combining physiological, transcriptomic, and metabolomic analyses, this study elucidates the regulatory mechanisms by which exogenous GABA enhances salt tolerance in common bean seedlings. Exogenous GABA synergistically integrates the GABA shunt and 2-oxoacid metabolism, thereby increasing citrate and aspartate levels. This metabolic shift drives the efficient accumulation of BCAAs and proline, providing the essential material basis for ABA biosynthesis, amino acid metabolism, and the synthesis of stress-responsive proteins. Simultaneously, GABA alleviates the salt-induced inhibition of the PLIP1 enzyme, promoting the hydrolysis of ester bonds to generate LPL. These molecules act as lipid messengers to amplify ABA signaling, which, in turn,

activates the core ABA signaling module (PYR/PYL-PP2C-ABF) to initiate downstream defense responses. Furthermore, the coordinated upregulation of *Perid* and associated genes facilitates lignin biosynthesis, reinforcing the physical barrier of the cell wall. This structural fortification restricts the transmembrane transport of Na^+ at its source, effectively mitigating ion toxicity. In terms of secondary metabolism, GABA directs flavonoid metabolic flux toward rutin—a derivative with superior water solubility and transport capacity—thereby significantly enhancing ROS scavenging efficiency. These multi-pathway synergies collectively counteract the physiological drought and ion toxicity induced by salinity, markedly alleviating the growth inhibition of common bean seedlings. This study not only clarifies the metabolic and molecular mechanisms underlying GABA-mediated salt tolerance but also identifies PLIP1 and its associated metabolic pathways as key targets for breeding broadly adaptable, salt-tolerant crops. These findings provide a robust theoretical foundation for ensuring stable crop yields under environmental stress.

Declarations

Abbreviations

GABA Gamma -Aminobutyric acid

WGCNA Weighted Gene Co-expression Network Analysis

SOD Superoxide Dismutase

POD Peroxidase

CAT Catalase

MDA Malondialdehyde

PLIP1 Phospholipase A1 PLIP1, chloroplasti

LPL lysophospholipids

ABA Absciscic Acid

ROS Reactive Oxygen Species

F3H flavanone 3-hydroxylase

Pn Net photosynthetic rate

Tr transpiration rate

Gs stomatal conductance

Ci intercellular CO₂ concentration

CHS Chalcone synthase

CCoAOMT Caffeoyle-CoA O-methyltransferase

CCR Cinnamoyl-CoA reductase

CAD Cinnamaldehyde dehydrogenase

Perid Peroxidase

PRODH Proline dehydrogenase

GS Glutamine synthase

GDH Glutamate dehydrogenase;

BCAT Branched-chain amino acid transaminase.

Ethics approval and consent to participate

All experimental procedures and plant materials used in this study comply with the relevant institutional, national, and international guidelines and legislation. No ethical approval or consent was required for this study, as the research was conducted on cultivated common bean (*Phaseolus vulgaris* L.) materials, which do not involve human subjects, vertebrate animals, or endangered plant species.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and/or analysed during the current study are available in the NCBI BioProject repository, under accession number PRJNA1452203, accessible at <https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1452203>.

Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Authors' contribution

Y. L. analyzed and interpreted the experimental data related to the effects of GABA on salt tolerance in common bean, and was a major contributor in writing the manuscript. D.J.L. conceived and designed the research project, curated the physiological indices (including MDA content, chlorophyll content, and antioxidant enzyme activities), and revised the draft. G.J.F. developed the experimental methodology, participated in data visualization, and assisted in manuscript revision. X.X.Y. acquired the funding support (Grant No. YQ2024C046) and supervised the entire research process.

T.F.Z. and C.L. validated the experimental findings and provided technical support for the lab work. Z.S.Y. performed the formal analysis and managed the data resources. Z.X.S. conducted the sample preparation and participated in the investigation. All authors read and approved the final manuscript.

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