

Multi-omics dissection reveals that fumarate serves as a metabolic hub linking Pro-Ca application to salt tolerance in rice tillers

Jin-Ji Tu

Guangdong Ocean University

Wen-Xin Song

Sun Yat-sen University

Wan-Xin Chen

Sun Yat-sen University

Zi-Yu Lu

Sun Yat-sen University

Yi Liu

Sun Yat-sen University

Qi-Cen Song

Sun Yat-sen University

Zi-Xun Huang

Sun Yat-sen University

Rui Deng

Guangdong Ocean University

Yi-Xi Dai

Guangdong Ocean University

Wen-Kang Huang

Guangdong Ocean University

Jing-Lin Wei

Hezhou University

Ying liu

Guangdong Ocean University

Nai-Jie Feng

Guangdong Ocean University

Quan-Sheng Qiu

Lanzhou University

Dian-Feng Zheng

zhengdf@gdou.edu.cn

Guangdong Ocean University
Ying-Bin Xue
Guangdong Ocean University

Research Article

Keywords: metabolome, rice, salt stress, tillering, transcriptome

Posted Date: September 14th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10755445/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.
[Read Full License](#)

Additional Declarations: No competing interests reported.

1 **Multi-omics dissection reveals that fumarate serves as**
2 **a metabolic hub linking Pro-Ca application to salt**
3 **tolerance in rice tillers**

4 Jin-Ji Tu¹, Wen-Xin Song², Wan-Xin Chen², Zi-Yu Lu², Yi Liu², Qi-Cen Song², Zi-Xun
5 Huang², Rui Deng¹, Yi-Xi Dai¹, Wen-Kang Huang¹, Jing-Lin Wei⁴, Ying Liu¹, Nai-Jie
6 Feng¹, Quan-Sheng Qiu³, Dian-Feng Zheng^{1*} and Ying-Bin Xue^{1*}

7 ¹South China Branch of National Saline-Alkali Tolerant Rice Technology Innovation
8 Center/College of Coastal Agriculture Sciences, Guangdong Ocean University,
9 Zhanjiang 524088, China

10 ²School of Agriculture and Biotechnology, Sun Yat-sen University, Shenzhen 518107,
11 Guangdong, China

12 ³MOE Key Laboratory of Cell Activities and Stress Adaptations, School of Life
13 Sciences, Lanzhou University, Lanzhou 73000, China

14 ⁴Hezhou University, Hezhou 542899, Guangxi, China

15 ***Corresponding author: Dian-Feng Zheng**, Email: zhengdf@gdou.edu.cn; **Ying-Bin**
16 **Xue**, Email: ybx@gdou.edu.cn

17

18 **Abstract**

19 **Background**

Soil salinization is a major abiotic constraint limiting rice growth and productivity worldwide. Rice (*Oryza sativa* L.) is moderately salt-sensitive, and salt stress significantly inhibits tillering, a key determinant of panicle number and final yield. Prohexadione calcium (Pro-Ca) is a novel plant growth regulator that inhibits gibberellin biosynthesis and has been shown to alleviate salt stress in rice seedlings. However, the molecular mechanisms by which Pro-Ca regulates salt tolerance in rice tillers remain poorly understood. In this study, we investigated the regulatory effects of exogenous Pro-Ca on salt-stressed tillers of the indica rice variety 9311 using integrated physiological, transcriptomic, and metabolomic approaches.

Results

Exogenous Pro-Ca treatment significantly alleviated salt-induced inhibition of tillering, increasing tiller number by 64.7%–137.5% compared with salt stress alone, while its effect on stem basal width was relatively modest. Pro-Ca enhanced antioxidant enzyme activities (SOD, APX, POD, CAT) by 4.9%–30.6%, reduced MDA accumulation by 15.2%–26.0%, and partially restored soluble sugar and protein levels. Ion analysis revealed that Pro-Ca decreased Na⁺ content by 50.7%, increased K⁺ content by 13.7%, and elevated the K⁺/Na⁺ ratio by approximately 1.3-fold, while IAA and SA contents increased by 40.0% and 22.5%, respectively. Transcriptomic analysis showed that Pro-Ca shifted the transcriptional response from passive damage repair (photosynthesis inhibition, cell wall degradation) toward active defense signaling, with significant enrichment of salicylic acid response, ethylene-activated signaling, and defense-related pathways. Metabolomic profiling revealed that Pro-Ca redirected metabolic flux from amino acid and energy metabolism toward alpha-linolenic acid metabolism, while Z-score analysis identified N-Carbamoylputrescine as a consistently upregulated key metabolite and 4-hydroxy-2-nonenal as a downregulated oxidative stress marker. Integrated multi-omics analysis pinpointed N-Carbamoylputrescine and Vanillin as central hubs linking gene expression changes with metabolic adjustments, and pathway integration revealed that the TCA cycle functions as a carbon–nitrogen metabolic hub, with fumarate significantly elevated across three interconnected

pathways. Furthermore, Pro-Ca activated cutin, suberine, and wax biosynthesis pathways, suggesting physical barrier reinforcement.

Conclusion

Our findings demonstrate that exogenous Pro-Ca enhances salt tolerance in rice tillers through a multi-layered synergistic strategy: reconstructing ion homeostasis by reducing Na⁺ accumulation while preserving K⁺ levels and coordinately elevating IAA (growth promotion) and SA (defense activation) contents; reprogramming the transcriptome from passive repair to active defense signaling, coupled with metabolic reprogramming of polyamine biosynthesis, lipid peroxidation alleviation, and TCA cycle maintenance; and reinforcing physical barriers through cutin, suberine, and wax biosynthesis. This study provides novel insights into the molecular mechanisms by which Pro-Ca regulates salt tolerance in rice tillers and offers a theoretical basis for the application of plant growth regulators in saline-alkaline rice production.

Keywords: metabolome; rice; salt stress; tillering; transcriptome

1 Abstract

Soil salinization represents one of the most significant abiotic stresses impacting global agricultural production, posing a serious threat to food security and sustainable agricultural development [1]. Over 10.7% of the world's land area, equivalent to nearly 1.4 billion hectares, is affected by salinization, with more than 25% of irrigated agricultural land experiencing constraints due to salt stress [2, 3]. This salt stress induces osmotic stress, ion stress from excessive Na^+ accumulation, and oxidative damage resulting from increased levels of reactive oxygen species (ROS), ultimately inhibiting plant growth and diminishing crop yields [4-6].

Rice (*Oryza sativa* L.) serves as the staple crop for over half of the global population, occupying more than 164 million hectares [7]. Nonetheless, rice exhibits moderate sensitivity to salt, with salt stress markedly hindering its growth and development. This stress results in reduced tillering and photosynthesis, ultimately diminishing both grain yield and quality [8, 9]. The tillering stage is critical, as it influences plant structure, effective panicle formation, and final yield [10]. The tiller node, a unique organ in Gramineous plants, serves as a vital site for the development of tiller buds and adventitious roots, functioning as a central hub for nutrient storage and tiller regulation [11, 12]. Research indicates that salt stress significantly decreases the number of tillers in rice, with the inhibition of tillers being more pronounced than that of the main stem [13]. However, current investigations into the response mechanisms of tiller nodes under salt stress remain limited, and the molecular regulatory network governing the adaptation of tiller nodes to salt stress is not yet fully understood.

Prohexadione calcium (Pro-Ca) is a novel cyclohexane carboxylic acid plant growth regulator that inhibits the conversion of GA_{20} to active GA_1 by suppressing the activities of GA_{20}ox and GA_3ox within the gibberellin biosynthesis pathway. Consequently, it delays vegetative growth while promoting reproductive development [14]. Recent studies have increasingly demonstrated that calcium dicyclate significantly enhances plant stress resistance. In rice, exogenous application of calcium dicyclate has been shown to effectively mitigate the inhibitory effects of salt stress on seedling

growth [15, 16]. During the tillering stage, foliar application of calcium dicyclate can significantly alleviate the adverse effects of salt stress on tillering growth, enhance the activities of superoxide dismutase (SOD) and peroxidase (POD) in the tillering leaves and stems, and reduce salt-induced damage to cell membranes [17, 18]. During the grain-filling period, calcium dicyclate regulates the source-reservoir relationship, facilitating the transport of photosynthetic products from the source (leaves) to the reservoir (grains) and ultimately enhancing rice yield [19]. Recent studies have demonstrated that calcium cyclidine maintains Na^+ and K^+ homeostasis by up-regulating the expression of E3 ubiquitin ligase genes and improves photosynthetic efficiency by down-regulating the expression of light-harvesting complex genes (*Lhcb1*, *Lhcb2*, *Lhcb3*, *Lhcb5*, *Lhcb6*) [20]. Additionally, calcium dicyclate mitigates the detrimental effects of salt stress by sustaining the levels of abscisic acid (ABA) and promoting the synthesis of oxylipin [21]. Zuo et al. utilized the random forest machine learning method to identify that the photosynthetic performance index (PIabs) and malondialdehyde (MDA) content were the most reliable predictors of rice biomass under the combined treatment of salt stress and calcium dicyclate [22].

Despite numerous reports detailing the physiological effects of calcium dicyclate in mitigating salt stress in rice, the transcriptional and metabolic regulatory mechanisms within the critical organ of tillers remain inadequately understood. Integrated analyses of transcriptomics and metabolomics offer a robust approach to elucidate the molecular mechanisms underlying plant stress responses [23-25]. In recent years, several studies integrating transcriptomic and metabolomic data related to salt stress in rice have been published in journals. However, most of these investigations have concentrated on the seedling stage or root system, resulting in a notable absence of comprehensive reports on the transcriptomic and metabolic regulatory networks of tillers subjected to salt stress and exogenous regulators [26-28].

In this study, the indica rice variety 9311 served as the experimental material. Through the measurement of physiological indices, transcriptome analysis, and metabolomics analysis, we systematically investigated the regulatory effects of exogenous Pro-Ca on the tillering nodes of rice subjected to salt stress. Our objective

was to delineate the transcriptomic and metabolomic response patterns of 9311 tillers under salt stress and to elucidate the regulatory networks through which exogenous Pro-Ca modulates gene expression and metabolite accumulation in these tillers. Additionally, we aimed to identify the key metabolic pathways and core gene-metabolite regulatory nodes involved in Pro-Ca-mediated salt tolerance. The findings of this study will provide new insights into the molecular mechanisms by which Pro-Ca enhances the salt tolerance of rice and establish a theoretical foundation for the rational application of plant growth regulators in rice production on saline-alkali land.

2. Materials and Methods

2.1 Test materials

The 9311 rice variety (*Oryza sativa* L.) was used as the test material. It was provided by the Germplasm Resource Bank of the College of Coastal Agriculture, Guangdong Ocean University. The concentration of the original solution of the chemical reagent Pro-Ca (Yuanye, Batch No. S31049-100g, purity 90% (BR)) used in this experiment was 5%, and the concentration of the regulator was 100 mg·L⁻¹. The reagents were provided by the Chemical Control Laboratory of Guangdong Ocean University.

2.2 Experimental design

According to the schematic diagram of the test device, this experiment was conducted at the College of Coastal Agriculture, Guangdong Ocean University (21.27°N, 110.32°E). When the rice seedlings had grown three leaves and one heart, they were transplanted into plastic pots. After green-up (six leaves and one heart) and before tillering, the plant materials were divided into four groups. A total of four treatments were established: (1) 0 (distilled water + 0% NaCl), (2) S (distilled water + 0.3% NaCl), (3) EA (100 mg·L⁻¹ Pro-Ca + 0% NaCl), (4) EAS (100 mg·L⁻¹ Pro-Ca + 0.3% NaCl). The third treatment group Pro-Ca (100 mg·L⁻¹ Pro-Ca + 0% NaCl) and the fourth treatment group Pro-Ca + S (100 mg·L⁻¹ Pro-Ca + 0.3% NaCl) were sprayed with 100 mg·L⁻¹ Pro-Ca on the leaf surface. The other two groups were used as controls and normal water was sprayed on the surface of the leaves. Spray evenly on both sides of the leaves and leaf sheaths until there is no dripping water. After 48 hours of external

application of Pro-Ca, the second treatment group S (distilled water + 0.3% NaCl) and the fourth treatment group (4) Pro-Ca + S (100 mg·L⁻¹ Pro-Ca + 0.3% NaCl) underwent the first sodium chloride treatment. The second sodium chloride treatment was carried out 24 hours after the first treatment, with a total addition of approximately 0.3% NaCl. The other two treatment groups were sprayed with normal water on the leaf surface. The salt content of the water layer is detected by the handheld SKD1688-TR-6 EC instrument (Beijing Shunkeda Technology Co., LTD., China) to ensure that the salt content remains relatively stable.

Rice plants were grown in a controlled chamber at 28°C with a 12 h light/12 h dark photoperiod. Light intensity was set to 25,000 lux, and relative humidity was maintained at 75%. Each treatment plants 50 potted plants. The plants were transplanted into plastic pots (19 cm diameter × 15 cm bottom diameter × 18 cm height) with a transplanting depth of 2 cm. Each pot contained four plants, spaced 4 cm apart, in 3 kg of red soil. The soil properties were as follows: organic carbon 32.4 g·kg⁻¹, available phosphorus 4.0 mg·kg⁻¹, available potassium 48.4 mg·kg⁻¹, alkali-hydrolyzed nitrogen 37.1 mg·kg⁻¹, and pH 7.23. Water levels in each pot were kept consistent throughout the growth period.

2.3 Measurements and methods

2.3.1 Determination of soluble sugar, soluble protein and malondialdehyde contents

Weigh 0.5g of the sample (fresh leaves or root systems), wash it clean and place it in a pre-cooled mortar. Add 10ml of 0.05mol/L pre-cooled phosphate buffer (pH7.8) in three portions and grind it into a homogenate on an ice bath. Transfer it to a centrifuge tube and centrifuge at 4 °C and 12000g for 20 minutes. The supernatant is the crude protein extract. Take 1ml of the enzyme solution, add 5ml of Coomassim brilliant blue solution, shake well, and measure OD595 after 2 minutes of reaction.

$$\text{Protein content(mg/g)} = (C/\mu\text{g} \times V_T/\text{ml}) / (W/\text{g} \times V_s/\text{ml} \times 1000)$$

Fresh samples (0.5 g) were chopped and homogenized, or dried sample powder (5–100 mg) was weighed and placed into large test tubes. Distilled water (15 mL) was added, and the tubes were heated in a boiling water bath for 20 min. After cooling, the

extracts were filtered into 100 mL volumetric flasks. The residues were rinsed several times with distilled water, and the filtrates were made up to volume with distilled water. An aliquot of the sample extract (1.0 mL) was mixed with 5 mL of anthrone reagent, and the absorbance was measured following the anthrone colorimetric procedure. Each sample was analyzed in triplicate. The malondialdehyde (MDA) content was determined via the thiobarbituric acid method[29].

2.3.2 Determination of antioxidant enzyme activity

The activities of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX) were determined spectrophotometrically. The tillering nodes of rice were selected, frozen in liquid nitrogen, and then ground. An amount of 0.50 g was accurately weighed and placed in 10 mL of 0.05 mol·L⁻¹ pre-cooled phosphate buffer (pH 7.8), ground into a homogenate, and centrifuged at 12,000*g for 20 min at 4 °C. The supernatant obtained was used to determine the activity of antioxidant enzymes. SOD activity was assayed by monitoring the inhibition of photochemical reduction of nitroblue tetrazolium (NBT) [30]. The reaction mixture (3 mL) contained 50 mM phosphate buffer (pH 7.8), 13 mM methionine, 75 μM NBT, 2 μM riboflavin, 0.1 mM EDTA, and 50 μL of enzyme extract. The reaction was initiated by illuminating the mixture under fluorescent light (4000 lux) for 15 min at 25 °C, and the absorbance was read at 560 nm. One unit of SOD activity was defined as the amount of enzyme required to inhibit 50% of the NBT photoreduction. POD activity was determined using guaiacol as the substrate [31]. The reaction mixture (3 mL) consisted of 50 mM phosphate buffer (pH 7.0), 20 mM guaiacol, 40 mM H₂O₂, and 100 μL of enzyme extract. The increase in absorbance due to the oxidation of guaiacol was recorded at 470 nm for 3 min at 25 °C. One unit of POD activity was defined as the amount of enzyme causing a change of 0.01 in absorbance per minute. The activity of ascorbic acid peroxidase (APX) was measured by using the sulfenicylic acid method [32]. Catalase (CAT) activity was determined by spectrophotometry (Shanghai Yuanxi Instrument Co., Ltd., Shanghai, China) [33].

