BMC Genomics

RESEARCH

Open Access

Physiological and transcriptomic evidence revealed the role of exogenous GABA in enhancing salt tolerance in strawberry seedlings

Yunting Zhang[1†], Meiyi Deng[1†], Bangyu Lin[1†], Su Tian[1], Yan Chen[1], Shan Huang[1], Yuanxiu Lin[1], Mengyao Li[1], Wen He[1], Yan Wang[1], Yong Zhang[1], Qing Chen[1], Ya Luo[1], Xiaorong Wang[1], Xianjie Gu[2*] and Haoru Tang[1*]

Abstract

As one of the most salt-sensitive crops, strawberry production is severely limited by salt stress. γ--aminobutyric acid (GABA) has been reported to play an important role in the immune response of plants. In this study, the physiological and transcriptomic changes in strawberry seedlings treated with GABA under salt stress were investigated to explore the effect of GABA on salt tolerance. The results showed that exogenous GABA maintained high osmolyte levels, increased antioxidant capacity, and decreased the ROS levels in strawberry leaves under salt stress; the MDA was reduced by 3.27–31.46%, with 10 mM being the most significant effect; the total (Spd + Spm)/Put ratio was upregulated after GABA treatments. More strikingly, the plants treated with 10 mM GABA significantly increased chlorophyll content and net photosynthetic rate in salt-stressed plants, which was explained by the transcriptomic data showing that the expression levels of most of chlorophyll metabolism and photosynthesis-related genes were upregulated. Furthermore, 38 potential TFs belonging to the WRKY, AP2/ERF, and MYB families were identified that may be positively involved in GABA-induced salt tolerance. Co-expressed network analysis revealed that some of these TFs, such as RAP2.7, WRKY46, and MYB306, were significantly positively correlated with chlorophyll metabolism. These findings provide an important basis for the use of GABA in the breeding of strawberry resistant to salt stress.

Keywords Strawberry, GABA, Salt tolerance, Antioxidant capacity, Chlorophyll metabolism

[†]Yunting Zhang, Meiyi Deng and Bangyu Lin contributed equally to this work.

*Correspondence:
Xianjie Gu
heyggu@gmail.com
Haoru Tang
htang@sicau.edu.cn
[1]College of Horticulture, Sichuan Agricultural University, Chengdu 611130, China
[2]Mianyang Academy of Agricultural Sciences, Mianyang 621000, China

Introduction

Soil salinity is a seriously adverse environmental factor that influence seed germination, plant growth and development, particularly in arid and semi-arid areas [1]. About one billion hectares of land are salinized to various degrees, which accounts for 7% the earth's total land area, and this problem is persistently worsening. The main cause for this problem is due to natural geochemical processes (primary salinization), whereas approximately 30% of irrigated lands around the world are salt-affected through secondary human-made salinization [2, 3]. It was estimated that saline soil could cause a global agricultural loss up to $27 billion every year [4].

High salinity disrupts plant physiology through inducing ionic stress, osmotic stress, and secondary stress pathways [5]. To handle such detrimental impacts, plants have evolved a variety of intricate adaptive strategies. Several classes of compatible osmolytes such as sugars, polyols and amino acids are accumulated to adjust osmotic homeostasis for conserving water uptake [6, 7]. Additionally, plant could compartmentalize Na^+ in the vacuole, enhance Na^+ efflux by activating the salt overly sensitive pathway, and Ca^{2+}-dependent Na^+ efflux pathway etc., and limit Na^+ influx through some channels of preferential uptake of K^+ rather than Na^+ etc., which eventually aims to reduce cytoplasm Na^+ and maintain cellular ion homeostasis [8]. The secondary stresses triggered by ionic and osmotic stresses are mainly due to excessive accumulation of reactive oxygen species (ROS) such as hydroxyl radicals (·OH), superoxide anions (O_2^-), and hydrogen peroxide (H_2O_2). It has been reported that high levels of ROS are detrimental to macromolecules such as proteins, lipids, and DNA [9]. To relieve the oxidative damage, cells have developed antioxidants including nonenzymatic low-molecular-weight components (AsA, GSH, carotenoids) and enzymatic components (SOD, CAT, POD, and APX), to detoxify ROS and re-establish cellular redox homeostasis [10, 11]. In addition, the development of omics and technologies used for gene function studies has revealed many regulatory pathways (ABA-dependent/independent pathway and the calmodulin pathway) and candidate genes related to salt stress resistance in plants, which contributes to gain a better understanding of plant salt tolerance [5, 12].

γ-aminobutyric acid (GABA, $C_4H_9NO_2$), a non-proteinogenic amino acid, is first isolated from potato tubers [13]. Thereafter, it has been found to be ubiquitous throughout plant and animal kingdoms. GABA biosynthesis can possibly occur via three pathways: ABA shunt, polyamine degradation and non-enzymatic reactions of proline [14]. Accumulating evidence has highlighted that GABA acts as a metabolite and a signaling molecule and plays an important role in regulating plant growth, development, and senescence under normal and stressful conditions [15]. A rapid increase of endogenous GABA and exogenous supplement of GABA could alleviate abiotic stresses, and ultimately improve morphological and physiological traits [16, 17]. Exogenous GABA rescued the impaired growth phenotype caused by abiotic stresses via promoting photosynthesis, accumulation of compatible osmolytes and mineral nutrients, polyamine metabolism and ROS scavenging [18, 19]. Moreover, GABA strengthens the stress resistance in plants by orchestrating gene expression and integrating other stress-related pathways such as ABA, JA and ethylene signaling pathways [20, 21]. Recently, GABA has been reported to improve salinity stress tolerance in soybean seedlings by modulating mineral nutrition, osmolyte levels and the ascorbate-glutathione cycle [22]. Application of exogenous GABA activates the salicylic acid pathway, promotes phenylpropanoid biosynthesis and enhances the ROS scavenging ability, thereby improving disease resistance in fresh-cut ice plants [23].

Strawberry, an important fruit crop and widely cultivated worldwide, is considered as a salt-sensitive species. The plant growth and fruit yield are adversely affected under salt stress [24]. Hence, it is pivotal to develop useful strategies to enhance strawberry salt tolerance. In this study, different concentrations of GABA were used to analyze the mitigating effect of supplement GABA on strawberry plants under salt stress. The physiological and gene transcriptional changes were analyzed to interpret the physiological response and possible molecular mechanism of GABA-induced salt stress tolerance in strawberry. Our results provide a theoretical basis for further application of GABA in strawberry and other plant production.

Materials and methods

Plant materials and experimental design

Strawberry seedlings (*Fragaria* × *ananassa*, 'Benihoppe') from the College of Horticulture, Sichuan Agricultural University (Chengdu, China) were prepared for this study and planted in plastic pots (16 cm diameter, 14 cm height) filled with a mixture of nutrient soil, garden soil, and vermiculite (in volume proportion of 5:2:1) on 12 March 2022. The seedlings (one seedling per pot) in the tray were grown in the greenhouse under natural conditions of sunlight and ambient temperature and subjected to route management for one and a half months. Uniformly well-grown and healthy plants were selected for salinity treatments and GABA application (Table S1). For salinity treatments, 50 ml of 0 mM and 200 mM NaCl solution were used to irrigate soil every three days. Meanwhile, these strawberry plants were sprayed with GABA. Briefly, the 0 mM NaCl-treated seedlings were sprayed with distilled water as controls (CK). The 200 mM NaCl-treated seedlings were given different concentrations (0, 2.5, 5,

10, 15, 20) of GABA foliar sprays, which were designated as SG0, SG2.5, SG5, SG10, SG15 and SG20, respectively. Exogenous GABA was sprayed on both sides of leaves until they were completely wet. There were three biological replicates of seven plants for each treatment. After 21 days, the photosynthetic parameters were measured and subsequently the samples were collected for further analysis.

Gas exchange and chlorophyll content determination

The photosynthetic parameters including net photosynthetic rate (Pn), transpiration rate (Tr), stomatal conductance (Gs) and intercellular CO_2 concentration (Ci) were determined on the functional leaves (the second fully expanded leaves from the top) with five seedlings for each treatment using a LI-6400 portable photosynthesis system (Li-Cor Inc., Lincoln, NE, USA) from 9:30 to 11:30 a.m. These measurements were performed according to the following settings: 400 μmol·mol^{-1} of the CO_2 concentration with 500 μmol·s^{-1} of flow rate, 25 °C of leaf chamber temperature, 1000 μmol·m^{-2}·s^{-1} of light intensity with a red/blue LED light source. Subsequently, the same leaves were sampled to measure the chlorophyll contents with 80% acetone. The absorbance of the extract was detected at 663 nm, and 645 nm using a microplate reader (Varioskan LUX, Thermo Fisher Scientific, USA). The contents of chlorophyll a (Chl a), chlorophyll b (Chl b) and total chlorophyll (T-Chl) were respectively computed following the formula described by He et al. [25].

Hydrogen peroxide, malondialdehyde and proline determination

Hydrogen peroxide (H_2O_2) was measured using the commercial kit (Suzhou Grace Biotechnology Co. Ltd., Suzhou, China). Malondialdehyde (MDA) content was measured according to the thiobarbituric acid (TBA) method. an amount of 0.2 g of leaf sample was put into 2 mL of ice-cold 10% (w/v) trichloroacetic acid. The mixture was centrifugated at 5,000 g at 4 °C for 10 min, the 100 μL of upper phase was added to 300 μL of 0.67% (w/v) 2-thiobarbituric acid. After incubation for 10 min at 100 °C, the mixture was cooled to room temperature and then centrifuged again. Subsequently, the supernatant was used to determine the optical density at 450 nm, 532 nm, and 600 nm. Leaf sample (0.1 g) was extracted with 1 mL of 3% (w/v) sulphosalicylic acid, and heated at 93 °C for 10 min. After centrifugation, the supernatant was diluted 10-fold with extraction buffer. Then, 500 μL diluted supernatant with an addition of equal volume of glacial acetic acid and 2.5% (w/v) ninhydrin was heated at 93 °C for 30 min. subsequently, the amount of proline was measured at 520 nm.

Polyamines determination

A total of 0.1 g leaf sample was extracted with 2 mL of 70% (v/v) methanol, followed by centrifuging at 12 000 g for 20 min at 4 ℃. The supernatant was obtained for polyamines determination using an ELISA kit (Shanghai Enzyme-Linked Biotechnology Co. Ltd. Shanghai, China).