2.3.3 Extraction and Determination of Endogenous Hormones

Hormone determination was carried out on the roots of rice tillers, and four replicates were set up for each sample. Fifty milligrams of the ground sample were weighed, and 10 μ L of an internal standard mixed solution (100 mg/mL) and 1 mL of an extractant of methanol, water, and formic acid in a 15:4:1 volumetric ratio were added to the sample and mixed thoroughly. The mixture was vortexed for 10 min and then centrifuged at 4°C and 12,000 rpm for 5 min. The resulting supernatant was transferred to a new centrifuge tube for concentration. After concentration, the extract was redissolved in 100 μ L of 80% methanol/aqueous solution, filtered through a 0.22 μ m membrane filter, and transferred to a vial for LC-MS/MS analysis. It was carried out by Wuhan Metewei Biotechnology Co., LTD. (Wuhan, China) using the UPLC-ESI-MS /MS system, which consists of Nexera X2 ultra-performance liquid chromatograph (Shimadzu, Kyoto, Japan) and 4500 QTRAP triple quadrupole linear ion trap mass spectrometer (AB Sciex) It is composed of Framingham, Massachusetts, USA. Chromatographic separation was performed using an Agilent SB-C18 column (1.8 μ m, 2.1 mm $\times$ 100 mm; Agilent Technologies, Santa Clara, CA, USA). The mobile phase is Solvent A (water containing 0.1% formic acid, v/v) and Solvent B (acetonitrile containing 0.1% formic acid, v/v). The gradient elution procedure is: 0-9.0 min, 95% A $\rightarrow$ 5% A; 9.0-10.0 min, 5% A; 10.0-11.1 minutes, 5% A $\rightarrow$ 95% A; The flow rate was 0.35 mL min⁻¹, the column temperature was maintained at 40°C, and the injection volume was 4 μ L. Data acquisition was performed via ultra-performance liquid chromatography. Qualitative analysis of the mass spectrometry data was conducted using MWDB (Metware Database v5.0), which was constructed based on known standard substances. Quantitative analysis was conducted through the multi-reaction monitoring mode of triple quadrupole mass spectrometry. The mass spectrometry data were processed using Analyst 1.6.3 software. Each treatment group included 4 biological replicates.

2.3.4 Determination of sodium, potassium, calcium, and chlorine elements

The samples were transferred to digestion tubes (approximately 0.1 g each). A few drops of distilled water were added to moisten the samples, followed by 5 mL of 98%

sulfuric acid (H₂SO₄). The tubes were covered with bent funnels and allowed to stand overnight. The following day, the samples were placed in a digestion furnace at 280 °C for approximately 1 h until the digests turned brown. During this period, 4–5 drops of hydrogen peroxide (H₂O₂) were added three times to catalyze the oxidation until the digests became clear or colorless. The samples were then further digested for an additional 20 min in the digestion furnace. After cooling, the digests were diluted to a final volume of 50 mL with double-distilled water[34].

Potassium (K⁺) and sodium (Na⁺) concentrations were determined using a flame photometer (FP640, Shanghai Instruments and Electrical Analysis Co., China). Calcium (Ca²⁺) concentration was determined using an atomic absorption spectrophotometer (or an inductively coupled plasma optical emission spectrometer, ICP-OES) following the manufacturer's instructions. Chloride (Cl⁻) content was determined by silver nitrate titration (Mohr method) or by ion chromatography. Briefly, an aliquot of the digested solution was titrated with 0.01 mol/L AgNO₃ solution using potassium chromate (K₂CrO₄) as the indicator, and the Cl⁻ concentration was calculated from the AgNO₃ consumption. All measurements were performed with four biological replicates[35].

2.4 Total RNA extraction and transcriptome analysis

On the 7th day of NaCl stress, RNA was extracted from the junction of the rice roots and stems. Transcriptome sequencing was completed by Suzhou Panomik Biomedical Technology Co., Ltd. Sequencing was performed within 24 h of completion of each treatment, and three biological replicates from each treatment were analysed. Total RNA was qualitatively and quantitatively analysed using a Drop and Agilent2100 Bioanalyzer (Thermo Fisher Scientific, Massachusetts, USA) [36]. SOAPnuke was used to obtain clean read segments. Then, the clean read segments were compared with the reference gene sequence using Bowtie2. The differentially expressed genes (DEGs) with Log₂FC (absolute value) >1 and P<0.05 were screened by Phyper and analysed by KEGG [36]. The transcriptomic data generated in this study is available in the NCBI SRA database under the accession number PRJNA1370692. The temporary submission

ID is SUB15812931, and the data will be publicly accessible starting on November 10, 2029 at the following link: <https://www.ncbi.nlm.nih.gov/sra/PRJNA1370692>.

2.5 Metabolite analysis

The same materials were used for metabolite analysis and transcriptomics. LC-MS/MS technology was employed for non-targeted metabolomics analysis. The raw mass spectrometry data (raw files) were collected using LC-MS/MS and imported into Compound Discoverer 3.1 (Thermo Fisher Scientific, USA) for data processing. The metabolomics analysis process involved data preprocessing, statistical analysis, classification and annotation of metabolites, and functional annotation. Three biological replicates were analysed for each treatment. Metabolite extraction was carried out in accordance with the scheme described by Beltran A et al. (2012), which provided detailed methods for sample preparation and metabolite extraction. [37].

2.6 qRT-PCR analysis

To verify the reliability of the RNA sequencing data, real-time fluorescence quantitative polymerase chain reaction (RT-qPCR) was used to detect the expression levels of six randomly selected DEGs in the transcriptome and the internal reference gene UBQ5. Each gene was analysed in three biological replicates. Total RNA was reverse transcribed into single-stranded cDNA using TransScript® All-in-One First-Strand cDNA Synthesis SuperMix for qPCR (one-step gDNA removal). qRT-PCR was performed using an ABI Quant Studio 6 Flex thermal cycling apparatus (USA) and SYBR Green PCR kit (Trans, China), according to the manufacturers' instructions. Up18S rRNA was used as the internal control [38]. The relative expression was calculated using the $2^{-\Delta\Delta CT}$ method. All reactions were conducted in triplicate, and three biological replicates were analysed for each gene (Supplementary Table S1).

2.3.7 Statistical Analysis

Data were collated using Excel 2010. Statistical analyses were performed using SPSS 24.0. Pearson's bivariate correlation analysis was used to assess the relationships between variables. All statistical tests were conducted at a significance level of $p < 0.05$. Graphs were generated using Origin 21.

3 Results and Analysis

3.1 Effects of exogenous Pro-Ca on rice tillering under salt stress

The tillering responses of the rice variety 9311 to salt stress were assessed from 7 to 21 days, alongside the dynamics of stem basal width (Figure 1A). The results indicated that salt stress significantly inhibited tillering. At each time point, the number of tillers under salt stress (S) was lower than that of the control (0). Specifically, the degree of inhibition for 9311 from 7 to 21 days was 38.5%, 26.3%, and 19.0% lower than that of the control group, respectively (Figure 1B). Additionally, under salt stress, the stem basal width of 9311 at each time point was also reduced compared to the control, with inhibition rates of 16.6%, 17.4%, and 18.8% on the 7th, 14th, and 21st days, respectively (Figure 1C).

The application of prohexadione-calcium (Pro-Ca) under salt stress (EAS) significantly mitigated the inhibition induced by salt. In comparison to salt treatment alone (S), EAS resulted in increases in tiller number of 137.5% on day 7, 78.6% on day 14, and 64.7% on day 21 (Figure 1B). Regarding stem basal width, EAS partially countered the reduction caused by salt stress, with increases of 14.8% on day 7, 3.9% on day 14, and 5.1% on day 21 relative to S (Figure 1C). These findings demonstrate that exogenous Pro-Ca effectively alleviates the suppressive effects of salt stress on tillering, while its impact on stem basal width is relatively modest.

Fig 1. Effects of exogenous Pro-Ca on rice tillering under salt stress. A: Growth phenotype; B: Wide stem base; C: Tiller number. The experimental data are expressed as the mean and standard error (SE) of four biological replicates. 0 indicates that only distilled water is used for treatment, S represents salt stress treatment, EA represents Pro-Ca treatment, and EAS represents Pro-Ca treatment under salt stress. Significance analysis was conducted using the Duncan model, and different lowercase letters indicate significant differences ($P<0.05$). The base width of the tillering node was measured using a digital caliper to ensure that the measurement was carried out at the widest point of the tillering base. The number of tillers is recorded by counting all visible tillers that appear from the base of each plant. Five biological replicates were set for each treatment group, with three plants in each replicate.

3.2 The effect of exogenous application of Pro-Ca on antioxidant enzymes and oxidative damage in 9311 tillers under salt stress

The activities of superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), and peroxidase (POD) in rice variety 9311 were measured at 7, 14, and 21 days under control (0), salt stress (S), and EAS. The results demonstrated that salt stress significantly induced the activities of all four antioxidant enzymes. Compared with the control (0), SOD activity under salt stress (S) was increased by 20.2%, 47.1%, and 31.7% on the 7th, 14th, and 21st days, respectively (Figure 2A). APX activity was increased by 25.9%, 22.6%, and 36.0% (Figure 2B). POD activity was increased by 9.8%, 25.0%, and 20.3% (Figure 2C). CAT activity was increased by 36.5%, 29.5%, and 30.8% (Figure 2D). The application of Pro-Ca under salt stress can significantly increase the activity of antioxidant enzymes. Specifically, EAS increased SOD activity by 11.5% (day 7), 12.1% (day 14), and 19.0% (day 21) relative to S (Figure 2A). For APX, EAS increased activity by 30.6% (day 7), 17.0% (day 14), and 15.4% (day 21) (Figure 2B). POD activity was increased by 9.2% (day 7), 14.9% (day 14), and 4.9% (day 21) (Figure 2C). CAT activity was increased by 12.6% (day 7), 18.9% (day 14), and 6.2% (day 21) (Figure 2D). These results indicate that exogenous Pro-Ca enhances the antioxidant capacity of rice under salt stress by further upregulating the activities of key ROS-scavenging enzymes, thereby alleviating oxidative damage.

As shown in Figure 2E, salt stress (S) significantly increased MDA content compared with the control (0), with elevations ranging from 39.4% to 297.8% across 7–35 days, and the highest increase at day 35. Application of Pro-Ca under salt stress (EAS) effectively reduced MDA accumulation, achieving mitigation rates of 15.2%–26.0% relative to S alone. For soluble sugar (Figure 2F), salt stress induced substantial accumulation (27.4%–66.3% above control), while EAS lowered soluble sugar levels by 8.3%–15.9% compared to S. Similarly, soluble protein content (Figure 2G) was markedly increased by salt stress (49.2%–124.6% above control), and EAS mitigated this increase by 14.9%–20.9% relative to S. Collectively, these results indicate that

exogenous Pro-Ca alleviates salt-induced oxidative damage and osmotic imbalance by reducing MDA accumulation and partially restoring soluble sugar and protein levels.

Fig 2. The effect of exogenous application of Pro-Ca on antioxidant enzymes and oxidative damage in 9311 tillers under salt stress. A-D: Activities of superoxide dismutase (SOD), ascorbic acid peroxidase (APX), peroxidase (POD), and catalase (CAT). (E) Accumulation of MDA. (F) Soluble sugar content. (G) Soluble protein content data were expressed as mean $\pm$ SE (n=6). Different letters indicate significant differences among treatments at the same time point ($p < 0.05$).

3.3 The effect of exogenous Pro-Ca on the element content and hormone content of rice tillers under salt stress

One of the primary challenges plants encounter under salt stress is ion toxicity and the disruption of K^+/Na^+ homeostasis due to excessive Na^+ accumulation. This study demonstrated that exogenous Pro-Ca treatment (EAS) significantly altered the ion balance in tillers subjected to salt stress (Figure 3A). Compared to the S treatment, EAS treatment decreased Na^+ content by 50.7%, increased K^+ content by 13.7%, and elevated the K^+/Na^+ ratio by approximately 1.3 times. Additionally, EAS treatment resulted in a 55.1% reduction in Ca^{2+} content relative to the S treatment, suggesting that Pro-Ca may have mitigated passive Ca^{2+} influx by alleviating stress intensity. Notably, Cl^- content increased by 31.3% following EAS treatment, which may be associated with enhanced anionic segregation induced by Pro-Ca to maintain charge balance. These findings indicate that Pro-Ca effectively facilitates the reconstruction of ionic homeostasis in tillers under salt stress by reducing Na^+ and Ca^{2+} accumulation while preserving K^+ levels (Figure 3B). Furthermore, compared to the salt stress treatment (S), exogenous Pro-Ca treatment (EAS) significantly elevated the levels of IAA and SA in tillers (Figure 3C, D). The IAA and SA contents in the EAS treatment group increased by 40.0% and 22.5%, respectively, compared to the S treatment group ($P < 0.05$).

Fig 3. Effects of exogenous Pro-Ca on ion homeostasis and phytohormone levels in rice tillers under salt stress. A: Determination of Cl, Ca, Na and K element contents in tillering nodes of rice under EAS and S treatment groups; B: Bar stacking diagram analysis of the contents of the four elements; C: IAA hormone content; D: SA hormone content.

3.4 Transcriptomic Analysis

3.4.1 Transcriptome Sequencing Results and Identification of Differentially Expressed Genes

The transcriptome sequencing of tiller segments from 9311, treated with NaCl under four conditions: 0 (distilled water + 0% NaCl), S (distilled water + 0.3% NaCl), EA (100 mg·L⁻¹ Pro-Ca + 0% NaCl), and EAS (100 mg·L⁻¹ Pro-Ca + 0.3% NaCl), was conducted to elucidate the mitigation mechanism of Pro-Ca under salt stress. The analysis yielded between 37,335,738 and 55,668,350 high-quality valid reads for each sample. The Q20 and Q30 values of the sequencing data ranged from 98.41% to 98.61% and 95.37% to 95.71%, respectively (Appendix 1, S2), indicating that the quality of the transcriptome data was excellent and appropriate for subsequent in-depth analysis. Principal component analysis (PCA) demonstrated significant differences among the control group (CK), treatment group S, treatment group EA, and treatment group EAS of 9311 (Figure 5D). Differentially expressed gene (DEG) analysis identified 1,132 DEGs in the 9CK-VS-9S treatment group, comprising 695 upregulated and 437 downregulated genes (Figure 5A). In the 9S-VS-9EA treatment group, 1,434 DEGs were identified, with 1,168 upregulated and 266 downregulated genes (Figure 5B). The 9S-VS-9EAS treatment group revealed 269 DEGs, including 144 upregulated and 125 downregulated genes (Figure 5C). Notably, 47 genes were downregulated in the 9CK-VS-9S treatment group while being upregulated in the 9S-VS-9EAS treatment group, whereas 94 genes were upregulated in the 9CK-VS-9S treatment group and downregulated in the 9S-VS-9EAS treatment group. Additionally, both Os05g0225500 and Os03g0418000 were upregulated in both processing groups. A total of 53 common DEGs were identified across the three comparison groups (Figure 5F).

Fig 4. Distribution of Sequencing Data and Differentially Expressed Genes (DEGs). A: Volcano plot of differential genes for 9CK-VS-9S. B: Volcano plot of differential genes for 9CK-VS-9EA. C: Volcano plot of differential genes for 9S-VS-9EAS. D: Principal component analysis of DEGs. Venn diagrams of differentially expressed genes E: 9CK-VS-9S and 9S-VS-9EAS. Venn diagrams of differentially expressed genes F: 9CK-VS-9S, 9CK-VS-9EA, and 9S-VS-9EAS.

3.4.2 GO enrichment analysis and KEEGG analysis

To elucidate the molecular mechanisms by which Pro-Ca mitigates salt stress, we performed Gene Ontology (GO) enrichment analysis across three comparisons (Figure 4, Supplementary Table S3-6). The findings revealed that in the 9S-vs-9CK group, there was significant enrichment of terms associated with photosynthesis inhibition (chloroplast thylakoid membrane, photosynthesis), cell wall degradation (chitin binding, glucanase), and tobacco amine synthesis. Additionally, the activity of transcription factors was notably enriched, indicating that salt stress elicited a passive survival response (Figure 4B). In contrast, the 9EA-vs-9CK group exhibited only a modest upregulation of photosynthetic capture and chitin activity, resulting in limited effects (Figure 4A). The key finding is that the enrichment spectrum of the 9EAS-vs-9S group has undergone a fundamental transformation. Photosynthesis-related items have completely disappeared, while there has been significant enrichment in salicylic acid response (GO:0009751), the ethylene-activated signaling pathway (GO:0009873), and the disease resistance and defense signaling pathway (GO:0009870). Additionally, chitinase activity has been further enhanced (Figure 4C). This transformation suggests that Pro-Ca does not merely reverse salt damage; rather, it actively engages the endogenous immune network of rice. This engagement shifts cellular resources from passive damage repair to the regulation of defense hormone signals, thereby synergistically enhancing antioxidant enzyme activity, reducing MDA accumulation, and stabilizing osmotic balance (Figure 4).

To further investigate the metabolic regulatory network of Pro-Ca in mitigating salt stress, we performed KEGG pathway enrichment analysis across three comparative groups ($Q\text{value} < 0.05$). The findings revealed that in the 9S-vs-9CK group, photosynthetically related pathways were significantly inhibited, including the

photosynthetic antenna protein (ko00196), photosynthesis (ko00195), and global metabolic pathways (ko01100). Conversely, there was an induction of phenylpropanoid biosynthesis (ko00940) and zeaxanthin biosynthesis (ko00908). This suggests that salt stress leads to the inhibition of energy metabolism while activating secondary metabolism (Figure 4E). In contrast, following the application of Pro-Ca (9EA-vs-9CK), the enrichment profile underwent a substantial transformation, with photosynthetically related pathways no longer showing significance. Instead, glycerophospholipid metabolism (ko00564), cysteine and methionine metabolism (ko00270), amino acid and nucleotide sugar metabolism (ko00520), and riboflavin metabolism (ko00740) emerged as the primary significant pathways, with most genes in these categories being downregulated (all glycerophospholipid metabolism was downregulated). Cysteine metabolism exhibited a downregulation of 75% (Figure 4F). This shift indicates that Pro-Ca does not directly "repair" the photosynthetic system. Rather, it enhances cell membrane stability and REDOX balance by modifying membrane lipid composition, regulating sulfur amino acid metabolism, and facilitating the synthesis of antioxidative cofactors (riboflavin), thereby promoting metabolic adaptation to salt stress.

To further validate the findings of the KEGG enrichment analysis, representative differentially expressed genes were selected from glycerol phospholipid metabolism (ko00564), cysteine/methionine metabolism (ko00270), and starch/sucrose metabolism (ko00500). Their expression changes under salt stress (S) and salt stress combined with regulators (EAS) treatments were compared (Figure 4G). The results revealed that genes associated with glycerol phospholipid metabolism, including *Os01g0720400* and *Os03g0832800*, were significantly downregulated in EAS. This suggests that Pro-Ca may inhibit phospholipase activity or membrane lipid degradation, thereby mitigating lipid peroxidation under salt stress, which aligns with the observed reduction in MDA content. In cysteine/methionine metabolism, *Os05g0149400* (ACO5) was upregulated in EAS, while *Os03g0338000* (transaminase) was downregulated. This indicates that Pro-Ca may facilitate stress adaptation by modulating the interplay between ethylene

signaling and sulfur metabolism (Figure 4G). Furthermore, the downregulation of *Os05g0153500* (trehalose phosphatase) in starch/sucrose metabolism in EAS may contribute to the maintenance of osmotic regulator balance (Figure 4G). The transformation of these gene expression patterns further substantiates that Pro-Ca collaboratively establishes a defensive metabolic network under salt stress through membrane lipid remodeling, sulfur assimilation, and the reprogramming of glucose metabolism.

Fig 5. GO enrichment analysis and KEGG enrichment analysis. A: GO enrichment map of 9CK-VS-9EA. B: GO enrichment map of 9CK-VS-9S. C: GO enrichment map of 9S-VS-9EAS. D: The KEGG enrichment plot of 9CK-VS-9EA. E: KEGG enrichment plot of 9CK-VS-9S. F: KEGG enrichment map of 9S-VS-9EAS. G: The expression levels of key genes in glycerophospholipid metabolism (G1, G2), cysteine metabolism (C1, C2), and starch metabolism (S1), G1: *Os01g0720400*, G2: *Os03g0832800*, C1: *Os05g0149400*, C2: *Os03g0338000*, S1: *Os05g0153500*.

3.5 Metabolomics analysis

3.5.1 Overview of metabolomics data and screening of differential metabolites

Salt stress significantly altered the metabolic profile of rice tillers, while Pro-Ca treatment effectively mitigated stress-induced metabolic disorders. PCA analysis revealed (Figures 5A, C) a distinct trend of inter-group separation among the three sample groups, 9CK, 9S, and 9EAS, with good clustering of intra-group samples. This indicates that the metabolic differences between the groups were statistically significant and that the model's predictive capability was robust. The statistics of differential metabolites indicated (Figure 5-B) that a total of 1,040 differential metabolites were identified in the 9S-VS-9CK comparison group, of which 582 were up-regulated and 458 were down-regulated. In the 9S-VS-9EAS comparison group, the total number of differential metabolites decreased to 784, with 354 up-regulated and 430 down-regulated.

Based on the OPLS-DA model, with a variable importance in projection (VIP) threshold of >1 and a $\log_2FC$ of ≥ 1 , key differential metabolites were identified. In the 9S-VS-9CK comparison group, the volcano plot (Figure 5E) indicated that Coenzyme Q2 and 3-Methyloctadecane were significantly upregulated. Conversely, 2-Hydroxy-4-trifluoromethyl benzoic acid and Gluconic acid were significantly downregulated. In the 9S-VS-9EAS comparison group (Figure 5D), gamma-Pentachlorocyclohexene, Piperic acid, and Multifidol exhibited significant upregulation. In contrast, 2-Aminobenzoic acid, Indican, Velosimine, and L-leucyl-L-leucyl were significantly downregulated. Notably, Piperic acid displayed opposing regulatory trends across the two comparison groups, being downregulated in 9S vs 9CK and upregulated in 9S vs 9EAS. This suggests that exogenous Pro-Ca treatment may mitigate the inhibitory effects of salt stress on secondary metabolic pathways by reversing the accumulation direction of this metabolite. Among these metabolites, Coenzyme Q2, a crucial component of the mitochondrial electron transport chain, may be upregulated in response to enhanced energy metabolism under salt stress. The downregulation of Gluconic acid implies a reprogramming of the pentose phosphate pathway or glycolic acid metabolism in the context of salt stress. These key differential metabolites present significant targets for elucidating the metabolic network through which Pro-Ca regulates salt tolerance in rice.

Fig 6. Overview of metabolomics data and screening of differential metabolites. A, C: Sample OPLS-DA score plots for 9S-VS-9CK and 9S-VS-9EAS. B: Statistics detailing the number of differential metabolites. D, E: Scatter plots illustrating the charge ratio and p-value of differential metabolites for 9S-VS-9EAS and 9S-VS-9CK.

3.5.2 KEGG enrichment analysis and identification of key metabolites

KEGG pathway enrichment analysis revealed that, under salt stress, the differential metabolites of the 9311 tiller were predominantly enriched in amino acid metabolism and energy metabolism pathways. Treatment with exogenous calcium

cyclobrate (Pro-Ca) further reshaped the metabolic response network. In the 9S-VS-9CK comparison group, the differential metabolites were significantly enriched in pathways such as arginine biosynthesis, alanine, aspartate, and glutamate metabolism, phenylpropanoid biosynthesis, the citrate cycle, plant hormone signal transduction, and linoleic acid metabolism (Figure 6A). In the 9S-VS-9EAS comparison group, the differential metabolites were significantly enriched in alpha-linolenic acid metabolism, linoleic acid metabolism, phenylpropanoid biosynthesis, arginine and proline metabolism, and plant hormone signal transduction (Figure 6B). Both groups exhibited co-enrichment in arginine metabolism, phenylpropanoid biosynthesis, and plant hormone signal transduction pathways. This indicates that the arginine metabolic pathway and phenylpropanoid metabolic pathway were significantly activated under salt stress, while Pro-Ca treatment specifically enriched the alpha-linolenic acid metabolic pathway. These findings suggest that exogenous Pro-Ca may enhance the plant defense response by promoting the biosynthesis of jasmonic acid (JA) mediated by alpha-linolenic acid. Furthermore, the enrichment of the TCA cycle and photosynthetic biological carbon fixation pathways in the 9S-VS-9EAS group further indicates that Pro-Ca treatment aids in maintaining energy metabolism and carbon assimilation efficiency under salt stress.

Z-score analysis revealed that the metabolite profile of the 9311 tillers underwent significant reshaping under salt stress, with treatment using exogenous calcium procyclate (Pro-Ca) further modifying the metabolic response pattern. In the 9S-VS-9CK comparison group, N-Carbamoylputrescine, inositol cyclic phosphate, and gluconic acid were significantly upregulated. Conversely, L-glutamic acid, thymidine, and L-arginine were significantly downregulated (Figure 6C). In the 9S-VS-9EAS comparison group, N-Carbamoylputrescine, gluconic acid, 3-methyloctadecane, and tyramine were significantly upregulated, while 4-hydroxy-2-nonenal (4-HNE), gibberellin A91, linoleoyl ethanolamide, and 9-oxo-ODE were significantly downregulated (Figure 6D).

Fig 7. KEGG enrichment analysis and identification of key metabolites. A, B: KEGG enrichment analysis for 9S-VS-9CK and 9S-VS-9EAS; C, D: Z-score analysis of differential metabolites for 9S-VS-9CK and 9S-VS-9EAS.

3.6 Integrated Transcriptomic and Metabolomic Analysis Reveals Pro-Ca-Mediated Metabolic Reprogramming Under Salt Stress

Collectively, the multi-omics integration revealed a coordinated transcriptional-metabolic reprogramming in response to Pro-Ca application under salt stress, pinpointing N-Carbamoylputrescine and Vanillin as central hubs that link gene expression changes with metabolic adjustments, while also uncovering widespread transcriptional-metabolic decoupling in key pathways such as lignin biosynthesis and energy metabolism.

Correlation network analysis identified a core metabolic module centered on N-Carbamoylputrescine, which was tightly correlated with multiple genes (*Os01g0944000*, *Os02g0216300*, *OsPAP10a*, *OsRCI2-9*), whereas transcription factors *ERF77*, *WRKY45*, and *ACO5* showed strong negative correlations with these metabolites (Figure 7A). Additionally, the pteridine compounds 7,8-Dihydroneopterin and 7,8-dihydromonapterin were highly correlated with *Os01g0944000*, *Os02g0216300*, and *OsPAP10a*, suggesting that this module may jointly regulate polyamine and nitric oxide (NO) signaling pathways to enhance salt tolerance.

Enzyme-substrate coupling analysis revealed a marked transcriptional-metabolic decoupling under salt stress (Figure 7B). Despite significant down-regulation of *OsCOMTL6* ($\log_2FC = -1.77$), its substrates (Sinapyl alcohol, Coniferaldehyde) and product (SAH) all increased, implying post-transcriptional or allosteric compensation in lignin monomer methylation. Similarly, while *RCH10* was down-regulated, other chitinase genes (*OsXIP*, *C10122*, *C10150*) were up-regulated, yet the common substrate N-Acetyl-D-glucosamine remained nearly unchanged, indicating functional redundancy within this enzyme family. Moreover, the sharp drop

in *Os05g0155000* ($\log_2\text{FC} = -4.81$) contrasted with a significant increase in its substrate Nadide ($\log_2\text{FC} = 1.00$), further supporting decoupling in NAD^+ -dependent energy metabolism.

Canonical correlation analysis (CCA) demonstrated a strong concordance between the two omics layers, with CCA1 explaining 95.07% of the total covariance (Figure 7C). Metabolites such as Indican, N-acetylcitrulline, Cyclo(L-leucyl-L-leucyl), and Velosimine clustered at the negative end of CCA1, while genes including *OsACPI*, *Os01g0944000*, *OsIPS1*, and *OsPAP10a* showed identical loading directions, confirming that Pro-Ca-induced transcriptional changes are tightly coupled with metabolic shifts.

O2PLS analysis further pinpointed Vanillin as the most influential feature connecting the two datasets, with the highest score (0.28) on the common component (Figure 7D). Other key connectors included Tyromycic acid, Panaquinquecol 7, and genes *OsSPX1*, *OsJRL*, *OsIPS2*, *OsIPS1*, *OsCCD1*, and *OsACPI*. The prominent position of Vanillin, a hallmark of phenylpropanoid metabolism, highlights the importance of this pathway in Pro-Ca-mediated metabolic coordination under salt stress.

Fig 8. Combined analysis of transcriptomics and metabolomics. A: Correlation network diagram. Triangles represent differentially expressed mRNAs, while dots signify differentially expressed metabolites. The color depth indicates the magnitude of the fold change. The color of the dotted lines reflects the correlation value; darker colors correspond to higher correlation values. B: Enzyme-substrate association analysis. The length of the columns corresponds to the absolute value of $\log_2(\text{FC})$, indicating that substances with longer columns exhibit greater differences in their respective omics and have a more significant impact on enzymatic reactions. C: Typical correlation analysis. Within the same area, points farther from the plateau indicate closer mutual

heights, resulting in higher correlation. D: O2PLS analysis. The load value for the two groups indicates the strength of the association; a greater absolute value suggests a stronger connection between the elements and the other group being analyzed.

3.7 Integrated analysis of Pro-Ca-mediated metabolic pathways in response to salt stress

The integrated analysis of the four metabolic pathways, as illustrated in Figure 8D, reveals that in the tillers of rice subjected to salt stress and treated with Pro-Ca, the TCA cycle functions as a central hub linking carbon and nitrogen metabolism. Fumarate levels were significantly elevated across three pathways: the TCA cycle, phenylalanine metabolism, and alanine-aspartic acid-glutamic acid metabolism. This finding suggests that Pro-Ca may facilitate the provision of carbon skeletons necessary for amino acid metabolism and the synthesis of secondary metabolites by enhancing the replenishment reactions within the TCA cycle. Furthermore, the activation of biosynthetic pathways for keratin, cork, and wax indicates that Pro-Ca also contributes to the formation of physical barriers, thereby synergistically improving the salt tolerance of rice.

As illustrated in Figure 8A, the comparison between the 9S-VS-9EAS groups revealed a significant upregulation of the TCA cycle intermediate product fumarate. This pathway exhibits a close metabolic association with arginine biosynthesis as well as the metabolism of alanine, aspartic acid, and glutamic acid. The TCA cycle intermediates connect to L-aspartate through oxaloacetate, with L-aspartate serving as an upstream substrate for arginine biosynthesis. Additionally, the branches of the TCA cycle that include succinate and fumarate are directly linked to L-arginosuccinate in the context of arginine metabolism. As illustrated in Figure 8B, both L-Asparagine and L-Aspartate exhibited significant downregulation within the alanine, aspartate, and glutamate metabolism pathways. This pathway is interconnected with the metabolism of cyano amino acids, lysine biosynthesis, and the metabolism of glycine, serine, and threonine. Furthermore, it is directly linked to the TCA cycle via fumarate (Figure 8A). Within the phenylalanine metabolism pathway (Figure 8C), L-Phenylalanine undergoes gradual degradation through the trans-cinnamate pathway, ultimately catalyzed by

enzyme systems such as mhpC and mhpB to yield fumarate and succinate, which are subsequently imported into the TCA cycle. This degradation pathway establishes a direct connection between phenylalanine metabolism and the TCA cycle. The accumulation of fumarate and succinate in this pathway aligns with the observed upregulation of both compounds in the TCA cycle (Figure 8A). Figure 8E indicates that significant alterations have occurred in the biosynthetic pathways of keratin, xylem, and wax. This pathway initiates with C18 oleic acid, undergoing successive reactions such as ω -hydroxylation (catalyzed by cytochrome P450 enzymes including CYP94A1, CYP86A1, and CYP704B1), ω -oxidation, and epoxide hydrolysis, resulting in a series of hydroxylated fatty acids and dicarboxylic acid monomers. Notably, key intermediate products such as 18-hydroxyoleate and 9,10,18-trihydroxystearate have also experienced changes within this pathway.

Fig 9. Analysis of key pathways involved in rice tillering under salt stress following the exogenous application of Pro-Ca. A: TCA cycle and arginine biosynthesis, B: Metabolism of alanine, aspartic acid, and glutamate, C: Phenylalanine metabolism, D: Pathway summary, E: Effects on the biosynthesis of keratin, cutin, and wax. The spheres represent metabolites, with red indicating significant upregulation, blue indicating significant downregulation, green squares representing proteins, and blue boxes denoting metabolic processes.