DPPH and FRAP determination

An amount of 0.1 g sample was added to 1mL of 80% ethanol, followed by centrifugating at 10 000 g and 4 °C for 10 min. The exact was collected to perform the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay and the ferric reducing antioxidant power (FRAP) assay, according to the procedures described by Zhang et al. [26]. The extract was mixed with the DPPH-ethanol solution, or the working FRAP reagent. After incubation, the scavenging effects were respectively evaluated by absorption value at 517 nm and 593 nm.

SOD, CAT, and POD determination

Leaf powder of approximately 0.1 g was supplemented with 1 mL precooled phosphate buffer (100 mM, 7. 0 pH) with 4% (w/v) insoluble PVPP and 1 mM EDTA. After spinning, the clear liquid was obtained for determination of SOD activities by the nitroblue tetrazolium (NBT) photoreduction method [26] and POD activities by the guaiacol oxidation method [27]. About 0.1 g leaf powder was weighed and homogenized in 1 mL extract buffer. After centrifugating at 4℃ and 12,000 g for 10 min, the upper phase was collected to determine the CAT activity with the commercial kit (Suzhou Grace Biotechnology Co. Ltd., Suzhou, China).

Transcriptome sequencing and analysis

Total RNA extracted from three treatments of CK, SG0 (ST), SG10 (GS) was used to construct RNA-seq library, after quantification and qualification assessment. A total of nine (three biological replicates of each treatment) libraries were clustered and sequenced (150 bp, pair-end) by Novogene (Beijing, China) on the Illumina NovaSeq6000 sequencing platform.

Clean data (clean reads) were screened by eliminating the reads containing poly-N, adapters, and low-quality reads. The clean reads were aligned to the octoploid strawberry 'Yanli' reference genome using Hisat2 (v.2.0.5) software. Differentially expressed genes (DEGs) between two groups were identified using DESeq2 with the significant thresholds of adjusted p value ≤ 0.05 and|log2(foldchange)| ≥ 1. Gene ontology (GO) and DEGG analysis of DEGs were performed using the clusterProfiler R package. Padj less than 0.05 as the threshold for significant enrichment.

The PlantTFDB online tool v.5.0 (http://planttfdb.cbi.pku.edu.cn/, accessed on 27 September 2024) was used to confirm the transcription factors from DEGs. For the co-expression network of correlation analysis, the co-expressed pairs with the criterion of pearson correlation coefficients (PCC)|r|≥ 0.80 and $p \leq 0.05$ were considered as significantly correlated pairs. The co-expression network was visualized by Cytoscape software (v3.10.0).

qRT-PCR analysis

To validate the accuracy of RNA-seq data, eight genes were selected to conduct qRT-PCR with SYBR Premix (Takara, Japan) on a CFX96 real-time PCR system (Bio-Rad, Hercules, CA, USA), as described by Zhang et al. [28]. *FaACTIN* gene (AB116565.1) was applied for normalization of the gene expression. The relative expression level of target genes was evaluated using the $2^{-\Delta\Delta CT}$ method. The gene-specific primers designed by Primer 5 (Premier Biosoft, Palo Alto, CA, USA.) were listed in Table S2.

Statistical analysis

All data were analyzed by one-way ANOVA using SPSS22.0 statistical software, and all graphs were drawn using GraphPad Prism 9.5 software. Different lowercase letters above the histograms indicate significant differences (Duncan's test, $P < 0.05$).

Results

Effect of GABA on osmotic substances in strawberry leaves under salt stress

By comparison with CK, the SG0 treatment notably induced the proline accumulation. GABA application inhibited the increase of proline content in plants exposed to salt stress. Except for SG10, other GABA treatments maintained the higher ($P < 0.05$) proline level than CK (Fig. 1A). Intriguingly, SG0 treatment had no

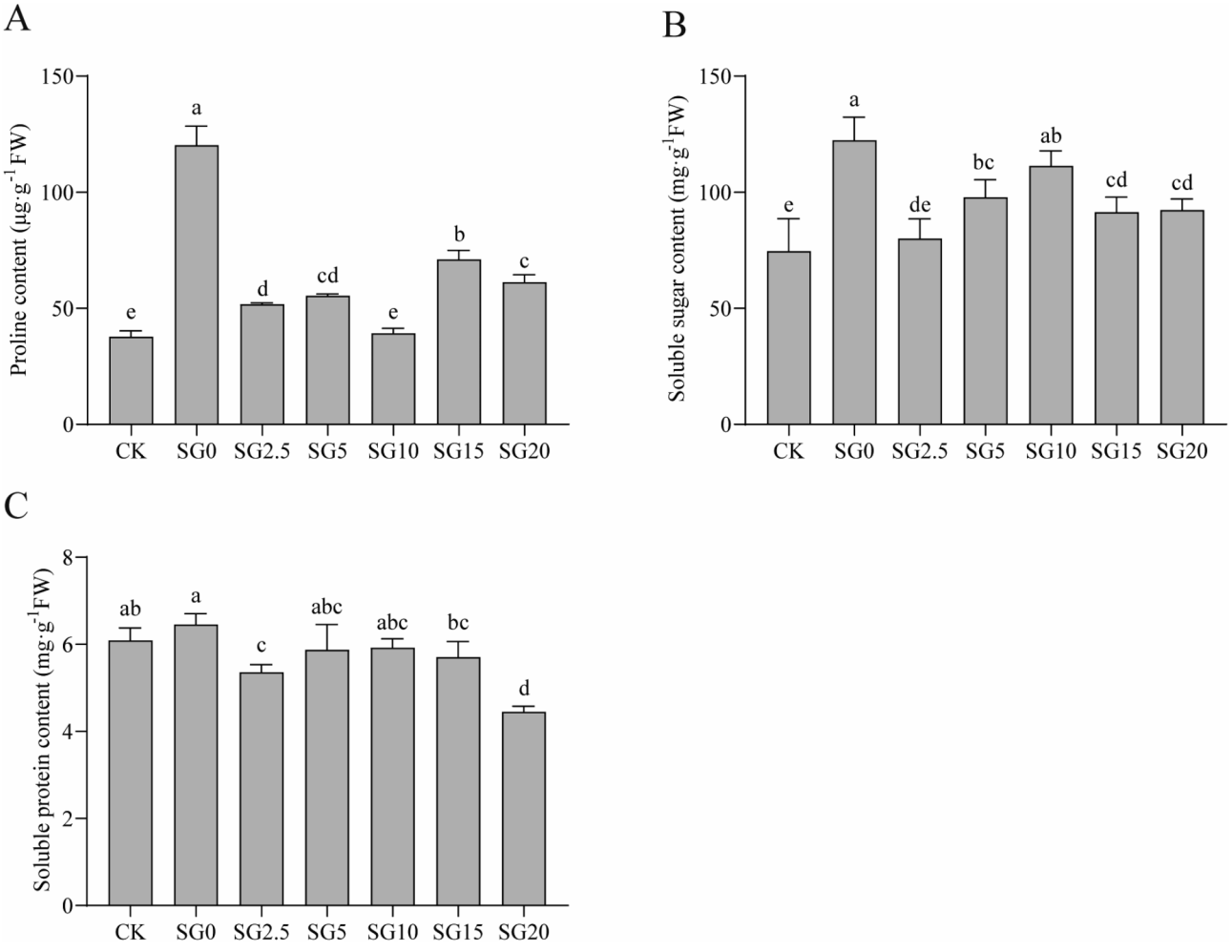

Fig. 1 Effect of GABA on osmotic substances in strawberry leaves under salt stress. (**A**) Proline content; (**B**) soluble sugar content; and (**C**) soluble protein content. Error bars represent SE (standard error) of three biological replicates. Different lowercase letters above the histograms indicate significant differences (Duncan's test, $P < 0.05$)

significant effect on soluble protein content. Compared to SG0, the addition of GABA at variable concentrations reduced soluble protein content, but only 2.5-, 15- and 20-mM GABA had significant effect (Fig. 1C). A dramatic increment in soluble sugar was observed after strawberry exposure to salt stress. The soluble sugar content in NaCl plus GABA-treated plants was lower than SG0 but higher than CK (Fig. 1B).

Effect of GABA on ROS and MDA in strawberry leaves under salt stress

The O_2^- production rate was significantly increased after plant subjected to salt stress, whereas GABA spray treatments at 5, 10, 15 and 20 mM decreased the O_2^- production rate by 6.55% ($P<0.05$), 0.32% ($P>0.05$), 47.75% ($P<0.05$) and 25.70% ($P<0.05$), respectively (Fig. 2A). A significant increment in H_2O_2 content was observed when strawberry seedlings were exposed to SG0 treatment. However, after foliar application of 2.5-, 5-, 10-, 15- and 20-mM GABA, the H_2O_2 content in plants was reduced by 11.68% ($P<0.05$), 6.56% ($P>0.05$), 17.24% ($P<0.05$), 14.77% ($P<0.05$) and 5.67% ($P>0.05$), respectively (Fig. 2B). Compared with the CK group, strawberry seedlings exposed to salt stress (SG0) significantly increased the MDA content, while the plants treated with GABA showed less increase. The MDA content in SG2.5, SG5, SG10, SG15 and SG20 treatments was decreased by 17.01% ($P<0.05$), 17.05% ($P<0.05$), 31.46% ($P<0.05$), 3.27% ($P>0.05$) and 21.16% ($P<0.05$), respectively as compared to SG0 treatment (Fig. 2C).