4 Discussion

4.1 Pro-Ca through enhanced antioxidant enzymes system ease salt stress induced the oxidative damage

The excessive accumulation of reactive oxygen species (ROS) in plant cells during salt stress is a primary contributor to oxidative damage [39]. In response to ROS toxicity, plants have developed an enzymatic antioxidant defense system that includes superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and ascorbic acid peroxidase (APX), among others [5]. This study demonstrated that the activities of

SOD, APX, POD, and CAT in the tillers of the 9311 variety under salt stress (S) treatment were significantly elevated compared to the control group, aligning with previous findings in rice [40]. Zhang et al. reported that the activities of SOD and POD in the leaves and stems of rice increased significantly during the tillering stage under salt stress [41]. This widespread enhancement in enzyme activity suggests that rice activates its intrinsic antioxidant defense mechanisms to mitigate excessive ROS production under salt stress [41]. Nevertheless, the increase in antioxidant enzyme activity is insufficient to entirely avert oxidative damage. The substantial rise in malondialdehyde (MDA) content observed under salt stress treatment in this study serves as clear evidence of this limitation. Additionally, Zuo et al. confirmed through random forest machine learning analysis that MDA content reliably predicts rice biomass negatively under the combined treatment of salt stress and calcium protriocyclate (PROCA), establishing it as a universal biomarker for assessing salt stress damage [42].

Following the exogenous injection of Pro-Ca, the activities of the four antioxidant enzymes in the EAS treatment group were significantly elevated compared to those in the S treatment group. This finding aligns closely with several published studies [43]. Zhang et al. confirmed that foliar application of Pro-Ca effectively enhances the activities of SOD and POD during the tillering stage of rice under salt stress [13]. Additionally, research by Li et al. demonstrated that Pro-Ca spraying could increase the APX activity of rice seedlings under salt stress by 84.2% and the SOD activity by 75.2% [44]. Variations in these results may be attributed to differences in treatment periods (seedling stage versus tillering stage), sampling locations (leaves versus tillering nodes), and intensities of salt stress. Nonetheless, all studies consistently indicate that Pro-Ca can further augment the enzymatic antioxidant capacity of rice under salt stress. Notably, in this study, the response amplitudes of different antioxidant enzymes to Pro-Ca varied. The response of APX was the most pronounced (15.4% - 30.6%), whereas the response of POD was comparatively modest (4.9% - 14.9%) [44]. This difference may be attributed to the distinct roles of various antioxidant enzymes within the reactive

oxygen species (ROS) clearance network. As a pivotal enzyme in the ascorbic acid-glutathione cycle, APX plays a central role in the elimination of H₂O₂ [45]. In addition to its function in H₂O₂ detoxification, POD is also involved in lignin synthesis and the reinforcement of cell walls [46]. The preferential activation of APX by Pro-Ca under salt stress indicates that it may primarily enhance ROS clearance efficiency by bolstering the ascorbic acid-glutathione cycle. Moreover, research by Li et al. demonstrated that Pro-Ca can upregulate the expression of antioxidant oxidase-related genes, such as *SOD2*, *PXMP2*, and *MPV17*, at the transcriptional level, thereby providing a molecular basis for the increased activity of antioxidant enzymes induced by Pro-Ca [15].

4.2 Pro-Ca synergistically alleviates salt stress damage by protecting tiller growth and development and reducing membrane lipid peroxidation

The number of tillers is a critical agronomic trait influencing rice yield, while tiller nodes serve as essential sites for the development of tiller buds and the formation of adventitious roots [10, 11]. In this study, under salt stress treatment, the tillering number of 9311 decreased by 38.5%, 26.3%, and 19.0% on days 7, 14, and 21, respectively, compared to the control. Additionally, the stem base width decreased by 16.6%, 17.4%, and 18.8% at the same time points. These results indicate that the inhibition of tillers due to salt stress is most pronounced during the initial stage of stress (7 days), with a gradual reduction in the extent of inhibition thereafter. This observation aligns with findings by Ruan et al., who reported that the inhibition of tillers in wheat under salt stress is more severe than that of the main stem [47]. The decline in tiller number may result from the direct inhibition of cell division and differentiation in tiller segments caused by salt stress [48]. Notably, the degree of inhibition in stem base width remained relatively stable across all time points, ranging from approximately 16% to 19%, in contrast to the progressively diminishing inhibition of tiller number. This suggests that the mechanisms regulating tiller number and stem base width under salt stress may differ.

Exogenous Pro-Ca treatment significantly mitigated the inhibition of tillering caused by salt stress. The tillering numbers in the EAS treatment group on days 7, 14, and 21 were 137.5%, 78.6%, and 64.7% higher than those in the S treatment group, respectively. This finding aligns with the research conducted by Zhang et al., which demonstrated that foliar application of Pro-Ca effectively alleviates the inhibitory effects of salt stress on the tillering growth of rice at all tillering positions [41]. The impact of Pro-Ca on promoting tillering was most pronounced at the early stage of stress (7 days), with an increase of 137.5%, before gradually diminishing. This decline may be attributed to the timing of Pro-Ca's role as an inhibitor of gibberellin synthesis [46]. Pro-Ca lowers the levels of active gibberellin by inhibiting the activities of GA20ox and GA3ox [46], thereby modifying the hormonal balance in plants and reallocating resources from internode elongation to the occurrence of tillering. However, the effect of Pro-Ca on stem base width appears to be relatively limited, suggesting that the response of this structural trait to plant growth regulators is both slow and constrained.

The excessive accumulation of reactive oxygen species (ROS) due to salt stress can damage unsaturated fatty acids in cell membranes, leading to membrane lipid peroxidation, with malondialdehyde (MDA) serving as the primary end product of this process [49]. In this study, the MDA content in the 9311 tillers subjected to salt stress increased continuously from 7 to 35 days. By day 35, the MDA level was 297.8% higher than that of the control group, indicating significant membrane lipid peroxidation damage resulting from salt stress. This trend aligns with the findings of Zhang et al., who reported that the levels of MDA and hydrogen peroxide (H_2O_2) in the leaves and stems of rice tillers significantly increased under salt stress [50]. Exogenous proline-calcium (Pro-Ca) treatment effectively mitigated MDA accumulation under salt stress. The MDA content in the EAS treatment group was 15.2% to 26.0% lower than that in the salt treatment group. This outcome is consistent with the results reported by Mei et al., who found that Pro-Ca reduced MDA levels in the main stem and tiller leaves of rice by 10.8% and 2.19%, respectively, under salt stress [45]. Furthermore, research by

Zuo et al. indicated that under the combined treatment of salt stress and Pro-Ca, MDA content emerged as one of the most reliable predictors of rice biomass, exhibiting a stable negative correlation with biomass [22]. The reduction of MDA content is the direct physiological consequence of Pro-Ca enhancing the activity of antioxidant enzymes. More efficient ROS clearance reduces the occurrence of membrane lipid peroxidation, thereby protecting the integrity of the cell membrane and providing the necessary cellular structural basis for the normal development of tillers.

This study revealed that under salt stress, the levels of soluble sugar and soluble protein significantly increased, aligning with the established response of plants to maintain cellular turgor pressure through the accumulation of osmotic regulatory substances [51]. In contrast, Pro-Ca treatment resulted in a reduction of both soluble sugar and soluble protein levels, decreasing by 8.3% to 15.9% and 14.9% to 20.9%, respectively, compared to the S treatment group. This seemingly paradoxical outcome may indicate a diminished requirement for osmotic regulation in plants subjected to Pro-Ca treatment. Given that Pro-Ca enhances antioxidant defense and regulates ion homeostasis [15, 20], the stress experienced by cells is alleviated, leading to a corresponding decrease in reliance on osmotic regulatory substances. A similar trend was observed in the study by Mei et al.: although Pro-Ca increased the soluble protein content in the main stem and tiller leaves, the increase was significantly less than that induced by salt stress itself [45].

4.3 Pro-Ca Reconstructs Ionic Homeostasis and Reprograms Hormonal Signals to Alleviate Salt Stress in Rice Tillers

Maintaining ionic homeostasis is fundamental to plant salt tolerance, as salinity disrupts the K^+/Na^+ balance and leads to cytotoxicity[52]. In the present study, Pro-Ca treatment reduced Na^+ accumulation by 50.7% while concurrently increasing K^+ content by 13.7%, resulting in a 1.3-fold elevation of the K^+/Na^+ ratio. This improvement is consistent with previous findings that Pro-Ca application decreases Na^+ content by 41.6–52.2% in rice seedlings[53], and by approximately 86–91% in leaf tissues[20]. Mechanistically, Pro-Ca has been shown to upregulate E3 ubiquitin ligase

genes, thereby facilitating Na^+ exclusion and sequestration. The 55.1% reduction in Ca^{2+} content observed in our study further supports this interpretation, as salt-induced Ca^{2+} overload is a hallmark of stress signaling[53]; its attenuation by Pro-Ca likely reflects a mitigation of stress intensity. Notably, the 31.3% increase in Cl^- content suggests that Pro-Ca may enhance anionic compartmentalization to maintain electroneutrality, a mechanism that warrants further investigation[52]. Collectively, these results demonstrate that Pro-Ca effectively re-establishes ionic equilibrium in tillers by reducing Na^+ and Ca^{2+} overload while preserving K^+ levels.

Beyond ionic regulation, phytohormones serve as critical mediators of stress adaptation. Our data showed that Pro-Ca elevated IAA and SA contents by 40.0% and 22.5%, respectively, under salt stress. The increase in IAA is particularly significant, as auxin promotes tiller bud outgrowth and organ development[54, 55], providing a hormonal basis for the observed tillering recovery. At the same time, the increase in SA, the key systemic acquired resistance regulator, suggests that Pro-Ca may have activated the defense signal network. Interestingly, the simultaneous rise of IAA and SA implies that Pro-Ca does not simply enforce a growth–defense trade-off, but rather enables a coordinated advancement of both developmental and defensive programs[56]. This dual activation is consistent with the notion that Pro-Ca maintains abscisic acid homeostasis and promotes oxylipin synthesis to alleviate salt stress[20]. Therefore, Pro-Ca orchestrates a multilayered protective strategy: at the ionic level, it restores K^+/Na^+ homeostasis and attenuates Ca^{2+} signaling overload; at the hormonal level, it concurrently boosts auxin-driven growth recovery and SA-mediated defense activation. This integrated regulation likely underpins the enhanced salt tolerance observed in Pro-Ca-treated rice tillers.

4.4 Pro-Ca Orchestrates Transcriptional Reprogramming from Passive Damage Repair to Active Defense Signaling

Transcriptomic reprogramming constitutes a fundamental layer of plant stress adaptation. In the 9S-vs-9CK comparison, salt stress elicited a passive survival response characterized by enrichment of photosynthesis inhibition, cell wall

degradation, and transcription factor activity—a pattern consistent with the well-documented salt-induced suppression of photosynthetic machinery in rice[28]. Upon Pro-Ca application (9EAS-vs-9S), the enrichment spectrum underwent a fundamental transformation: photosynthesis-related terms disappeared, while salicylic acid response (GO:0009751), ethylene-activated signaling (GO:0009873), and defense signaling (GO:0009870) became prominently enriched. This transcriptional shift indicates that Pro-Ca actively engages the endogenous immune network of rice, shifting cellular resources from passive damage repair to defense hormone signaling. KEGG analysis further revealed that Pro-Ca redirected enrichment from inhibited photosynthetic pathways (ko00196, ko00195) toward glycerophospholipid metabolism (ko00564), cysteine/methionine metabolism (ko00270), and riboflavin metabolism (ko00740), with most genes in these categories being downregulated[20]. This pattern aligns with recent evidence that Pro-Ca upregulates E3 ubiquitin ligase genes and downregulates light-harvesting complex genes to enhance photosynthetic efficiency[15].

At the pathway level, the downregulation of glycerophospholipid metabolism genes (*Os01g0720400*, *Os03g0832800*) in EAS suggests that Pro-Ca inhibits membrane lipid degradation, thereby mitigating lipid peroxidation under salt stress[57]. This transcriptional regulation is corroborated by the reduced MDA content observed in physiological measurements. In cysteine/methionine metabolism, upregulation of *Os05g0149400* (*ACO5*) and downregulation of *Os03g0338000* indicate that Pro-Ca modulates ethylene signaling and sulfur assimilation, linking antioxidant defense to hormonal signaling[52]. Furthermore, the downregulation of *Os05g0153500* (trehalose phosphatase) in starch/sucrose metabolism suggests refined osmotic adjustment, potentially redirecting carbon flux toward other compatible solutes[58]. Collectively, these transcriptional transformations demonstrate that Pro-Ca establishes a defensive metabolic network through three integrated mechanisms: membrane lipid remodeling, sulfur assimilation for glutathione-based antioxidant defense, and reprogramming of glucose metabolism for osmotic balance—transitioning from passive damage repair to active defense signaling in salt-stressed rice tillers.

4.5 Pro-Ca Reshapes the Metabolic Landscape of Salt-Stressed Rice Tillers

Metabolic reprogramming constitutes a critical layer of plant stress adaptation. KEGG analysis showed that salt stress predominantly enriched amino acid metabolism and the TCA cycle, whereas Pro-Ca treatment specifically redirected enrichment toward alpha-linolenic acid metabolism while preserving TCA cycle and carbon fixation pathways[59]. Z-score analysis further revealed that N-Carbamoylputrescine was upregulated under salt stress and further elevated by Pro-Ca[60], while 4-hydroxy-2-nonenal (4-HNE) was significantly downregulated in the EAS group[61]. The opposing regulation of Piperic acid, downregulated under salt stress but upregulated upon Pro-Ca treatment, suggests that Pro-Ca reverses salt-induced suppression of secondary metabolism[20]. These findings indicate that Pro-Ca coordinates a multi-layered metabolic strategy: reinforcing polyamine biosynthesis for antioxidant defense, alleviating lipid peroxidation, sustaining TCA cycle activity for energy supply, and activating jasmonic acid-mediated defense through alpha-linolenic acid metabolism[62].

4.6 Pro-Ca Reinforces Carbon-Nitrogen Metabolic Coupling and Physical Barrier Formation in Salt-Stressed Rice Tillers

Integrated pathway analysis revealed that Pro-Ca orchestrates metabolic adaptation through two complementary mechanisms: reinforcing TCA-centered carbon-nitrogen coupling and activating cutin/suberin/wax biosynthesis for physical barrier reinforcement. The TCA cycle functioned as a central hub linking carbon and nitrogen metabolism, with fumarate significantly elevated across three interconnected pathways: the TCA cycle, phenylalanine metabolism, and alanine-aspartate-glutamate metabolism. This pattern is consistent with recent metabolomic studies showing that the TCA cycle, along with alanine/aspartate/glutamate metabolism, is among the most highly correlated pathways in rice under saline-alkali stress[61, 62]. The accumulation of fumarate and succinate from phenylalanine degradation via the trans-cinnamate pathway, catalyzed by mhpC and mhpB, establishes a direct carbon flow from phenylalanine catabolism into the TCA cycle[63]. This metabolic linkage provides

anaplerotic carbon skeletons to sustain TCA cycle activity under energy-demanding stress conditions. Meanwhile, the downregulation of asparagine and aspartate in the alanine-aspartate-glutamate pathway, coupled with their connection to the TCA cycle through fumarate, indicates that Pro-Ca redirects nitrogen flux from storage amino acids toward arginine biosynthesis and polyamine production[64].

Concurrently, the activation of cutin, suberin, and wax biosynthesis pathways represents a second layer of Pro-Ca-mediated protection. This pathway, initiated from C18 oleic acid through successive ω -hydroxylation (catalyzed by *CYP94A1*, *CYP86A1*, and *CYP704B1*), ω -oxidation, and epoxide hydrolysis, generates hydroxylated fatty acids and dicarboxylic acid monomers for polymer formation. Transcriptomic and proteomic evidence has demonstrated that salt stress induces drastic alterations in cuticular wax and suberin layers, which prevent Na⁺-induced dehydration and osmotic stress, and that reinforcing these lipid barriers is a key mechanism for improving salinity tolerance in rice[65]. KEGG enrichment of down-regulated genes in “cutin, suberine and wax biosynthesis” has been consistently observed in salt-stress transcriptomic studies, and these lipophilic barriers play established roles in protecting plants from abiotic stresses[66]. Collectively, these findings indicate that Pro-Ca enhances salt tolerance through coordinated carbon-nitrogen metabolic reprogramming—sustaining TCA cycle activity via fumarate-mediated anaplerosis—while simultaneously reinforcing physical barriers through cutin/suberin/wax biosynthesis to restrict ion infiltration and water loss.

5 Conclusion

Through an integrated analysis of morphophysiology, ionomics, phytohormone profiling, transcriptomics, and metabolomics, this study elucidates the multilayered regulatory mechanisms by which exogenous prohexadione calcium (Pro-Ca) alleviates salt-induced damage in rice tillers.