Effect of GABA on antioxidant enzymes and total antioxidant capacity in strawberry leaves under salt stress

Salt stress enhanced the activities of antioxidant enzymes (SOD, POD, and CAT) in plants (Fig. 3A-C). The addition of 20 mM GABA significantly increased the SOD activity in strawberry plants under salt stress (Fig. 3A), while 2.5 mM and 10 mM GABA remarkably upregulated the

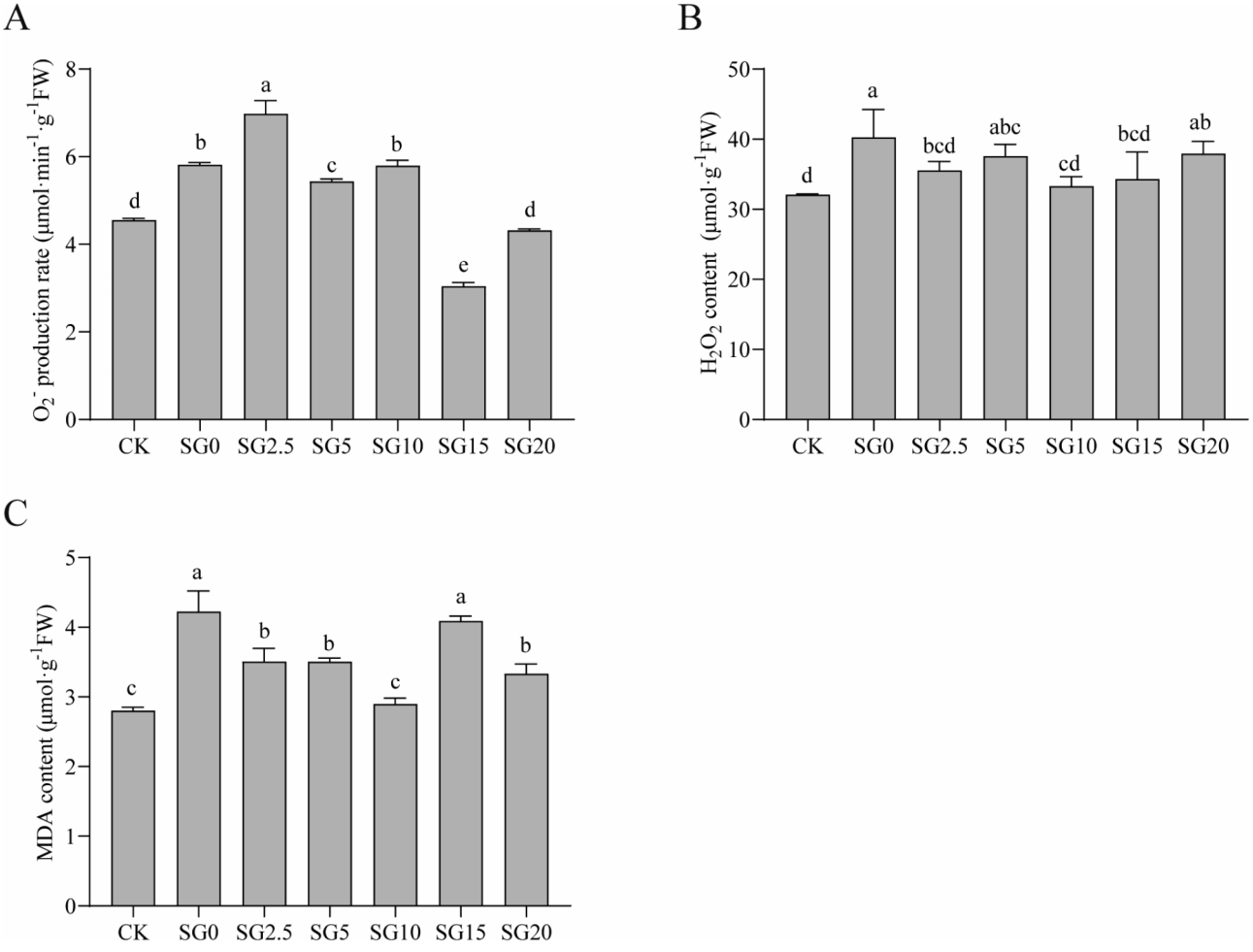

Fig. 2 Effect of GABA on ROS and MDA in strawberry leaves under salt stress. (**A**) O_2^- production rate; (**B**) H_2O_2 content; and (**C**) MDA content. Error bars represent SE (standard error) of three biological replicates. Different lowercase letters above the histograms indicate significant differences (Duncan's test, $P<0.05$)

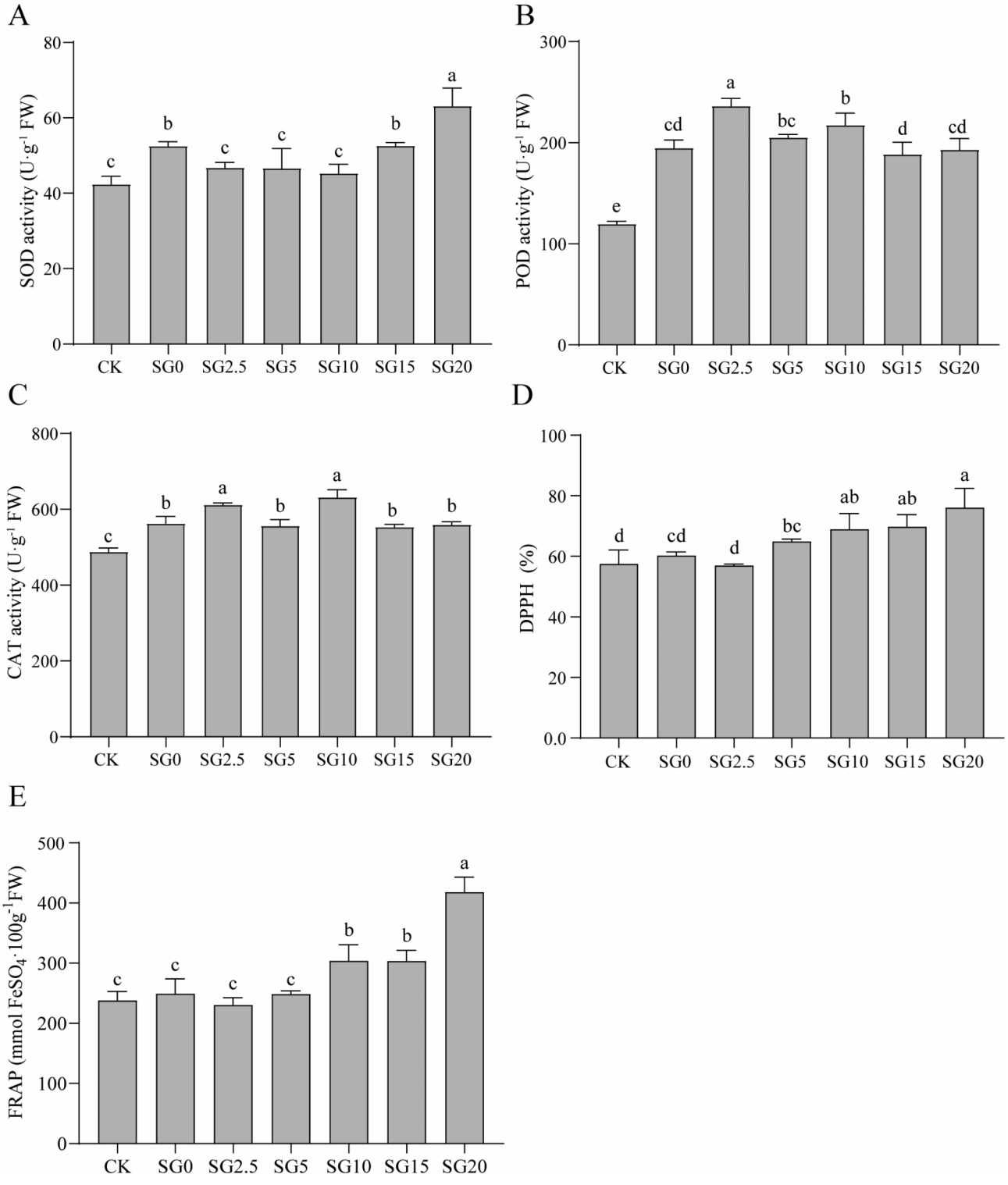

Fig. 3 Effect of GABA on antioxidant enzymes and total antioxidant capacity in strawberry leaves under salt stress. (**A**) Superoxide dismutase (SOD); (**B**) peroxidase (POD); (**C**) catalase (CAT); (**D**) DPPH (1,1-diphenyl-2-picryl-hydrazyl) radical scavenging activity; and (**E**) ferric reducing antioxidant power (FRAP). Error bars represent SE (standard error) of three biological replicates. Different lowercase letters above the histograms indicate significant differences (Duncan's test, $P < 0.05$)

POD and CAT activities in plants exposed to salt stress (Fig. 3B-C). The DPPH and FRAP in seedlings exposed to salt stress had no significant change when compared to the CK. The values of DPPH and FRAP displayed an upward trend with the increase of GABA concentration. Moreover, it was seen that DPPH and FRAP values in SG10, SG15 and SG20 treatments were notably higher than SG0 (Fig. 3D-E).

Effect of GABA on polyamines in strawberry leaves under salt stress

After salt stress (SG0), the level of spermine was significantly more than those of the untreated plants (CK). The adding of GABA at different concentrations except 5 mM had stronger effect on promoting spermine accumulation in plants (Fig. 4A). However, the plants under salt stress exhibited lower spermidine content than CK, while application of GABA inhibited the decrease of spermidine content in plant under salt stress (Fig. 4B). Additionally, compared with CK, the putrescine amount under SG0 was obviously upregulated. Under 5-, 10- and 15- mM GABA treatments, the putrescine content of strawberry leaves was significantly higher than that of SG0 plants (Fig. 4C). Additionally, the ratio of total (Spd + Spm)/ Put in plant was reduced under salt stress, while it was upregulated by applying 2.5, 10, 15, 20 mM GABA (Fig. 4D).

Effect of GABA on gas exchange and chlorophyll content in strawberry leaves under salt stress

Salt stress (SG0) remarkably reduced net photosynthetic rate (Pn), stomatal conductance (Gs), and transpiration rate (Tr) in comparison with CK. Foliar application of exogenous GABA (2.5, 5, 10, 15, 20 mM) affected the gas