(1) At the morphological and ion homeostatic level, Pro-Ca significantly mitigated salt-induced tiller inhibition, increasing tiller number by 64.7%–137.5%. This was accompanied by a 50.7% reduction in Na⁺ accumulation, maintenance of K⁺ levels, and an approximately 1.3-fold elevation of the K⁺/Na⁺ ratio, collectively reestablishing ionic equilibrium in tiller nodes. Pro-Ca also coordinately elevated the contents of IAA and SA, promoting growth and defense simultaneously.

(2) At the transcriptomic level, Pro-Ca redirected the salt-induced transcriptional response from passive repair signatures—such as photosynthetic suppression and cell wall degradation—toward active activation of salicylic acid-responsive, ethylene-signaling, and defense-related pathways, while modulating glycerophospholipid, sulfur, and carbohydrate metabolism to establish a defensive metabolic network.

(3) At the metabolomic level, the TCA cycle emerged as a central hub, with fumarate-mediated anaplerosis redirecting carbon skeletons from phenylalanine degradation and amino acid metabolism toward arginine and polyamine biosynthesis. Integrated network analysis identified N-Carbamoylputrescine and Vanillin as core gene-metabolite hubs, underscoring the central roles of polyamine synthesis and phenylpropanoid metabolism in Pro-Ca-mediated salt tolerance.

(4) In parallel, Pro-Ca activated the biosynthesis of cutin, suberine, and wax, reinforcing physical barriers that restrict Na⁺ infiltration and reduce water loss from tiller nodes.

Collectively, these findings demonstrate that Pro-Ca enhances salt tolerance in rice tillers through a coordinated, multi-target strategy involving ion homeostasis reconstruction, transcriptional reprogramming toward active defense, metabolic network rewiring, and physical barrier reinforcement. This study provides a mechanistic framework for the precise application of plant growth regulators in saline-alkaline rice production and offers new perspectives for chemical regulation of crop salt tolerance.

931

932 **List of abbreviations**

933 DEGs: differentially expressed genes

934 Tr: transpiration rate

935 SOD: Superoxide dismutase

936 MDA: Malondialdehyde

937 POD: peroxidase

938 qRT-PCR: quantitative real-time polymerase chain reaction

939 SA: salicylic acid

940 IAA: in auxin

941 SE: standard deviation

942 GO: gene ontology

943

944 **Declarations**

945 **Ethics approval and consent to participate**

946 Not applicable.

947 **Consent for publication**

948 Not applicable.

949 **Availability of data and materials**

The transcriptome data (BioProject accession: PRJNA1300947) and the metabolomics data (BioProject accession: PRJNA1370692; provisional submission ID: SUB15812931) have been deposited in the NCBI Sequence Read Archive (SRA). Both datasets are under embargo and will be publicly released on November 10, 2029. After that date, they can be accessed via the respective links: <https://www.ncbi.nlm.nih.gov/sra/PRJNA1300947> and <https://www.ncbi.nlm.nih.gov/sra/PRJNA1370692>. Prior to the release date, the data are restricted to the submitters and are not accessible to the public.

Competing interests

The authors declare that they have no competing interests.

Funding

This research was funded by Guangdong Provincial Department of Agriculture and Rural Affairs (2024KJ31); Guangdong Provincial Education Department Key Field Special Project for Colleges and Universities, No.2021ZdZX4027; Innovation Team Project of Universities in Guangdong Province, No.2021KCXTD011; Binhai Agricultural Engineering Technology Research Center (230420020).

Authors' contributions

J.T. responsible for manuscript writing, investigation, data collation and formal analysis. Y.X. contributed to methodology and conceptualization. D.Z. helps

969 conceptualize, obtain funding, manage and supervise projects. Q.Q., N.F. and Y.L. assist
970 in writing censorship and editing. W.S., W.C., Z.L., Y.L., Q.S., Z.H., R.D., W.H., Y.D.,
971 and J.W. assist in investigation. All the authors participated in the preparation, writing
972 and revision of the manuscript and adopted the submitted manuscript.

973 **Acknowledgements**

974 Not applicable.

975

- 977 1. Chinnusamy, Viswanathan, Jian-Kang Zhu. 2004. Plant salt tolerance. *Plant*
 978 *Responses to Abiotic Stress* Springer Berlin Heidelberg, 241–270.
 979 https://doi.org/10.1007/978-3-540-39402-0_10
- 980 2. Cai, K., H. Gao, X. Wu, S. Zhang, Z. Han, X. Chen, G. Zhang, F. Zeng. 2019. “The
 981 Ability to Regulate Transmembrane Potassium Transport in Root Is Critical for
 982 Drought Tolerance in Barley.” *Int J Mol Sci* 20: <https://doi.org/10.3390/ijms20174111>
- 983 3. Shabala, S., I. Pottosin. 2014. “Regulation of potassium transport in plants under
 984 hostile conditions: implications for abiotic and biotic stress tolerance.” *Physiol Plant*
 985 151: 257–279. <https://doi.org/10.1111/ppl.12165>
- 986 4. Miller, G., N. Suzuki, S. Ciftci-Yilmaz, R. Mittler. 2010. “Reactive oxygen species
 987 homeostasis and signalling during drought and salinity stresses.” *Plant Cell Environ*
 988 33: 453–467. <https://doi.org/10.1111/j.1365-3040.2009.02041.x>
- 989 5. Gill, S. S., N. Tuteja. 2010. “Reactive oxygen species and antioxidant machinery in
 990 abiotic stress tolerance in crop plants.” *Plant Physiol Biochem* 48: 909–930.
 991 <https://doi.org/10.1016/j.plaphy.2010.08.016>
- 992 6. Yang, Y., Y. Guo. 2018. “Elucidating the molecular mechanisms mediating plant
 993 salt-stress responses.” *New Phytol* 217: 523–539. <https://doi.org/10.1111/nph.14920>
- 994 7. Deng, R., Y. Li, N. J. Feng, D. F. Zheng, A. Khan, Y. W. Du, J. Q. Zhang, et al.
 995 2025. “Integrative analysis of transcriptome and metabolome reveal molecular
 996 mechanism of tolerance to salt stress in rice.” *BMC Plant Biol* 25: 335.
 997 <https://doi.org/10.1186/s12870-025-06300-8>
- 998 8. Zhang, R., Y. Wang, S. Hussain, S. Yang, R. Li, S. Liu, Y. Chen, H. Wei, Q. Dai, H.
 999 Hou. 2022. “Study on the Effect of Salt Stress on Yield and Grain Quality Among
 1000 Different Rice Varieties.” *Front Plant Sci* 13: 918460.
 1001 <https://doi.org/10.3389/fpls.2022.918460>
- 1002 9. Razzaque, M. A., N. M. Talukder, M. S. Islam, A. K. Bhadra, R. K. Dutta. 2009.
 1003 “The effect of salinity on morphological characteristics of seven rice (*Oryza sativa*)
 1004 genotypes differing in salt tolerance.” *Pak J Biol Sci* 12: 406–412.
 1005 <https://doi.org/10.3923/pjbs.2009.406.412>
- 1006 10. Zhao, S., S. Jang, Y. K. Lee, D. G. Kim, Z. Jin, H. J. Koh. 2020. “Genetic Basis of
 1007 Tiller Dynamics of Rice Revealed by Genome-Wide Association Studies.” *Plants*
 1008 (*Basel*) 9: <https://doi.org/10.3390/plants9121695>
- 1009 11. Wang, Y., J. Lu, T. Ren, S. Hussain, C. Guo, S. Wang, R. Cong, X. Li. 2017.
 1010 “Effects of nitrogen and tiller type on grain yield and physiological responses in rice.”
 1011 *AoB Plants* 9: plx012. <https://doi.org/10.1093/aobpla/plx012>

1012 12. Liu, X., Q. Hu, J. Yan, K. Sun, Y. Liang, M. Jia, X. Meng, et al. 2020. “ζ-Carotene
1013 Isomerase Suppresses Tillering in Rice through the Coordinated Biosynthesis of
1014 Strigolactone and Absciscic Acid.” *Mol Plant* 13: 1784–1801.
1015 <https://doi.org/10.1016/j.molp.2020.10.001>

1016 13. Zhang, R., D. Zheng, N. Feng, L. Linfeng, J. Ma, X. Yuan, J. Huang, L. Huang.
1017 2024. “Effect of salt stress on different tiller positions in rice and the regulatory effect
1018 of prohexadione calcium.” *PeerJ* 12: e18357. <https://doi.org/10.7717/peerj.18357>

1019 14. Rademacher, W. 2000. “GROWTH RETARDANTS: Effects on Gibberellin
1020 Biosynthesis and Other Metabolic Pathways.” *Annu Rev Plant Physiol Plant Mol Biol*
1021 51: 501–531. <https://doi.org/10.1146/annurev.arplant.51.1.501>

1022 15. Li, Y., H. Zhou, N. Feng, D. Zheng, G. Ma, S. Feng, M. Liu, M. Yu, X. Huang, A.
1023 Huang. 2023. “Physiological and transcriptome analysis reveals that prohexadione-
1024 calcium promotes rice seedling's development under salt stress by regulating
1025 antioxidant processes and photosynthesis.” *PLoS One* 18: e0286505.
1026 <https://doi.org/10.1371/journal.pone.0286505>

1027 16. Zhang, Q., J. Ya, A. Khan, S. Duan, W. Huang, R. Deng, J. Zhang, et al. 2026.
1028 “Differential regulation of rice seedling morphogenesis, carbon metabolism, and
1029 energy charge by Pro-Ca and GA₃ under salt stress.” *BMC Plant Biol* 26: 322.
1030 <https://doi.org/10.1186/s12870-026-08156-y>

1031 17. Zhang, R., D. Zheng, N. Feng, Q. S. Qiu, H. Zhou, F. Meng, X. Huang, A. Huang,
1032 Y. Li. 2023. “Prohexadione-calcium alleviates the leaf and root damage caused by salt
1033 stress in rice (*Oryza sativa* L.) at the tillering stage.” *PLoS One* 18: e0279192.
1034 <https://doi.org/10.1371/journal.pone.0279192>

1035 18. Mei, W., S. Yang, J. Xiong, A. Khan, L. Zhao, X. Du, J. Huo, et al. 2025.
1036 “Prohexadione-Calcium Reduced Stem and Tiller Damage and Maintained Yield by
1037 Improving the Photosynthetic and Antioxidant Capacity of Rice (*Oryza sativa* L.)
1038 Under NaCl Stress.” *Plants (Basel)* 14: <https://doi.org/10.3390/plants14020188>

1039 19. Deng, R., D. Zheng, N. Feng, A. Khan, J. Zhang, Z. Sun, J. Li, et al. 2025.
1040 “Prohexadione Calcium Improves Rice Yield Under Salt Stress by Regulating Source-
1041 Sink Relationships During the Filling Period.” *Plants (Basel)* 14:
1042 <https://doi.org/10.3390/plants14020211>

1043 20. Deng, R., Y. Li, N. J. Feng, D. F. Zheng, Y. W. Du, A. Khan, Y. B. Xue, J. Q.
1044 Zhang, Y. N. Feng. 2024. “Integrative Analyses Reveal the Physiological and
1045 Molecular Role of Prohexadione Calcium in Regulating Salt Tolerance in Rice.” *Int J*
1046 *Mol Sci* 25: <https://doi.org/10.3390/ijms25169124>

1047 21. Zhong, Wenji, Peng Deng, Naijie Feng, Dianfeng Zheng, Youwei Du, Rui Deng,
1048 Zhiyuan Sun, Shaobiao Duan, Xixi Yang, Nana Yue. 2026. “Integrated transcriptomic
1049 and metabolomic analysis of prohexadione calcium-mediated alleviation of salt stress

1050 in oilseed rape (*Brassica napus* L.) seedlings.” *BMC Plant Biology* 26: 904.
1051 <https://doi.org/10.1186/s12870-026-08401-4>
1052 22. Zuo, Guanqiang, Wanqi Mei, Naijie Feng, Dianfeng Zheng. 2025.
1053 “Photosynthetic performance index (PIabs) and malondialdehyde (MDA) content
1054 determine rice biomass under combined salt stress and prohexadione-calcium
1055 treatment.” *BMC Plant Biology* 25: 823. <https://doi.org/10.1186/s12870-025-06826-x>
1056 23. Du, You-Wei, Ling Liu, Nai-Jie Feng, Dian-Feng Zheng, Mei-Ling Liu, Hang
1057 Zhou, Peng Deng, Ya-xing Wang, Hui-Min Zhao. 2023. “Combined transcriptomic
1058 and metabolomic analysis of alginate oligosaccharides alleviating salt stress in rice
1059 seedlings.” *BMC Plant Biology* 23: 455. <https://doi.org/10.1186/s12870-023-04470-x>
1060 24. Huo, Jingxin, Minglong Yu, Naijie Feng, Dianfeng Zheng, Rui Zhang, Yingbin
1061 Xue, Aaqil Khan, et al. 2024. “Integrated transcriptome and metabolome analysis of
1062 salinity tolerance in response to foliar application of choline chloride in rice (*Oryza*
1063 *sativa* L.).” *Frontiers in Plant Science* Volume 15 - 2024:
1064 <https://doi.org/10.3389/fpls.2024.1440663>
1065 25. Tong, Haiyang, Chao Wang, Xiaoqian Han, Qihao Sun, Enxi Luo, Chao Yang,
1066 Guo Xu, et al. 2025. “Multi-omics-Based Construction of ncRNA-Gene-Metabolite
1067 Networks Provides New Insights Into Metabolic Regulation Under Salt Stress in
1068 Rice.” *Rice* 18: 50. <https://doi.org/10.1186/s12284-025-00811-6>
1069 26. Radwan, N. S., S. F. Lamlo, A. H. Emwas, M. Jaremko, N. R. Abdelsalam.
1070 2026. “Cultivar-specific metabolomic and morpho-physiological responses reveal
1071 distinct salt tolerance mechanisms in rice (*Oryza sativa* L.).” *BMC Plant Biol* 26:
1072 <https://doi.org/10.1186/s12870-025-08086-1>
1073 27. Shen, X., S. Dai, M. Chen, Y. Huang. 2024. “Spermidine augments salt stress
1074 resilience in rice roots potentially by enhancing OsbZIP73's RNA binding capacity.”
1075 *BMC Plant Biol* 24: 786. <https://doi.org/10.1186/s12870-024-05492-9>
1076 28. Han, Y., C. Wu, X. Ji, M. Yang, H. Zhu, Z. Pei, M. Qu, L. Qu, Z. Li, S. Yan. 2025.
1077 “Transcriptome Analysis Reveals Differences in Molecular Mechanisms Between
1078 Salt-Tolerant and Salt-Sensitive Rice (*Oryza sativa* L.) Varieties Under Salt Stress.”
1079 *Curr Issues Mol Biol* 47: <https://doi.org/10.3390/cimb47100832>
1080 29. Kittipornkul, Piyatida, Chairat Treesubsuntorn, Paitip Thiravetyan. 2020. “Effect
1081 of exogenous catechin and salicylic acid on rice productivity under ozone stress: the
1082 role of chlorophyll contents, lipid peroxidation, and antioxidant enzymes.”
1083 *Environmental Science and Pollution Research* 27: 25774–25784.
1084 <https://doi.org/10.1007/s11356-020-08962-3>
1085 30. Dey, S., S. Sen Raychaudhuri. 2024. “Methyl jasmonate improves selenium
1086 tolerance via regulating ROS signalling, hormonal crosstalk and phenylpropanoid

1087 pathway in *Plantago ovata*.” *Plant Physiol Biochem* 209: 108533.
 1088 <https://doi.org/10.1016/j.plaphy.2024.108533>
 1089 31. Yang, S., Y. Tao, M. Liu, H. Zhou, G. Chen, J. Xu, H. Zhang, J. Mo. 2026.
 1090 “Transcriptome and metabolome analysis demonstrate critical contribution of
 1091 flavonoid biosynthesis and galactose metabolism to salt resistance of rice roots.” *Sci*
 1092 *Rep* <https://doi.org/10.1038/s41598-026-60030-x>
 1093 32. Wang, X., Z. Wu, Q. Zhou, X. Wang, S. Song, S. Dong. 2021. “Physiological
 1094 Response of Soybean Plants to Water Deficit.” *Front Plant Sci* 12: 809692.
 1095 <https://doi.org/10.3389/fpls.2021.809692>
 1096 33. Srivastava, A. K., D. Singh. 2020. “Assessment of malathion toxicity on
 1097 cytophysiological activity, DNA damage and antioxidant enzymes in root of *Allium*
 1098 *cepa* model.” *Sci Rep* 10: 886. <https://doi.org/10.1038/s41598-020-57840-y>
 1099 34. Gao, Yunyun, Guoxing Zhang, Shun Yao Jiang, Yong-Xin Liu. 2024. “Wekemo
 1100 Bioincloud: A user-friendly platform for meta-omics data analyses.” *iMeta* 3: e175.
 1101 <https://doi.org/https://doi.org/10.1002/imt2.175>
 1102 35. López-Serrano, Lidia, Ángeles Calatayud, Salvador López-Galarza, Ramón
 1103 Serrano, Eduardo Bueso. 2021. “Uncovering salt tolerance mechanisms in pepper
 1104 plants: a physiological and transcriptomic approach.” *BMC Plant Biology* 21: 169.
 1105 <https://doi.org/10.1186/s12870-021-02938-2>
 1106 36. Chen, Y., Y. Chen, C. Shi, Z. Huang, Y. Zhang, S. Li, Y. Li, et al. 2018.
 1107 “SOAPnuke: a MapReduce acceleration-supported software for integrated quality
 1108 control and preprocessing of high-throughput sequencing data.” *Gigascience* 7: 1–6.
 1109 <https://doi.org/10.1093/gigascience/gix120>
 1110 37. Beltran, A., M. Suarez, M. A. Rodríguez, M. Vinaixa, S. Samino, L. Arola, X.
 1111 Correig, O. Yanes. 2012. “Assessment of compatibility between extraction methods
 1112 for NMR- and LC/MS-based metabolomics.” *Anal Chem* 84: 5838–5844.
 1113 <https://doi.org/10.1021/ac3005567>
 1114 38. Jain, M., A. Nijhawan, A. K. Tyagi, J. P. Khurana. 2006. “Validation of
 1115 housekeeping genes as internal control for studying gene expression in rice by
 1116 quantitative real-time PCR.” *Biochem Biophys Res Commun* 345: 646–651.
 1117 <https://doi.org/10.1016/j.bbrc.2006.04.140>
 1118 39. Monsur, Mahmuda Binte, Juel Datta, Md Motiar Rohman, Mirza Hasanuzzaman,
 1119 Akbar Hossain, Mohammad Sohikul Islam, Maham Asif Bukhari, et al. 2022. Saline
 1120 Toxicity and Antioxidant Response in *Oryza sativa*: An Updated Review. *Managing*
 1121 *Plant Production Under Changing Environment* Springer Nature Singapore, 79–102.
 1122 https://doi.org/10.1007/978-981-16-5059-8_4
 1123 40. Du, X. L., N. J. Feng, D. F. Zheng, Y. Lin, H. Zhou, J. H. Li, X. H. Yang, J. X.
 1124 Huo, W. Q. Mei. 2025. “Effects of exogenous Uniconazole (S3307) on oxidative

1125 damage and carbon metabolism of rice under salt stress.” *BMC Plant Biol* 25: 541.
 1126 <https://doi.org/10.1186/s12870-025-06467-0>

1127 41. Zhang, R., D. Zheng, N. Feng, Q. S. Qiu, H. Zhou, M. Liu, Y. Li, et al. 2023.
 1128 “Prohexadione calcium enhances rice growth and tillering under NaCl stress.” *PeerJ*
 1129 11: e14804. <https://doi.org/10.7717/peerj.14804>

1130 42. Zuo, G., W. Mei, N. Feng, D. Zheng. 2025. “Photosynthetic performance index
 1131 (PIabs) and malondialdehyde (MDA) content determine rice biomass under combined
 1132 salt stress and prohexadione-calcium treatment.” *BMC Plant Biol* 25: 823.
 1133 <https://doi.org/10.1186/s12870-025-06826-x>

1134 43. Ya, Jiangyuan, Dianfeng Zheng, Qicheng Zhang, Wenkang Huang, Qianqian
 1135 Shang, Hang Zhou, Naijie Feng, et al. 2026. “Effects of simulated seawater intrusion-
 1136 induced salt stress and the recovery process on growth, antioxidant system, and
 1137 photosynthesis of rice, and the alleviating effect of Pro-Ca.” *BMC Plant Biology* 26:
 1138 907. <https://doi.org/10.1186/s12870-026-08668-7>

1139 44. Li, Yao, Hang Zhou, Naijie Feng, Dianfeng Zheng, Guohui Ma, Shengjie Feng,
 1140 Meiling Liu, Minglong Yu, Xixin Huang, Anqi Huang. 2023. “Physiological and
 1141 transcriptome analysis reveals that prohexadione-calcium promotes rice seedling’s
 1142 development under salt stress by regulating antioxidant processes and
 1143 photosynthesis.” *PLoS One* 18: e0286505.
 1144 <https://doi.org/10.1371/journal.pone.0286505>

1145 45. Noctor, Graham. 2025. “Hydrogen peroxide, ascorbate, and glutathione: building
 1146 the Foyer–Halliwell–Asada pathway.” *Planta* 261: 132.

1147 46. Rademacher, Wilhelm. 2000. “Growth retardants: effects on gibberellin
 1148 biosynthesis and other metabolic pathways.” *Annual review of plant biology* 51: 501–
 1149 531.

1150 47. Ruan, Yuefeng, Yuncai Hu, Urs Schmidhalter. 2008. “Insights on the role of
 1151 tillering in salt tolerance of spring wheat from detillering.” *Environmental &*
 1152 *Experimental Botany* 64: 33–42.

1153 48. Ogawa, Daisuke, Kiyomi Abe, Akio Miyao, Mikiko Kojima, Hitoshi Sakakibara,
 1154 Megumi Mizutani, Haruka Morita, Yosuke Toda, Tokunori Hobo, Yutaka Sato. 2011.
 1155 “RSS1 regulates the cell cycle and maintains meristematic activity under stress
 1156 conditions in rice.” *Nature communications* 2: 278.

1157 49. Zheng, Chao, Jian-Ping Chen, Xiao-Wei Wang, Ping Li. 2025. 'Reactive Oxygen
 1158 Species in Plants: Metabolism, Signaling, and Oxidative Modifications', *Antioxidants*,
 1159 p. 617.

1160 50. Zhang, Rongjun, Dianfeng Zheng, Naijie Feng, Quan-Sheng Qiu, Hang Zhou,
 1161 Meiling Liu, Yao Li, Fengyan Meng, XiXin Huang, Anqi Huang. 2023.

1162 “Prohexadione calcium enhances rice growth and tillering under NaCl stress.” *PeerJ*
1163 11: e14804.

1164 51. Xiao, F., H. Zhou. 2022. “Plant salt response: Perception, signaling, and
1165 tolerance.” *Front Plant Sci* 13: 1053699. <https://doi.org/10.3389/fpls.2022.1053699>

1166 52. Deng, Rui, Yao Li, Nai-Jie Feng, Dian-Feng Zheng, You-Wei Du, Aaqil Khan,
1167 Ying-Bin Xue, Jian-Qin Zhang, Ya-Nan Feng. 2024. 'Integrative Analyses Reveal the
1168 Physiological and Molecular Role of Prohexadione Calcium in Regulating Salt
1169 Tolerance in Rice', *International Journal of Molecular Sciences*, p. 9124.

1170 53. Liu, Meiling, Naijie Feng, Dianfeng Zheng, Rongjun Zhang. 2024. 'Prohexadione
1171 Calcium and Gibberellin Improve Osmoregulation, Antioxidant Response and Ion
1172 Homeostasis to Alleviate NaCl Stress in Rice Seedlings', *Agronomy*, p. 1318.

1173 54. Mei, Wanqi, Shaoxia Yang, Jian Xiong, Aaqil Khan, Liming Zhao, Xiaole Du,
1174 Jingxin Huo, et al. 2025. 'Prohexadione-Calcium Reduced Stem and Tiller Damage
1175 and Maintained Yield by Improving the Photosynthetic and Antioxidant Capacity of
1176 Rice (*Oryza sativa* L.) Under NaCl Stress', *Plants*, p. 188.

1177 55. Yang, J., Z. Yuan, Q. Meng, G. Huang, C. Périn, C. Bureau, A. C. Meunier, et al.
1178 2017. “Dynamic Regulation of Auxin Response during Rice Development Revealed
1179 by Newly Established Hormone Biosensor Markers.” *Front Plant Sci* 8: 256.
1180 <https://doi.org/10.3389/fpls.2017.00256>

1181 56. Swain, R., S. Sahoo, M. Behera, G. R. Rout. 2023. “Instigating prevalent abiotic
1182 stress resilience in crop by exogenous application of phytohormones and nutrient.”
1183 *Front Plant Sci* 14: 1104874. <https://doi.org/10.3389/fpls.2023.1104874>

1184 57. Xue, Y., C. Zhou, N. Feng, D. Zheng, X. Shen, G. Rao, Y. Huang, W. Cai, Y. Liu,
1185 R. Zhang. 2024. “Transcriptomic and Lipidomic Analysis Reveals Complex
1186 Regulation Mechanisms Underlying Rice Roots' Response to Salt Stress.” *Metabolites*
1187 14: <https://doi.org/10.3390/metabo14040244>

1188 58. Ge, L. F., D. Y. Chao, M. Shi, M. Z. Zhu, J. P. Gao, H. X. Lin. 2008.
1189 “Overexpression of the trehalose-6-phosphate phosphatase gene OsTPP1 confers
1190 stress tolerance in rice and results in the activation of stress responsive genes.” *Planta*
1191 228: 191–201. <https://doi.org/10.1007/s00425-008-0729-x>

1192 59. Qian, G., M. Wang, X. Wang, K. Liu, Y. Li, Y. Bu, L. Li. 2023. “Integrated
1193 Transcriptome and Metabolome Analysis of Rice Leaves Response to High Saline-
1194 Alkali Stress.” *Int J Mol Sci* 24: <https://doi.org/10.3390/ijms24044062>

1195 60. Saha, J., K. Giri. 2017. “Molecular phylogenomic study and the role of exogenous
1196 spermidine in the metabolic adjustment of endogenous polyamine in two rice cultivars
1197 under salt stress.” *Gene* 609: 88–103. <https://doi.org/10.1016/j.gene.2017.02.001>

1198 61. Sultana, Most Sharmin, Chisato Sakurai, Md Sanaullah Biswas, László Szabados,
1199 Jun'ichi Mano. 2024. “Accumulation of reactive carbonyl species in roots as the

1200 primary cause of salt stress-induced growth retardation of *Arabidopsis thaliana*.”
1201 *Physiologia Plantarum* 176: e14198. <https://doi.org/https://doi.org/10.1111/ppl.14198>
1202 62. Han, C., G. Chen, D. Zheng, N. Feng. 2023. “Transcriptomic and metabolomic
1203 analyses reveal that ABA increases the salt tolerance of rice significantly correlated
1204 with jasmonic acid biosynthesis and flavonoid biosynthesis.” *Sci Rep* 13: 20365.
1205 <https://doi.org/10.1038/s41598-023-47657-w>
1206 63. Bai, G., H. Zhang, Y. Li, D. H. Yang, M. Fei, T. Pang, Y. Fu, et al. 2025.
1207 “Integration of metabolic and transcriptomic analyses for revealing the galactose
1208 metabolism of tobacco (*Nicotiana tabacum*) under salt stress.” *Front Plant Sci* 16:
1209 1614515. <https://doi.org/10.3389/fpls.2025.1614515>
1210 64. Wang, Haixia, Xingying Yu, Jianhao Tang, Qi Zhao, Ruifang Yang, Jianjiang Bai,
1211 Liming Cao, Ruoyu Xiong. 2026. 'Effects of Prohexadione Calcium on Lodging
1212 Resistance and Yield in High-Quality japonica Rice', *Agronomy*, p. 974.
1213 65. Min, C. W., C. Lee, R. Gupta, G. H. Lee, J. S. Kim, N. W. Park, J. H. Chin, K. H.
1214 Jung, S. T. Kim. 2026. “Comprehensive DIA-MS Proteomics of Root Basal Nodes
1215 Elucidates Mechanisms of Salt Tolerance in Rice.” *Proteomics* e70138.
1216 <https://doi.org/10.1002/pmic.70138>
1217 66. Zhu, J., Z. Wang, H. Chen, M. Chen, X. Zhao, C. Mao, Y. Kong, et al. 2025.
1218 “Overexpression of OsDUF846.2 enhances the sensitivity of rice to salt and heat
1219 stresses.” *BMC Plant Biol* 25: 1359. <https://doi.org/10.1186/s12870-025-07426-5>
1220

Figures

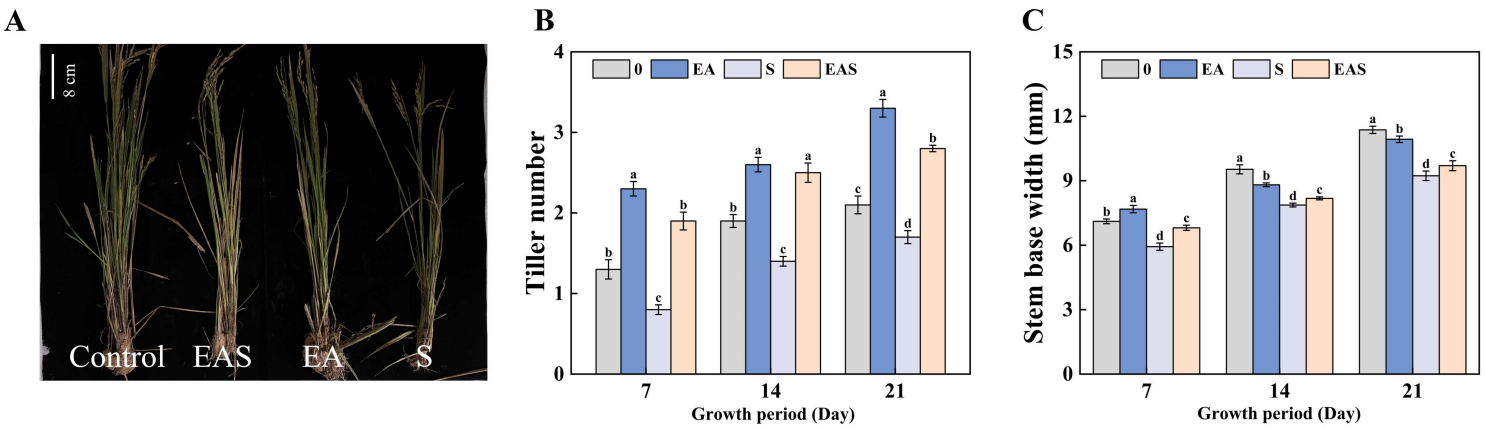


Figure 1

Effects of exogenous Pro-Ca on rice tillering under salt stress. A: Growth phenotype; B: Wide stem base; C: Tiller number. The experimental data are expressed as the mean and standard error (SE) of four biological replicates. 0 indicates that only distilled water is used for treatment, S represents salt stress treatment, EA represents Pro-Ca treatment, and EAS represents Pro-Ca treatment under salt stress. Significance analysis was conducted using the Duncan model, and different lowercase letters indicate significant differences ($P<0.05$). The base width of the tillering node was measured using a digital caliper to ensure that the measurement was carried out at the widest point of the tillering base. The number of tillers is recorded by counting all visible tillers that appear from the base of each plant. Five biological replicates were set for each treatment group, with three plants in each replicate.