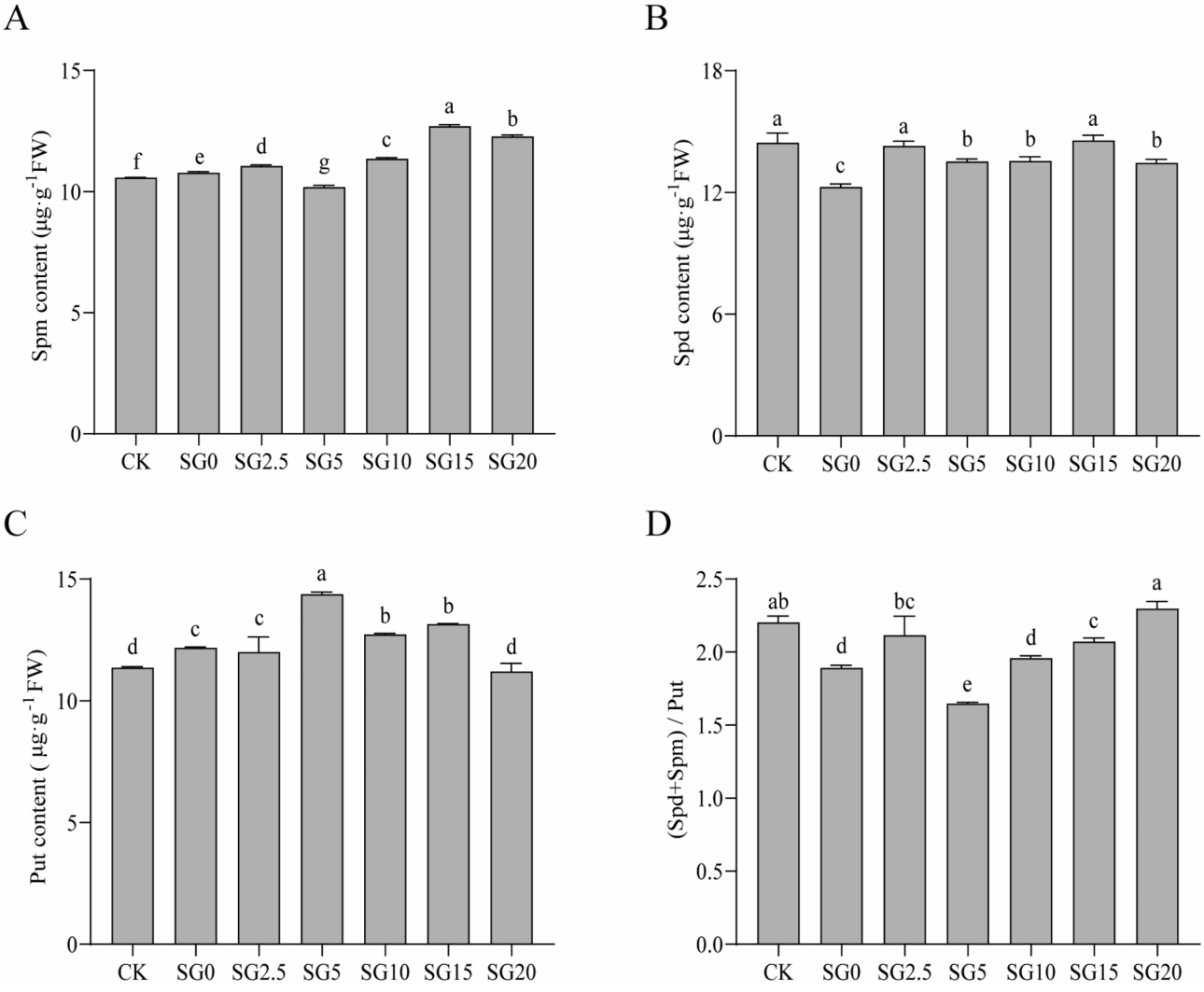

Fig. 4 Effect of GABA on polyamines in strawberry leaves under salt stress. (**A**) Spermine (Spm); (**B**) spermidine (Spd); (**C**) putrescine (Put); and (**D**) the ratio of total contents of Spd and Spm to Put (Spd + Spm)/Put. Error bars represent SE (standard error) of three biological replicates. Different lowercase letters above the histograms indicate significant differences (Duncan's test, $P < 0.05$)

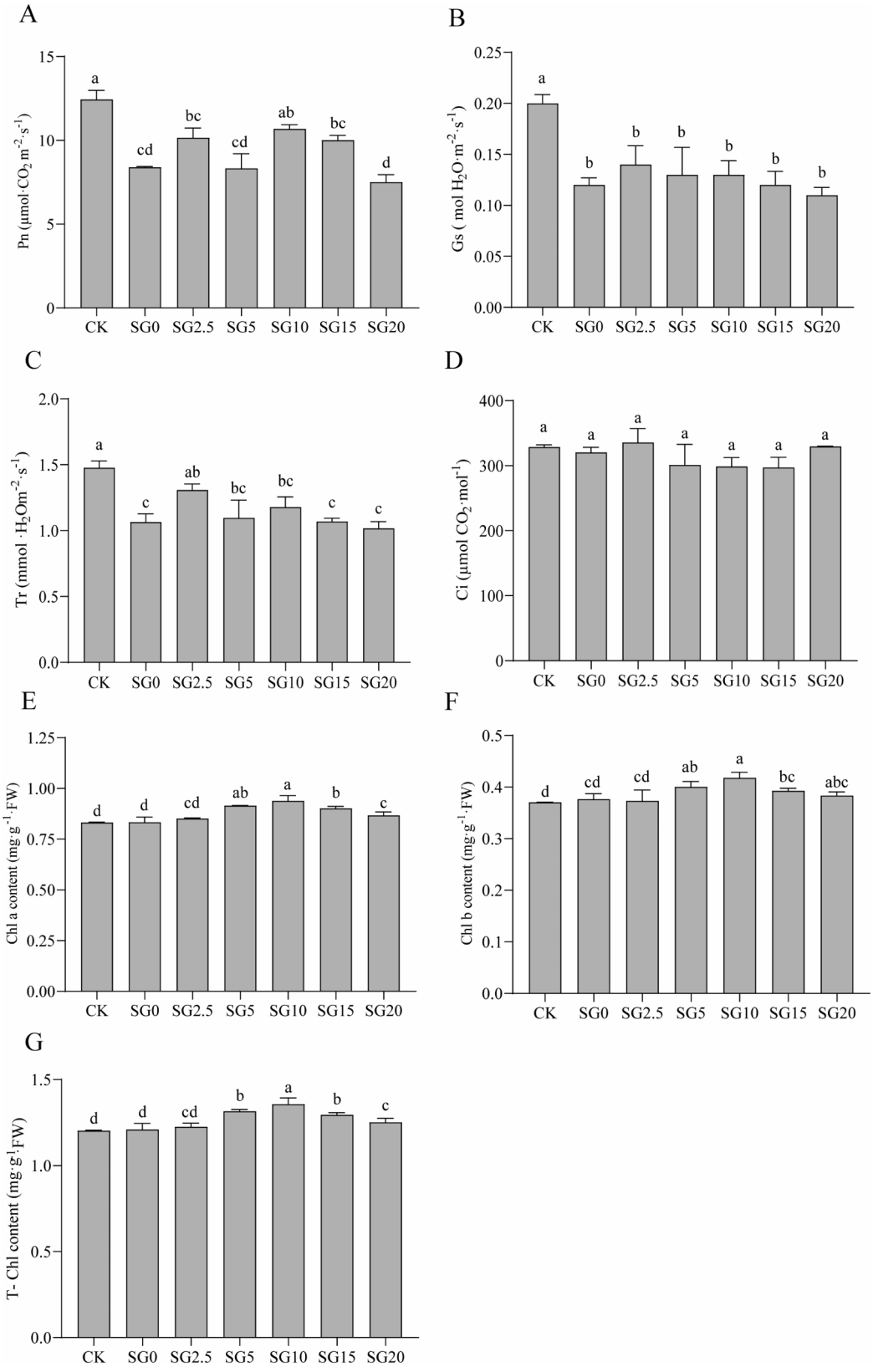

Fig. 5 Effect of GABA on gas exchange and chlorophyll content in strawberry leaves under salt stress. (**A**) Net photosynthetic rate (Pn); (**B**) stomatal conductance (Gs); (**C**) transpiration rate (Tr); (**D**) intercellular CO_2 concentration (Ci); (**E**) chlorophyll a (Chl a), (**F**) chlorophyll b (Chl b); and (**G**) total chlorophyll (T-Chl). Error bars represent SE (standard error) of three biological replicates. Different lowercase letters above the histograms indicate significant differences (Duncan's test, $P < 0.05$)

exchange parameters of plants under salt stress (Fig. 5A-D). The net photosynthetic rate of plants treated with 2.5 mM (SG2.5), 10 mM (SG10), 15 mM GABA (SG15) was increased by 21.10% ($P>0.05$), 27.29% ($P<0.05$), 19.31% ($P>0.05$), respectively, compared to SG0, whereas 5 mM (SG5) and 20 mM (SG20) GABA treatments reduced the net photosynthetic rate by 0.83% ($P>0.05$)and 10.61% ($P>0.05$) (Fig. 5A). With the increase of GABA dosage, the chlorophyll a, chlorophyll b and total chlorophyll contents in plant leaves exhibited an upward trend first and then downward trend. Under SG10 treatment, the contents of the chlorophyll a, chlorophyll b and total chlorophyll were highest among all treatments (Fig. 5E-G). These findings indicated that 10 mM GABA effectively mitigated the inhibition of photosynthesis in plant exposed to salt condition.

Transcriptome assembly

Our results showed that GABA spray treatment at 10 mM, being the optimal concentration, significantly improved the salt stress tolerance of seedlings, as evidenced by changes in various physiological indicators. To gain a deep insight into GABA-induced mitigation of salt stress on transcriptomic level. Nine cDNA libraries were constructed with leaves from three biological replicates of CK, SG0 (ST), SG10 (GS) treatments for RNA-seq. A total of 61.32 G of clean data were generated. The Q20 and Q30 were greater than 98.34% and 94.86%, respectively. The GC content was 45.41-46.24%. Approximately 88.91% of clean reads from each sample were aligned to the reference genome (Table S3). The replicates of samples showed a linear correlation (average $R^2>0.8$) (Fig. S1). The outcomes suggested that the transcriptome sequencing data could be used for downstream analysis.

Differentially expressed genes identification

A total of 10,875 DEGs were screened in ST vs. CK comparison pair, 5240 GEGs of which was upregulated, and 5635 DEGs of which was downregulated. A total of 2945 DEGs were identified in GS vs. CK comparison pair. Of these, 1386 DEGs were upregulated and 1559 DEGs were downregulated. In GS vs. ST comparison pair, 3887 DEGs were expressed, among which 1902 DEGs were upregulated and 1985 DEGs were downregulated (Fig. 6A). Shared and unique DEGs in three comparison pairs are depicted in the Venn diagram. The largest number of common DEGs (2713) were detected between the ST vs. CK and GS vs. ST comparison pairs. The unique DEGs in GS vs. CK was far fewer than that in ST vs. CK owing to the addition of exogenous GABA (Fig. 6B). The DEGs were hierarchically clustered based on their expression pattern across three treatments. The GEGs in GS and CK treatments was more closely clustered together in comparison to ST treatments (Fig. 6C).

Differentially expressed genes annotation

GO and KEGG pathway enrichment analyses of the DEGs were conducted to further explore the DEGs' biological functions. The first ten significantly enriched GO terms of biological process (BP), cell component (CC), and molecular function (MF) were respectively selected (Fig. S2). In BP category, "photosynthesis" (GO:0015979) was the top one enriched GO term in ST vs. CK, GS vs. CK and GS vs. ST. In CC category, "thylakoid part" (GO:0044436) was the top one enriched Go term in three pairwise comparisons. In MF category, "serine-type endopeptidase inhibitor activity" (GO:0004867), "chitinase activity" (GO:0004568) and "oxidoreductase activity, acting on diphenols and related substances as donors, oxygen as acceptor" (GO:0016682) was the most significantly enriched in ST vs. CK, GS vs. CK and GS vs. ST, respectively.