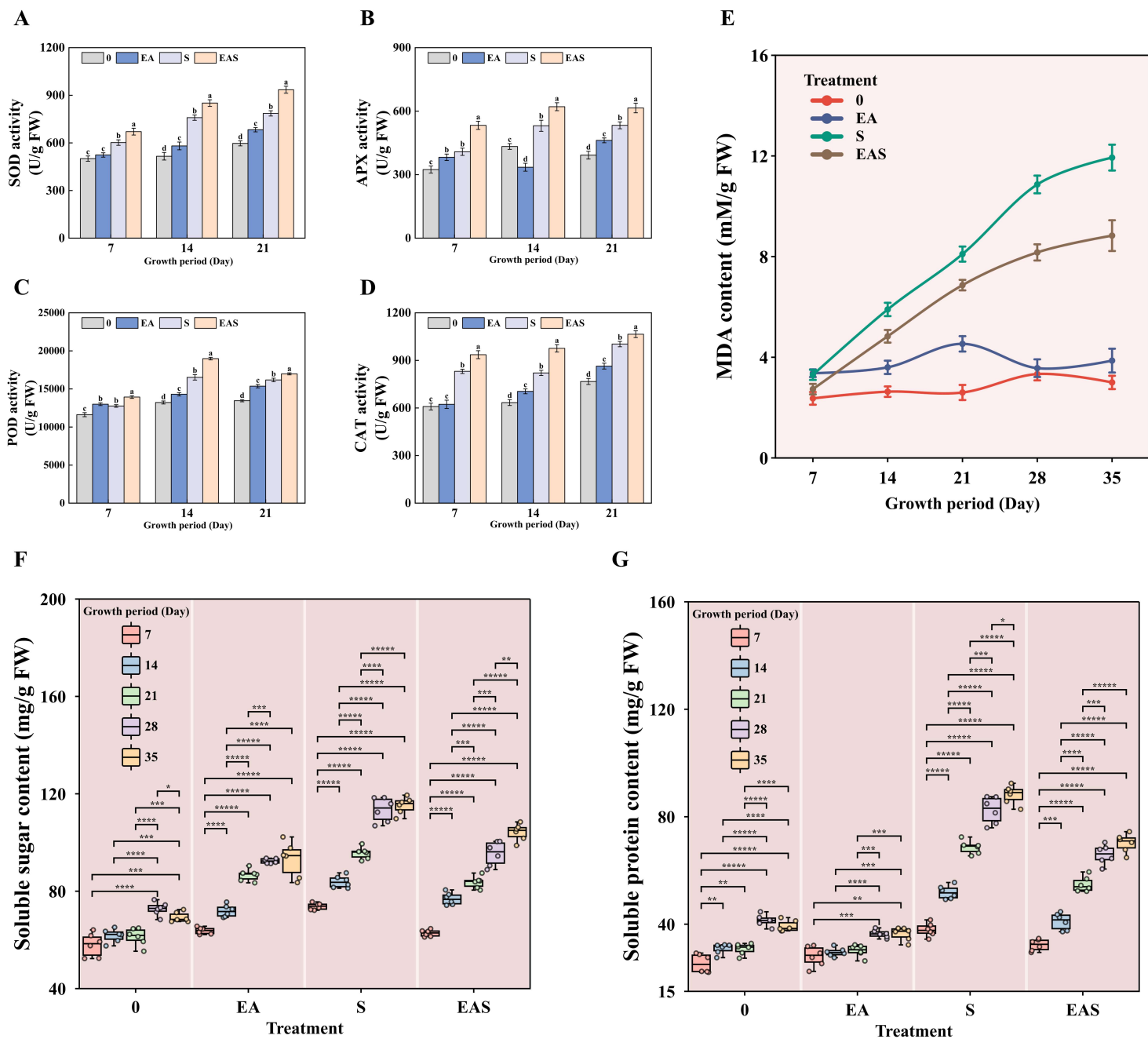


Figure 2

The effect of exogenous application of Pro-Ca on antioxidant enzymes and oxidative damage in 9311 tillers under salt stress. A-D: Activities of superoxide dismutase (SOD), ascorbic acid peroxidase (APX), peroxidase (POD), and catalase (CAT). (E) Accumulation of MDA. (F) Soluble sugar content. (G) Soluble protein content data were expressed as mean $\pm$ SE (n=6). Different letters indicate significant differences among treatments at the same time point ($p < 0.05$).

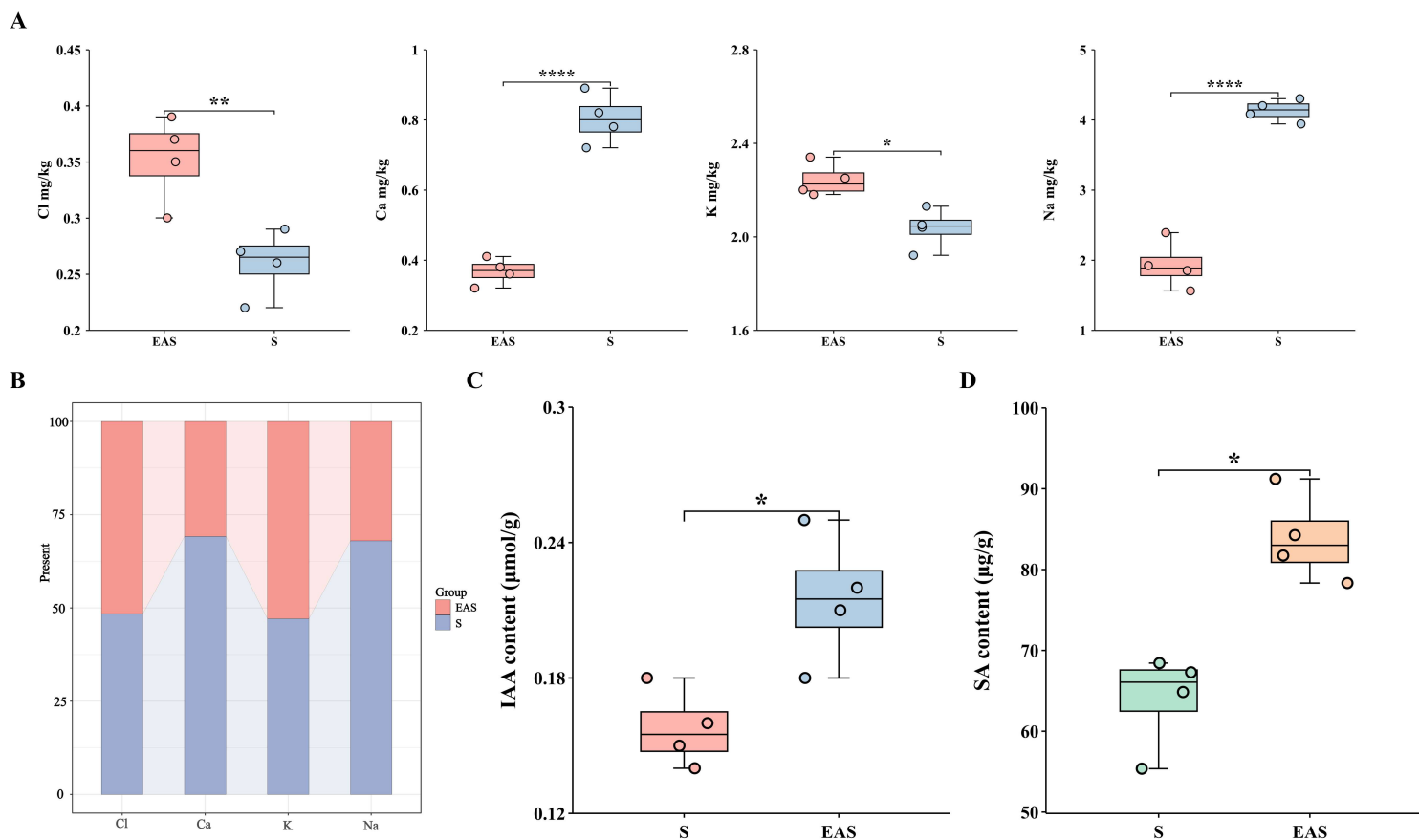


Figure 3

Effects of exogenous Pro-Ca on ion homeostasis and phytohormone levels in rice tillers under salt stress. A: Determination of Cl, Ca, Na and K element contents in tillering nodes of rice under EAS and S treatment groups; B: Bar stacking diagram analysis of the contents of the four elements; C: IAA hormone content; D: SA hormone content.

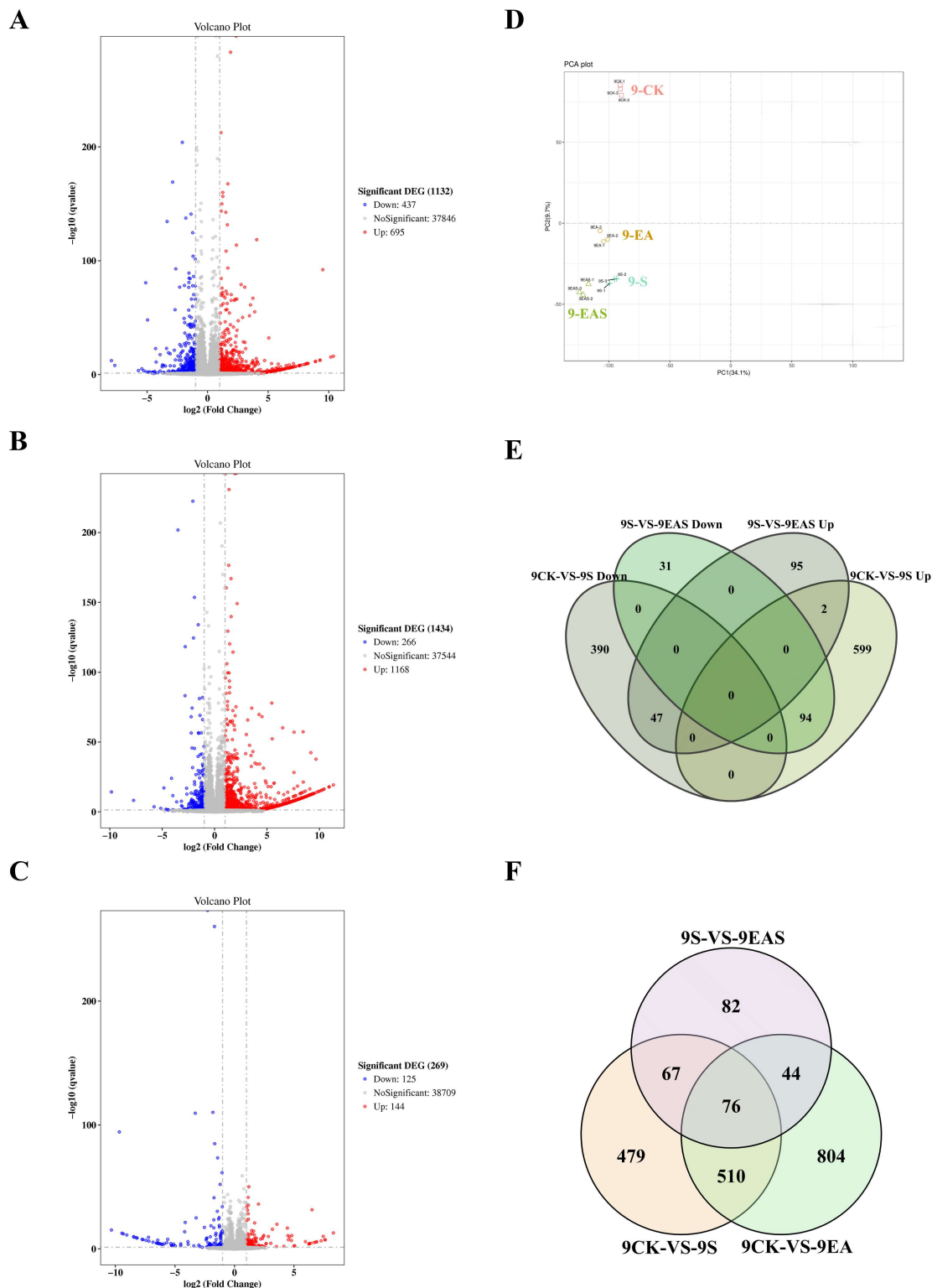


Figure 4

Distribution of Sequencing Data and Differentially Expressed Genes (DEGs). A: Volcano plot of differential genes for 9CK-VS-9S. B: Volcano plot of differential genes for 9CK-VS-9EA. C: Volcano plot of differential genes for 9S-VS-9EAS. D: Principal component analysis of DEGs. Venn diagrams of differentially expressed genes E: 9CK-VS-9S and 9S-VS-9EAS. Venn diagrams of differentially expressed genes F: 9CK-VS-9S, 9CK-VS-9EA, and 9S-VS-9EAS.

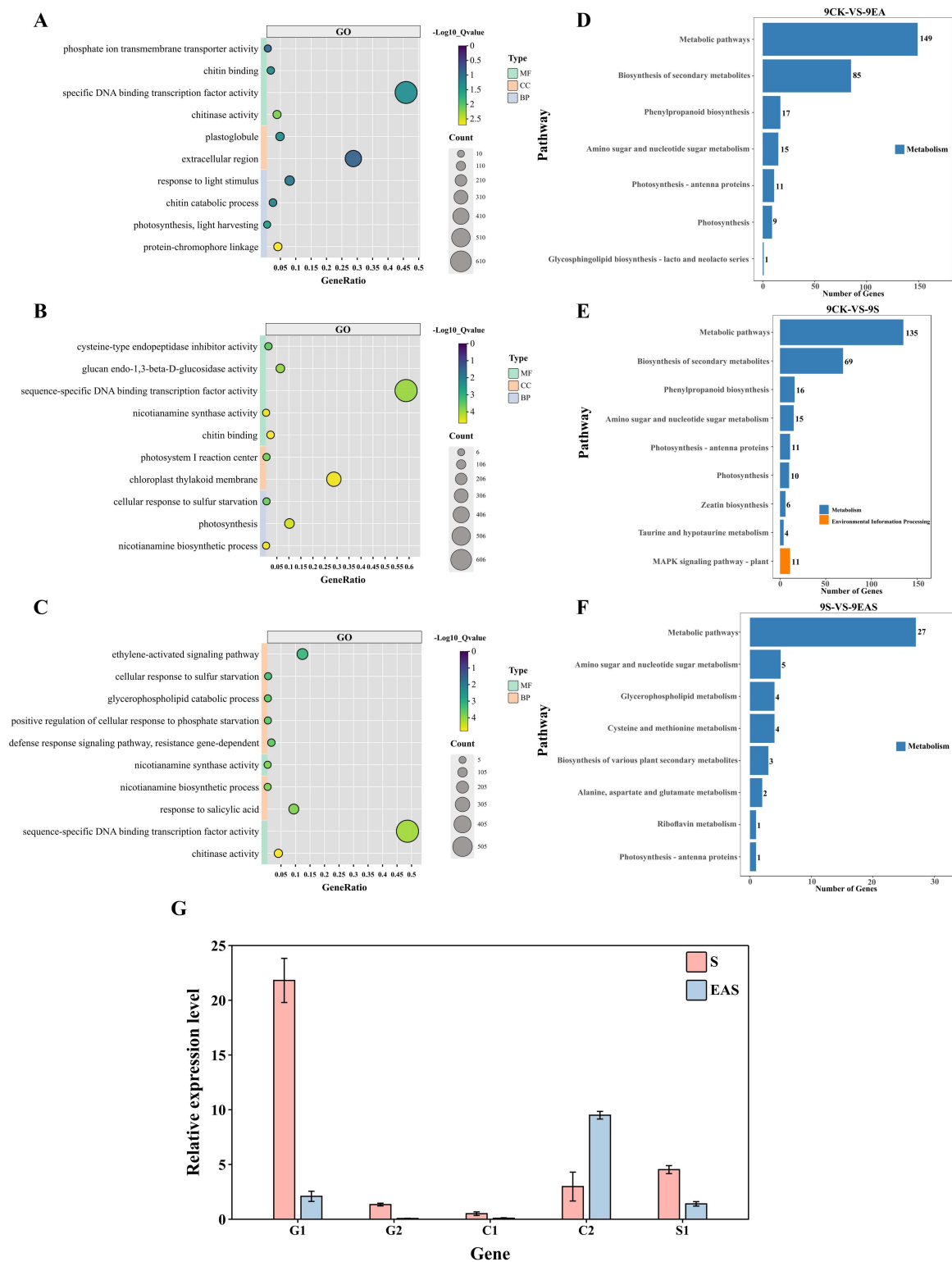


Figure 5

GO enrichment analysis and KEGG enrichment analysis. A: GO enrichment map of 9CK-VS-9EA. B: GO enrichment map of 9CK-VS-9S. C: GO enrichment map of 9S-VS-9EAS. D: The KEGG enrichment plot of 9CK-VS-9EA. E: KEGG enrichment plot of 9CK-VS-9S. F: KEGG enrichment map of 9S-VS-9EAS. G: The expression levels of key genes in glycerophospholipid metabolism (G1, G2), cysteine metabolism (C1,

C2), and starch metabolism (S1), G1: *Os01g0720400*, G2: *Os03g0832800*, C1: *Os05g0149400*, C2: *Os03g0338000*, S1: *Os05g0153500*.