The KEGG enrichment analysis showed DEGs in ST vs. CK, GS vs. CK and GS vs. ST were significantly enriched in 21, 10 and 16 metabolic pathways, respectively (Fig. 7). Five pathways of "Photosynthesis", "Photosynthesis-antenna proteins", "Carbon fixation in photosynthetic organisms" "Glyoxylate and dicarboxylate metabolism", and "Pentose phosphate pathway" simultaneously existed significant enrichment in three comparison pairs. Moreover, "Sesquiterpenoid and triterpenoid biosynthesis", "Anthocyanin biosynthesis" and "Isoflavonoid biosynthesis" pathways were simultaneously abundant in ST vs. CK and GS vs. CK. Strikingly, many of DEGs in ST vs. CK and GS vs. ST were annotated to the pathways of amino acid and carbohydrate metabolism, which were probably important for GABA-mediated salt stress response.

Differentially expressed genes involved in chlorophyll and photosynthesis

In total, 13 DEGs, including 1 HEMB, 4 HEMF, 1 CHLD/H/I, 1 CRD, 1 DVR, 1 CHLG, 2 CBR (NYC1/NOL), 1 SGRL and 1 PPH, were identified to be related to chlorophyll synthesis, chlorophyll cycle and chlorophyll degradation, respectively (Fig. 8A, Table S4). Based on the heatmap, the expression levels of all these DEGs were downregulated in ST compared to CK and GS treatments. HEMB and CHLD/H/I were most highly expressed in GS, while other genes were more highly expressed in CK than in ST and GS. Furthermore, 91 DEGs, including 2 light-harvesting chlorophyll (LHC) protein complex genes (LHCA5 and LHCA6), 44 photosystem I (PSI) subunit genes (PSAs) and 45 photosystem II (PSII) subunit genes (PSBs), were identified (Fig. 8B, Table S5). It was found that the expression of most of these photosynthesis-related genes was downregulated in strawberry leaves from ST compared to those from CK and GS. Only one PSBR subunit gene (FxaYL_342g0196150) of PSII was upregulated in ST compared to CK and GS. Notably,

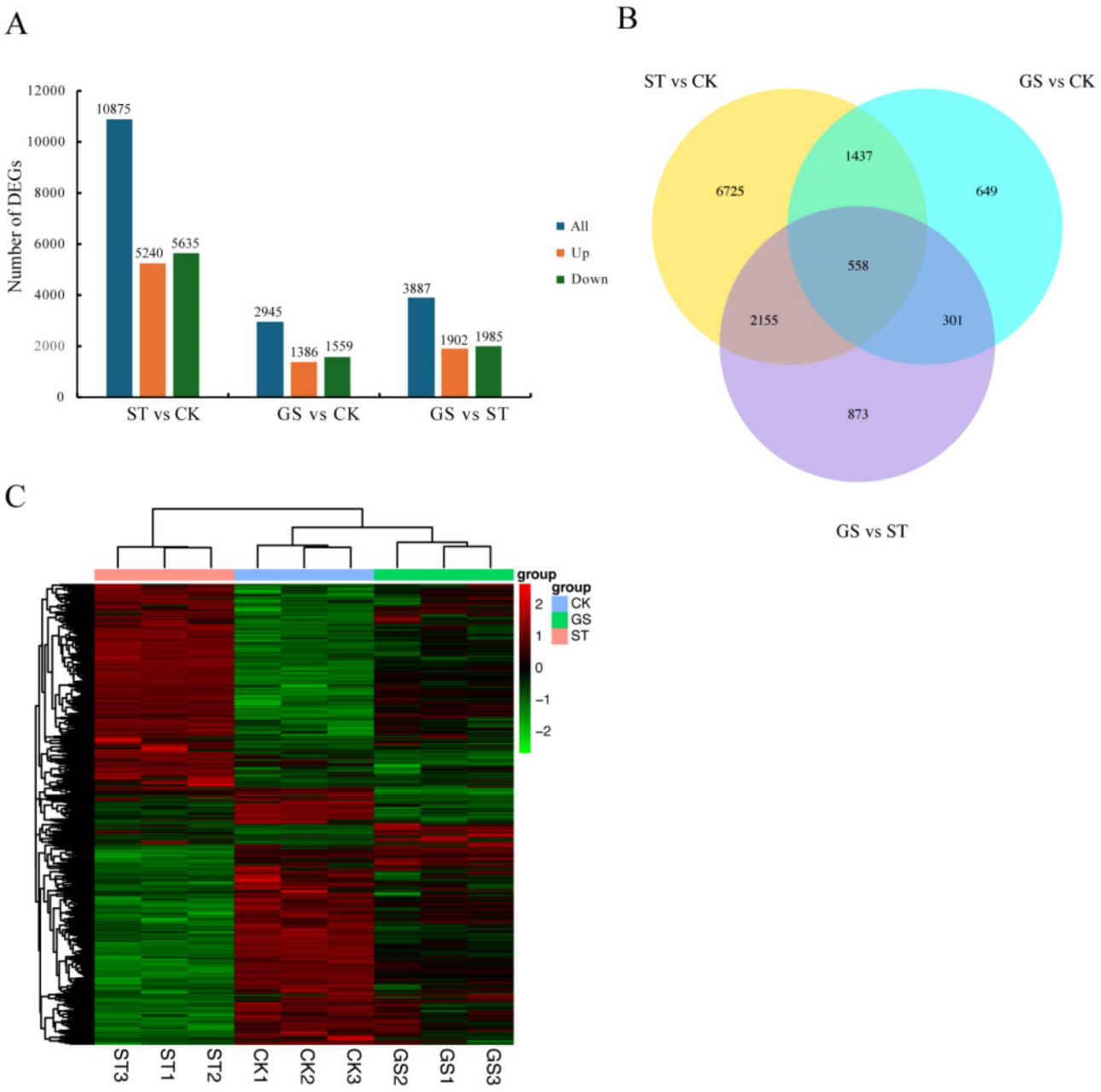

Fig. 6 Differential expressed genes (DEGs) comprehensive analysis in strawberry leaves under different treatments. (**A**) The Number of up- or down-regulated DEGs by pairwise comparison. (**B**) Venn diagram showing the commonly and uniquely regulated DEGs in the three comparison groups. (**C**) Heat map showing the expression pattern of the DEGs in the different samples

most of these photosynthesis-related genes was down-regulated in GS vs. CK, but upregulated in GS vs. ST.

Differentially expressed transcription factors and correlation analysis

To identify the putative transcription factors that positively participated in GABA-induced salt resistance in strawberry, the top 3 differentially expressed transcription factors (MYBs, AP2/ERFs and WRKYs) with log2 (foldchange) < -1 in ST vs. CK and ST vs. GS were identified, which included 10 AP2/ERFs, 14 MYBs and 14 WRKYs (Fig. S3, Table S6). Furthermore, the network of correlation analysis was conducted to ascertain the relationship between these TFs, chlorophyll metabolites and structural genes related to chlorophyll metabolism in strawberry leaves (Fig. 9, Table S7). As a result, 428 significantly correlated pairs ($|r| > 0.8$, $p < 0.05$) were observed. A very strong positive correlation was found between chlorophyll a, chlorophyll b and total chlorophylls. In addition, 9 structural genes containing CHLD,

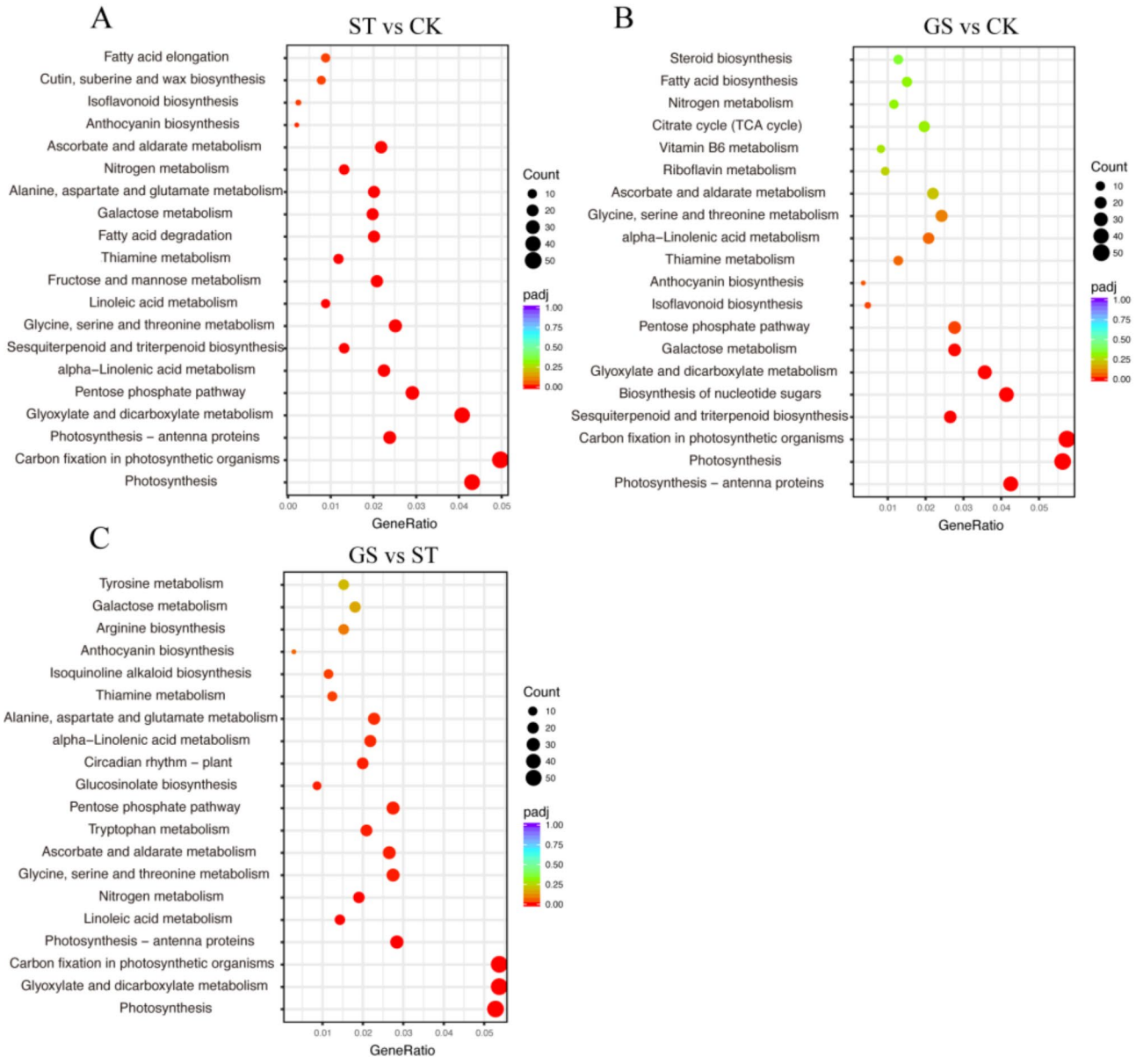

Fig. 7 Top 20 enriched KEGG pathways of the DEGs in different comparison groups. (**A**) ST vs. CK; (**B**) GS vs. CK; and (**C**) GS vs. ST. The abscissa is the ratio of the number of genes in the KEGG pathway analysis to the total number of differentially expressed genes. The ordinate is the KEGG pathway. The size of the dots represents the number of genes annotated to the KEGG pathway, and the colors from red to purple represent the significance level of the enrichment

HEMF, HEMB, SGRL, DVR, and CRD were highly positively correlated with some TFs such as RAP2.7, WRKY46, WRKY51, WRKY70 and MYB306.

Verification of DEGs by qRT-PCR

Eight DEGs related to chlorophyll metabolism and transcription factor genes, CHLD (FxaYL_311g0364060), DVR (FxaYL_421g0540370), CHLG (FxaYL_622g0081720), WRKY40 (FxaYL_212g0611500), WRKY51 (FxaYL_111g0825870), RAP2.7 (FxaYL_741g0919280), MYB306 (FxaYL_611g0097510), DIV1 (FxaYL_311g0370900), were used for verification (Fig. S4). These genes were found to show a largely consistent expression trend between the qRT-PCR and RNA-seq data, indicating the reliability of the transcriptome data.

Discussion

When exposed to salt stress, plants typically accumulate various osmolytes, such as soluble proteins, soluble sugars, and proline, to increase the concentration of cell fluid to withstand the stress [29], which was also confirmed

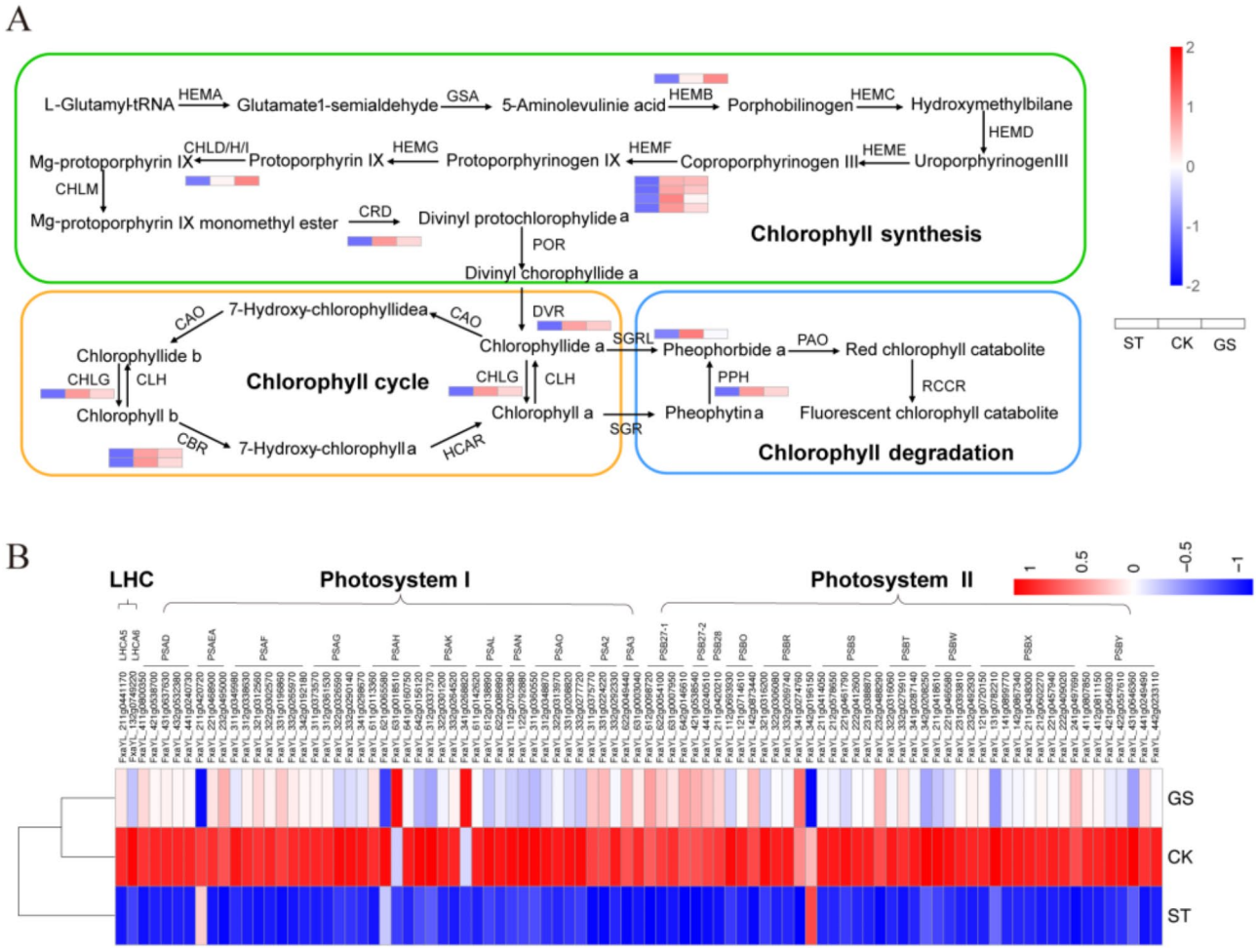

Fig. 8 Expression patterns of DEGs involved in photosynthesis. (**A**) Expression patterns of differentially expressed structure genes involved in chlorophyll metabolism. (**B**) Expression patterns of DEGs involved in light reaction of photosynthesis

in the present work. However, the contents of soluble proteins, soluble sugars, and proline were decreased in strawberry leaves treated with GABA plus NaCl compared to salt stress, but remained at relatively high levels compared to the control (Fig. 1). These results indicated that GABA may maintain a positive osmotic pressure to meet the water balance when plant cell was subjected to salt stress. A similar report was made on melatonin application in maize under drought stress [30].

Salinity-induced ionic and osmotic stresses impair plant metabolism, leading to accumulation of the ROS and membrane lipid peroxidation damage [31]. Multiple studies have demonstrated that GABA plays a vital role as an antioxidant in detoxifying reactive oxygen and free radicals in living organisms [32]. However, whether GABA can directly eliminate ROS has not been demonstrated. In the present study, significantly higher levels of O_2^-, H_2O_2 and MDA were observed after salt stress compared to the control. However, exogenous application of varying concentrations of GABA apparently reduced the contents of ROS and MDA in strawberry leaves, with 10 mM being the most significant effect, suggesting that GABA could effectively protect the cell membrane from oxidative damage under salt stress (Fig. 2). Plants are known to have evolved a complex network of non-enzymatic and enzymatic defense systems against oxidative damage by scavenging excess stress-induced ROS [33]. In this study, the activities of SOD, CAT and POD in strawberry leaves were significantly increased under salt stress compared to the control (Fig. 3A-C). Several studies have reported the salt-induced up-regulation of antioxidant enzymes to help plants resist unfavorable conditions [34–36], which corroborate our findings. After the application of different concentrations of GABA, the plants were able to maintain high levels of activity of these enzymes under salt stress (Fig. 3A-C). Especially, the 20 mM dose of GABA was identified as the most effective in enhancing SOD activity (Fig. 3A), whereas 2.5 mM and 10 mM GABA treatments were more beneficial in improving POD and CAT activities (Fig. 3B-C). The use of GABA

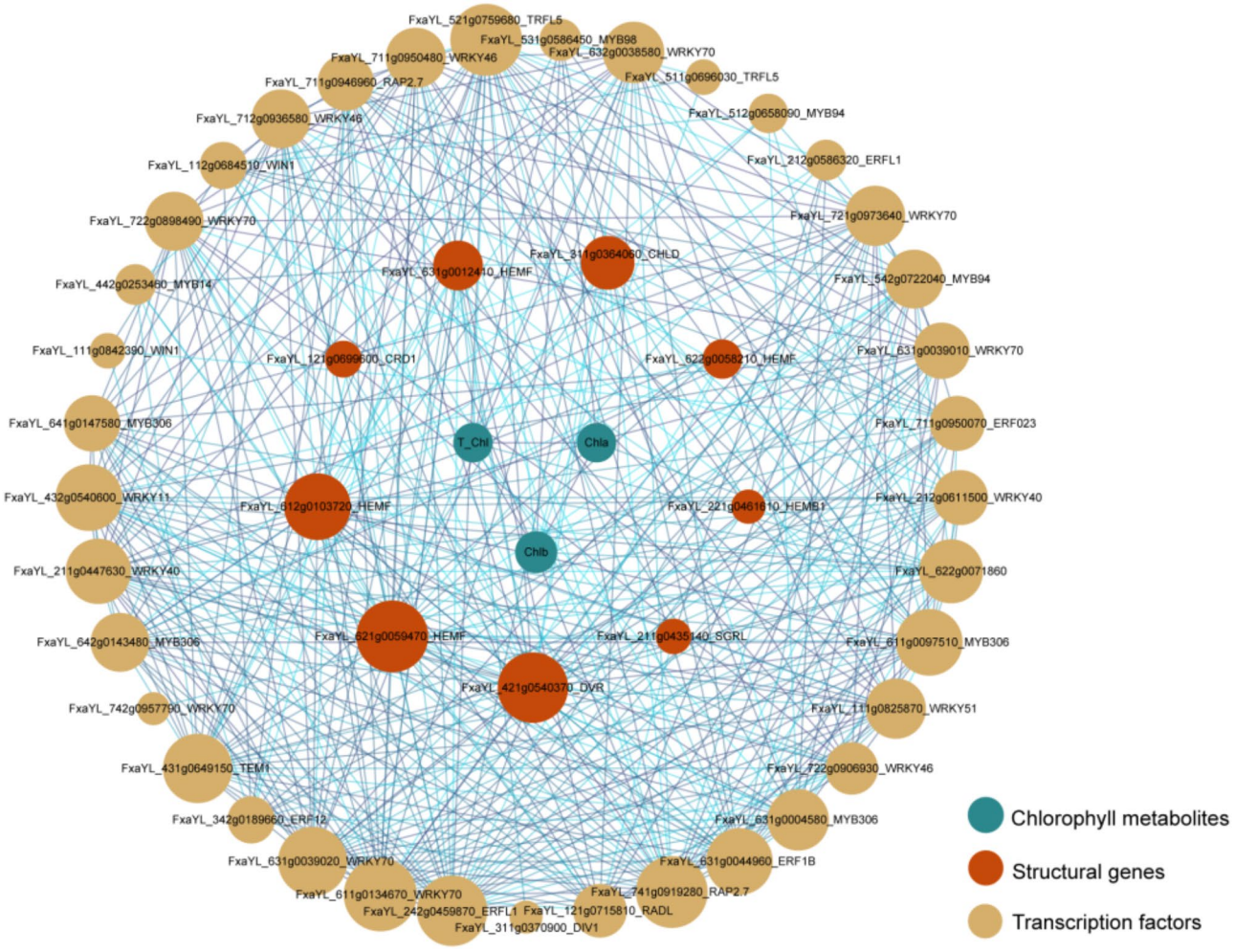

Fig. 9 Network of correlation analysis among chlorophyll metabolites, DEGs related to chlorophyll metabolism, and some differentially expressed transcription factors. The bluer the line, the stronger the positive correlation between the two indicators