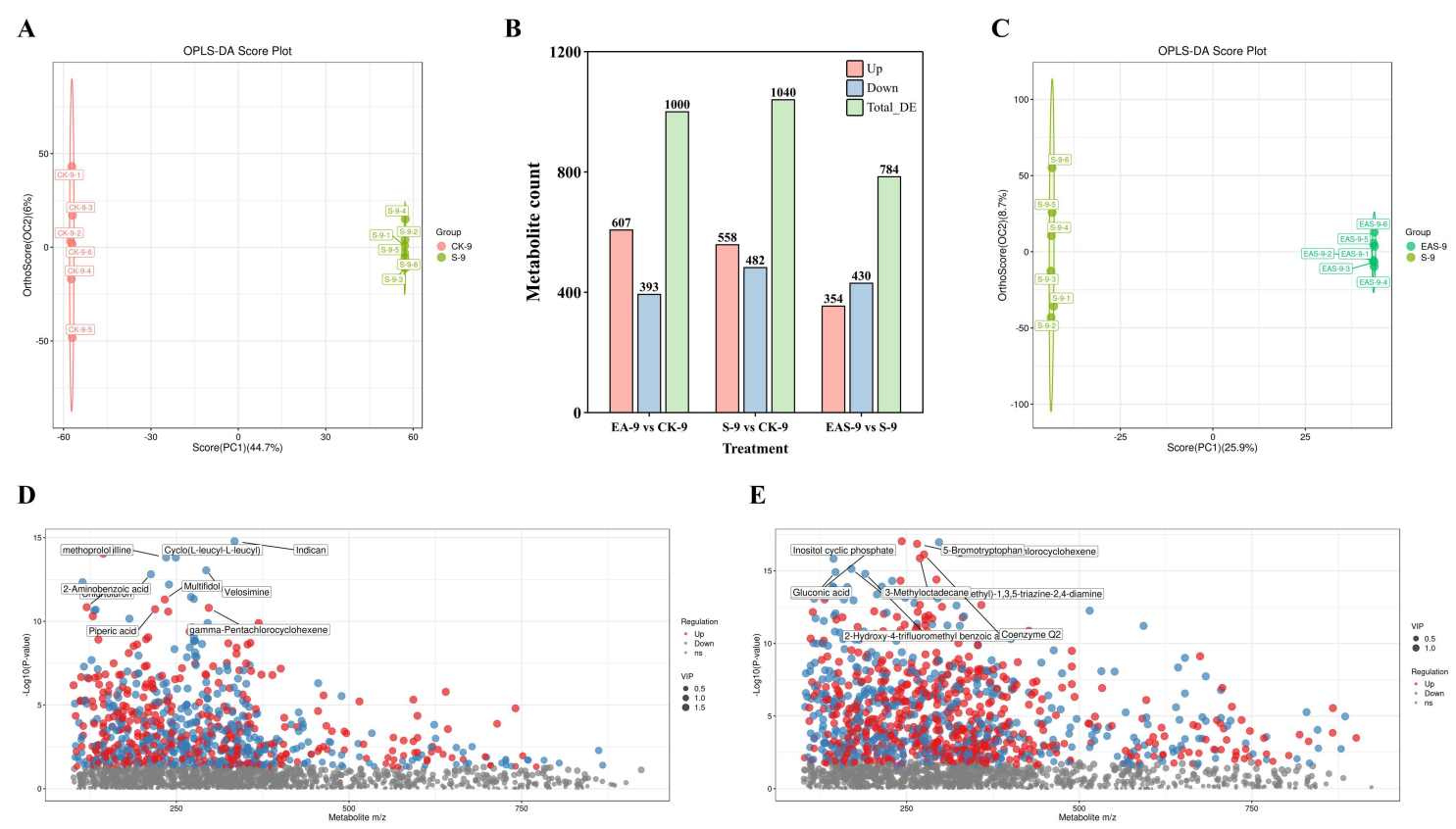


Figure 6

Overview of metabolomics data and screening of differential metabolites. A, C: Sample OPLS-DA score plots for 9S-VS-9CK and 9S-VS-9EAS. B: Statistics detailing the number of differential metabolites. D, E: Scatter plots illustrating the charge ratio and p-value of differential metabolites for 9S-VS-9EAS and 9S-VS-9CK.

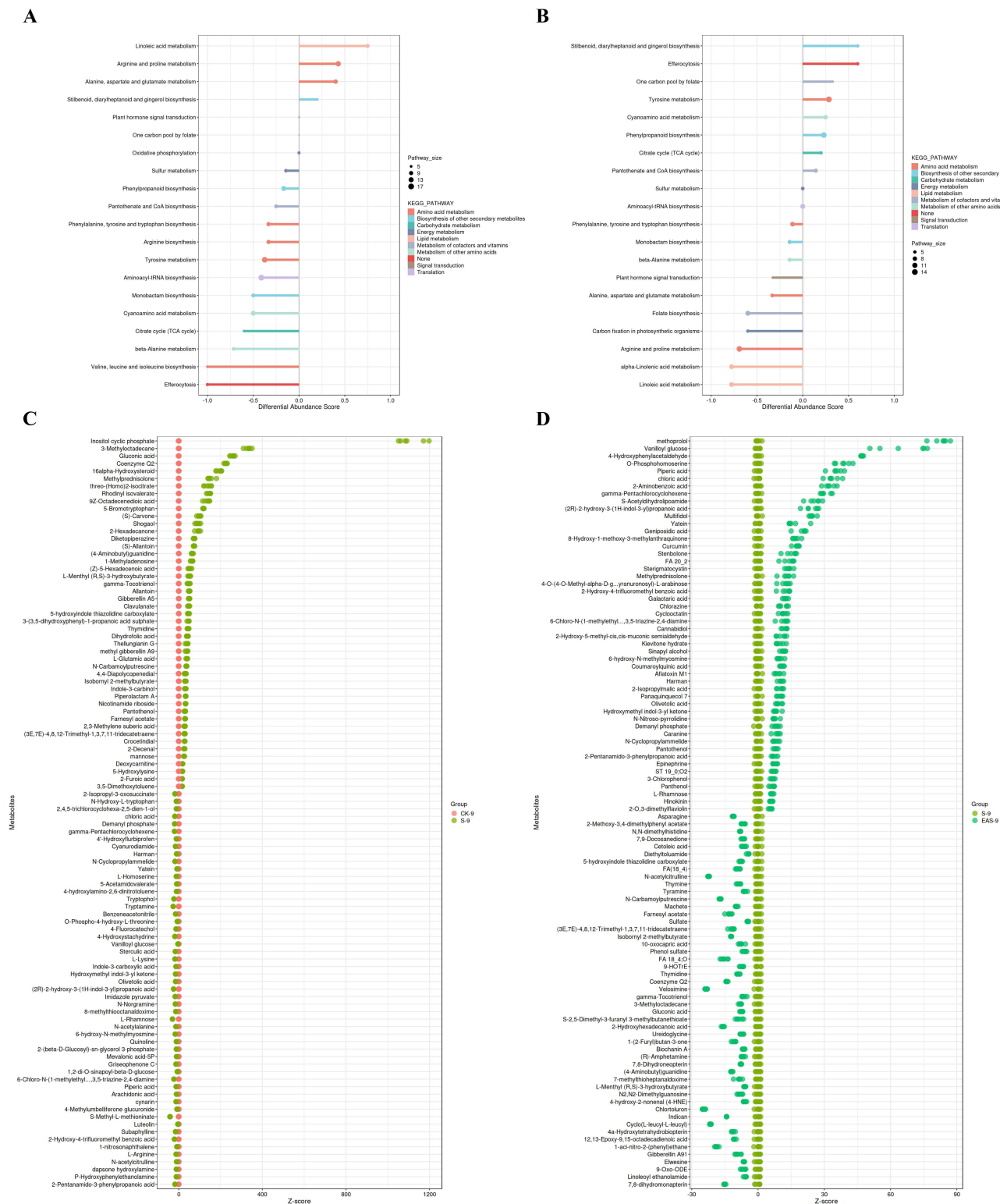


Figure 7

KEGG enrichment analysis and identification of key metabolites. A, B: KEGG enrichment analysis for 9S-VS-9CK and 9S-VS-9EAS; C, D: Z-score analysis of differential metabolites for 9S-VS-9CK and 9S-VS-9EAS.

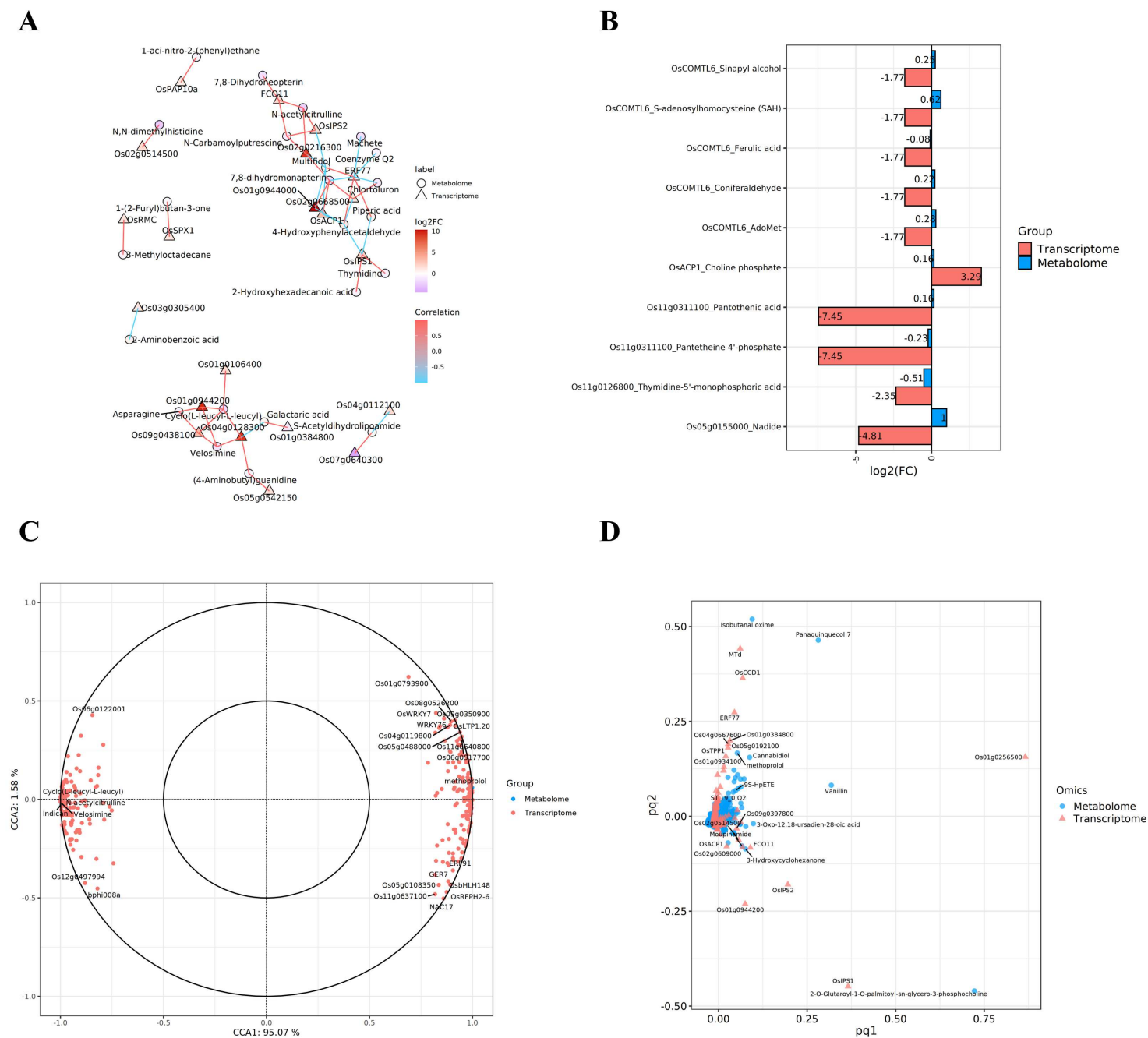


Figure 8

Combined analysis of transcriptomics and metabolomics. A: Correlation network diagram. Triangles represent differentially expressed mRNAs, while dots signify differentially expressed metabolites. The color depth indicates the magnitude of the fold change. The color of the dotted lines reflects the correlation value; darker colors correspond to higher correlation values. B: Enzyme-substrate association analysis. The length of the columns corresponds to the absolute value of $\log_2(FC)$, indicating that substances with longer columns exhibit greater differences in their respective omics and have a more significant impact on enzymatic reactions. C: Typical correlation analysis. Within the same area, points farther from the plateau indicate closer mutual heights, resulting in higher correlation. D: O2PLS

analysis. The load value for the two groups indicates the strength of the association; a greater absolute value suggests a stronger connection between the elements and the other group being analyzed.

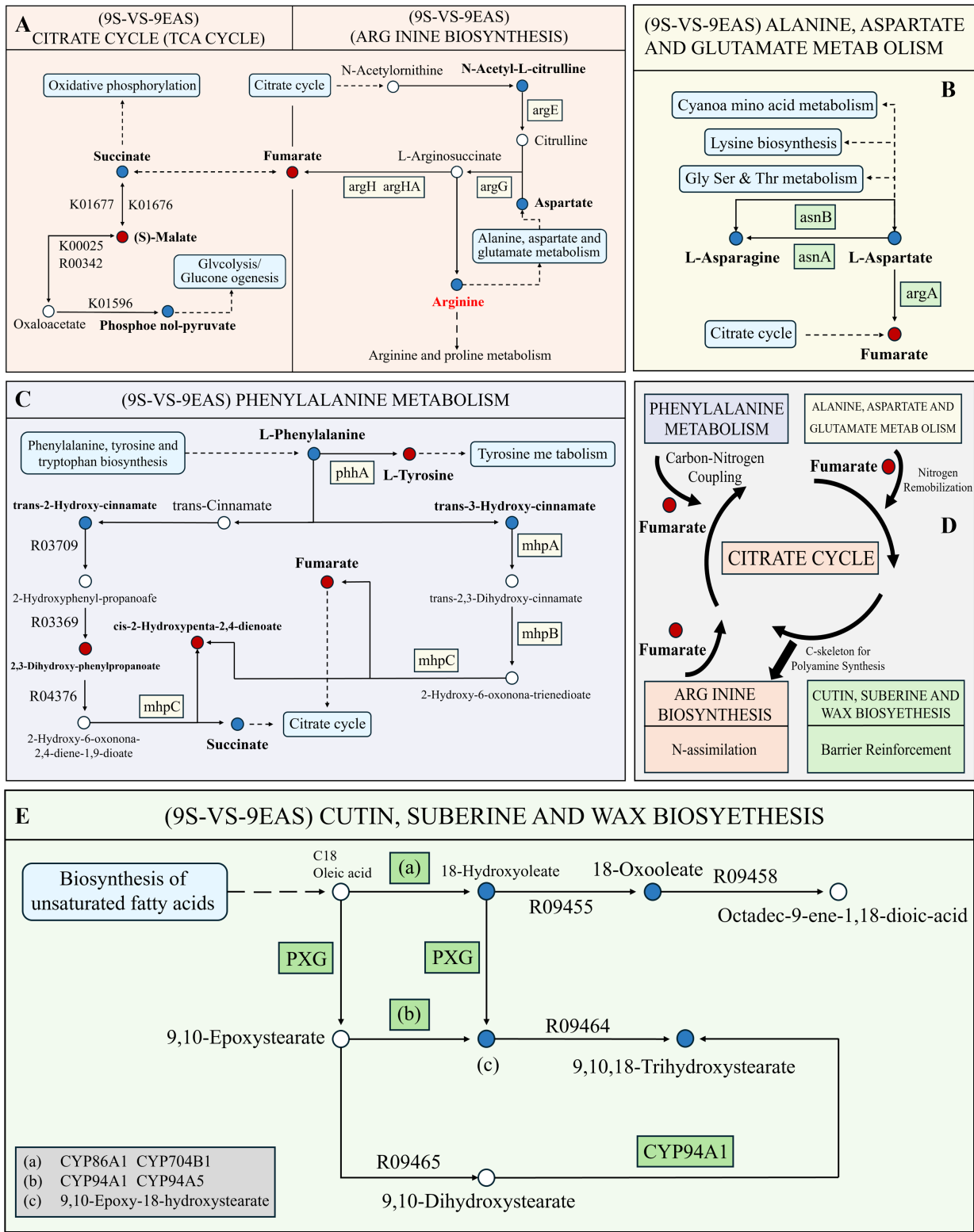


Figure 9

Analysis of key pathways involved in rice tillering under salt stress following the exogenous application of Pro-Ca. A: TCA cycle and arginine biosynthesis, B: Metabolism of alanine, aspartic acid, and

glutamate, C: Phenylalanine metabolism, D: Pathway summary, E: Effects on the biosynthesis of keratin, cutin, and wax. The spheres represent metabolites, with red indicating significant upregulation, blue indicating significant downregulation, green squares representing proteins, and blue boxes denoting metabolic processes.

Supplementary Files

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

- [Additionalfile1.docx](#)