is also associated with the control of APX, PPO and other antioxidant enzyme activities in some crops, such as maize [37], mungbean [17]. Total antioxidant activity can be assessed using the DPPH and FRAP parameters [38]. Higher concentrations of GABA (10, 15 and 20 mM) notedly increased the total antioxidant capacity (DPPH and FRAP), compared with the salt-treated plant alone (Fig. 3D and E). Our previous study also found that higher doses (10 mM and 15 mM) of GABA were more effective in increasing DPPH in postharvest strawberries [39]. These results suggest that exogenous GABA protects strawberries from salinity-induced oxidative burst damage through the promotion of the antioxidant system.

The polyamines in plants mainly include spermidine (Spd), spermine (Spm) and their precursor putrescine (Put), and are considered to be one of the prominent regulators during salt stress through modulation of ROS homeostasis [40]. On the one hand, polyamines inactive ROS due to their unique polycationic structure. On the other hand, polyamines generate ROS resulted from polyamines' catabolism [41]. The alteration of endogenous polyamines during salt stress dependes on the plant genotypes, the plant tissues and their developmental stages, the duration and intensity of the stress treatment [42, 43]. Our results showed that spm and put were increased in strawberry leaves under salt stress, while spd was decreased. The GABA treatments upregulated polyamines in salt-stressed strawberry leaves in a dose-dependent manner (Fig. 4A-C). Several studies have shown that GABA, the catabolic products of polyamines, enhances tolerance to various stresses by upregulating the PAs levels [44]. Moreover, higher concentrations of GABA (10, 15 and 20 mM) can increase the ratio (Spd + Spm)/Put in plants under salinity treatment (Fig. 4D). Some authors indicated that conversion of Put to Spd and Spm, and maintaining higher levels of Spd and Spm, were required for plant salt-tolerance [45–47].

Salt stress usually impairs plant growth by reducing chlorophyll content and photosynthetic capacity.

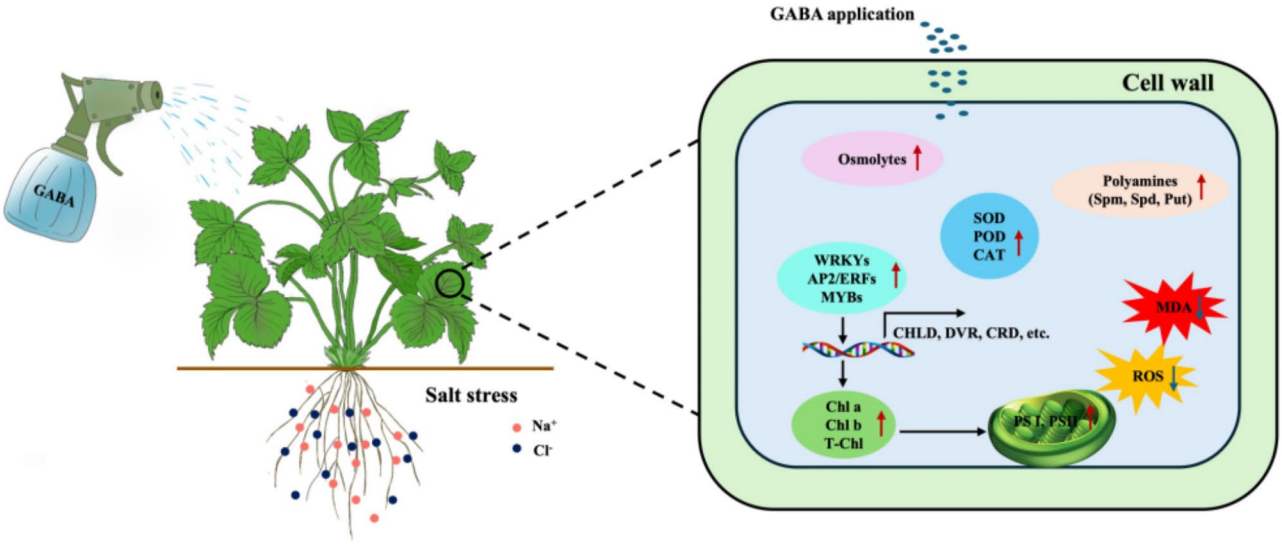

Fig. 10 A working model of exogenous GABA treatment to enhance the salt tolerance in strawberry. Briefly, the exogenous application of GABA significantly alleviated the physiological and metabolic damage caused by salt stress through promoting the accumulation of osmolytes and polyamines, increasing the activities of antioxidant enzymes, and decreasing ROS and MDA. Moreover, GABA can improve photosynthetic capacity by regulating some TFs and genes involved in chlorophyll metabolism

However, the application of exogenous GABA can mitigate these inhibitory effects of salt stress [37, 48]. The results of this study outlined that exogenous GABA promoted chlorophyll production and inhibited the decrease of net photosynthetic rate under salt stress, with 10 mM GABA treatment showing the strongest ability. Similar observations were found in maize [49], *Vicia faba* [50], and lettuce [51]. For example, the chloroplast ultrastructure and SPAD value in maize were improved after GABA application, thereby significantly contributing to the photosynthetic performance of the leaves under salt stress [49]. A putative mechanism for this protective effect is likely to be that GABA can activate the antioxidant system to protect photosystem against oxidative damage in plant [17, 37]. Transcriptome analysis showed that GABA induced the expression of a large number of genes related to chlorophyll metabolism and photosynthesis (involving in PSII, PSI and light harvesting complex) in salt-treated leaves (Fig. 8), which was coincided with their physiological response. Transcription factors (TFs) act as upstream regulators to modulate the expression of target genes of metabolic pathways in the plant stress response [52]. In the present study, 38 potential positive transcription factors (TFs) that may be involved in GABA-induced salt tolerance in strawberry leaves were screened from the top 3 differentially expressed transcription factors (MYBs, AP2/ERFs and WRKYs), such as RAP2.7, WRKY51, MYB306 (Fig. S3. The subsequent correlation analysis revealed a significant correlation between chlorophyll metabolites, chlorophyll-related genes and some selected TFs, indicating that these TFs may be positively involved in GABA-induced salt tolerance by regulating chlorophyll metabolism (Fig. 9).

Conclusion

In summary, 10 mM exogenous GABA effectively alleviated salt stress of strawberry seedlings through multiple mechanisms (Fig. 10), including maintaining oxidative homeostasis and reducing membrane lipid peroxidation by enhancing antioxidant capacity (SOD, POD, CAT, DPPH and FRAP), mediating polyamines accumulation, and improving photosynthetic capacity by inhibiting the degradation of photosynthetic pigments and increasing net photosynthetic rate. Transcriptomic analysis showed that most of the genes involved in chlorophyll metabolism and photosynthesis were upregulated by GABA spraying in salt-stressed strawberry. In addition, the expression levels of 38 TFs classified into WRKY, MYB and AP2/ERF families were elevated and highly positively correlated with chlorophyll metabolism related genes, indicating that these TFs may be positively involved in GABA-induced the adaptability of strawberry to salt stress by regulating chlorophyll metabolism, but the underlying mechanisms need further investigation.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12864-025-11368-5.

Supplementary Material 1

Supplementary Material 2

Author contributions
Y. Z. (Yunting Zhang) and M. D. designed the experiment, analyzed the data and wrote the manuscript. B. L., S.T., Y. C. and S. H. conducted data curation, validation and formal analysis. Y. L. (Yuanxiu Lin), M. L., W. H. and Y. W. prepared the figures. Y. Z. (Yong Zhang), Q. C., Y. L. (Ya Luo), X. W., X. G., and H. T. revised the manuscript. X. G. and H. T supervised this study.

Funding
This work was supported by the National Natural Science Foundation of China (Grant No. 32302479), the Natural Science Foundation of Sichuan Province (Grant No. 2023NSFSC1243) and the Double Support Project of Discipline Construction of Sichuan Agricultural University (Grant No. 03573134).

Data availability
The raw data of RNA-seq were deposited in the NCBI SRA database (accession: PRJNA1204972).

Declarations

Ethic approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 17 December 2024 / Accepted: 14 February 2025
Published online: 25 February 2025

References

1. Haj-Amor Z, Araya T, Kim D-G, Bouri S, Lee J, Ghiloufi W, Yang Y, Kang H, Jhariya MK, Banerjee A. Soil salinity and its associated effects on soil microorganisms, greenhouse gas emissions, crop yield, biodiversity and desertification: A review. Sci Total Environ. 2022;843:156946.
2. Hopmans JW, Qureshi A, Kisekka I, Munns R, Grattan S, Rengasamy P, Ben-Gal A, Assouline S, Javaux M, Minhas P. Critical knowledge gaps and research priorities in global soil salinity. Adv Agron. 2021;169:1–191.
3. Singh A. Soil salinity: A global threat to sustainable development. Soil Use Manag. 2022;38(1):39–67.
4. Shankar V, Evelin H. Strategies for reclamation of saline soils. Microorganisms Saline Environments: Strategies Funct 2019:439–49.
5. Yang Y, Guo Y. Elucidating the molecular mechanisms mediating plant salt-stress responses. New Phytol. 2018;217(2):523–39.
6. Rajasheker G, Jawahar G, Jalaja N, Kumar SA, Kumari PH, Punita DL, Karumanchi AR, Reddy PS, Rathnagiri P, Sreenivasulu N. Role and regulation of osmolytes and ABA interaction in salt and drought stress tolerance. Plant signaling molecules. Elsevier; 2019. pp. 417–36.
7. Jogawat A. Osmolytes and their role in abiotic stress tolerance in plants. Mol Plant Abiotic Stress: Biology Biotechnol 2019:91–104.
8. Amin I, Rasool S, Mir MA, Wani W, Masoodi KZ, Ahmad P. Ion homeostasis for salinity tolerance in plants: A molecular approach. Physiol Plant. 2021;171(4):578–94.
9. Miller G, Suzuki N, Ciftci-Yilmaz S, Mittler R. Reactive oxygen species homeostasis and signalling during drought and salinity stresses. Plant Cell Environ. 2010;33(4):453–67.
10. Del Río LA. ROS and RNS in plant physiology: an overview. J Exp Bot. 2015;66(10):2827–37.
11. Zhang Y, Li Y, He Y, Hu W, Zhang Y, Wang X, Tang H. Identification of NADPH oxidase family members associated with cold stress in strawberry. FEBS Open Bio. 2018;8(4):593–605.
12. Zhou H, Shi H, Yang Y, Feng X, Chen X, Xiao F, Lin H, Guo Y. Insights into plant salt stress signaling and tolerance. J Genet Genomics 2023.
13. Steward F, Thompson J, Dent C. γ-aminobutyric acid, a constituent of the potato tuber. Science 1949(110):439–40.
14. Khan MIR, Jalil SU, Chopra P, Chhillar H, Ferrante A, Khan NA, Ansari MI. Role of GABA in plant growth, development and senescence. Plant Gene. 2021;26:100283.
15. Gahlowt P, Tripathi DK, Singh SP, Gupta R, Singh VP. GABA in plants: developmental and stress resilience perspective. Physiol Plant. 2024;176(1):e14116.
16. Priya M, Sharma L, Kaur R, Bindumadhava H, Nair RM, Siddique K, Nayyar H. GABA (γ-aminobutyric acid), as a thermo-protectant, to improve the reproductive function of heat-stressed Mungbean plants. Sci Rep. 2019;9(1):7788.
17. Ullah A, Ali I, Noor J, Zeng F, Bawazeer S, Eldin SM, Asghar MA, Javed HH, Saleem K, Ullah S. Exogenous γ-aminobutyric acid (GABA) mitigated salinity-induced impairments in Mungbean plants by regulating their nitrogen metabolism and antioxidant potential. Front Plant Sci. 2023;13:1081188.
18. Zarbakhsh S, Shahsavar AR. Exogenous γ-aminobutyric acid improves the photosynthesis efficiency, soluble sugar contents, and mineral nutrients in pomegranate plants exposed to drought, salinity, and drought-salinity stresses. BMC Plant Biol. 2023;23(1):543.
19. Seifikalhor M, Aliniaeifard S, Bernard F, Seif M, Latifi M, Hassani B, Didaran F, Bosacchi M, Rezadoost H, Li T. γ-Aminobutyric acid confers cadmium tolerance in maize plants by concerted regulation of polyamine metabolism and antioxidant defense systems. Sci Rep. 2020;10(1):3356.
20. Li M, Zhang X, Li J, Ali M, Wang Y, Liu X, Li F, Li X. GABA primes defense responses against *Botrytis cinerea* in tomato fruit by modulating ethylene and JA signaling pathways. Postharvest Biol Technol. 2024;208:112665.
21. Liu C, Wang H, Zhang X, Ma F, Guo T, Li C. Activation of the ABA signal pathway mediated by GABA improves the drought resistance of Apple seedlings. Int J Mol Sci. 2021;22(23):12676.
22. Qian Z, Lu L, Zihan W, Qianyue B, Chungang Z, Shuheng Z, Jiali P, Jiaxin Y, Shuang Z, Jian W. Gamma-aminobutyric acid (GABA) improves salinity stress tolerance in soybean seedlings by modulating their mineral nutrition, osmolyte contents, and ascorbate-glutathione cycle. BMC Plant Biol. 2024;24(1):365.
23. Wang H, Yin X, Li J, Sun Y, Cheng F, Zhu D. γ-aminobutyric acid (GABA) treatment improves disease resistance and preserves fresh-cut *Mesembryanthemum crystallinum* L. LWT 2025:117398.
24. Ferreira JF, Liu X, Suarez DL. Fruit yield and survival of five commercial strawberry cultivars under field cultivation and salinity stress. Sci Hort. 2019;243:401–10.
25. He W, Wang Y, Chen Q, Sun B, Tang H-R, Pan D-M, Wang X-R. Dissection of the mechanism for compatible and incompatible graft combinations of *Citrus grandis* (L.) Osbeck ('hongmian Miyou'). Int J Mol Sci. 2018;19(2):505.
26. Zhang Y, Li S, Deng M, Gui R, Liu Y, Chen X, Lin Y, Li M, Wang Y, He W. Blue light combined with Salicylic acid treatment maintained the postharvest quality of strawberry fruit during refrigerated storage. Food Chemistry: X. 2022;15:100384.
27. Liang L, Tang W, Lian H, Sun B, Huang Z, Sun G, Li X, Tu L, Li H, Tang Y. Grafting promoted antioxidant capacity and carbon and nitrogen metabolism of bitter gourd seedlings under heat stress. Front Plant Sci. 2022;13:1074889.
28. Zhang Y, Jiang L, Li Y, Chen Q, Ye Y, Zhang Y, Luo Y, Sun B, Wang X, Tang H. Effect of red and blue light on anthocyanin accumulation and differential gene expression in strawberry (*Fragaria× ananassa*). Molecules. 2018;23(4):820.
29. Yang Y, Guo Y. Unraveling salt stress signaling in plants. J Integr Plant Biol. 2018;60(9):796–804.
30. Huang B, Chen Y-E, Zhao Y-Q, Ding C-B, Liao J-Q, Hu C, Zhou L-J, Zhang Z-W, Yuan S, Yuan M. Exogenous melatonin alleviates oxidative damages and protects photosystem II in maize seedlings under drought stress. Front Plant Sci. 2019;10:677.
31. Hasanuzzaman M, Raihan MRH, Masud AAC, Rahman K, Nowroz F, Rahman M, Nahar K, Fujita M. Regulation of reactive oxygen species and antioxidant defense in plants under salinity. Int J Mol Sci. 2021;22(17):9326.
32. Ansari MI, Jalil SU, Ansari SA, Hasanuzzaman M. GABA shunt: a key-player in mitigation of ROS during stress. Plant Growth Regul. 2021;94:131–49.
33. Kerchev PI, Van Breusegem F. Improving oxidative stress resilience in plants. Plant J. 2022;109(2):359–72.
34. Wei J, Liang J, Liu D, Liu Y, Liu G, Wei S. Melatonin-induced physiology and transcriptome changes in banana seedlings under salt stress conditions. Front Plant Sci. 2022;13:938262.
35. Jiang D, Lu B, Liu L, Duan W, Chen L, Li J, Zhang K, Sun H, Zhang Y, Dong H. Exogenous melatonin improves salt stress adaptation of cotton seedlings by regulating active oxygen metabolism. PeerJ. 2020;8:e10486.
36. Zhang T, Shi Z, Zhang X, Zheng S, Wang J, Mo J. Alleviating effects of exogenous melatonin on salt stress in cucumber. Sci Hort. 2020;262:109070.
37. Aljuaid BS, Ashour H. Exogenous γ-aminobutyric acid (GABA) application mitigates salinity stress in maize plants. Life. 2022;12(11):1860.

38. Rumpf J, Burger R, Schulze M. Statistical evaluation of DPPH, ABTS, FRAP, and Folin-Ciocalteu assays to assess the antioxidant capacity of lignins. Int J Biol Macromol. 2023;233:123470.
39. Zhang Y, Lin B, Tang G, Chen Y, Deng M, Lin Y, Li M, He W, Wang Y, Zhang Y. Application of γ-aminobutyric acid improves the postharvest marketability of strawberry by maintaining fruit quality and enhancing antioxidant system. Food Chemistry: X. 2024;21:101252.
40. Saha J, Brauer EK, Sengupta A, Popescu SC, Gupta K, Gupta B. Polyamines as redox homeostasis regulators during salt stress in plants. Front Environ Sci. 2015;3:21.
41. Wang W, Paschalidis K, Feng J-C, Song J, Liu J-H. Polyamine catabolism in plants: a universal process with diverse functions. Front Plant Sci. 2019;10:561.
42. Li Z, Geng W, Tan M, Ling Y, Zhang Y, Zhang L, Peng Y. Differential responses to salt stress in four white clover genotypes associated with root growth, endogenous polyamines metabolism, and sodium/potassium accumulation and transport. Front Plant Sci. 2022;13:896436.
43. Liu J-H, Inoue H, Moriguchi T. Salt stress-mediated changes in free polyamine titers and expression of genes responsible for polyamine biosynthesis of Apple *in vitro* shoots. Environ Exp Bot. 2008;62(1):28–35.
44. Gupta S, Kant K, Kaur N, Jindal P, Naeem M, Khan MN, Ali A. Polyamines: rising stars against metal and metalloid toxicity. Plant Physiol Biochem 2024:109030.
45. Zapata PJ, Serrano Ma, Pretel MT, Amorós A, Botella MÁ. Polyamines and ethylene changes during germination of different plant species under salinity. Plant Sci. 2004;167(4):781–8.
46. Li S, Jin H, Zhang Q. The effect of exogenous spermidine concentration on polyamine metabolism and salt tolerance in Zoysiagrass (*Zoysia Japonica* Steud) subjected to short-term salinity stress. Front Plant Sci. 2016;7:1221.
47. Liu J, Yu B-j, Liu Y-l. Effects of spermidine and spermine levels on salt tolerance associated with Tonoplast H+-ATPase and H+-PPase activities in barley roots. Plant Growth Regul. 2006;49:119–26.
48. Shelp BJ, Bown AW, Zarei A. 4-Aminobutyrate (GABA): a metabolite and signal with practical significance. Botany. 2017;95(11):1015–32.
49. Wang Y, Cao H, Wang S, Guo J, Dou H, Qiao J, Yang Q, Shao R, Wang H. Exogenous γ-aminobutyric acid (GABA) improves salt-inhibited nitrogen metabolism and the anaplerotic reaction of the Tricarboxylic acid cycle by regulating GABA-shunt metabolism in maize seedlings. Ecotoxicol Environ Saf. 2023;254:114756.
50. Shomali A, Aliniaeifard S, Didaran F, Lotfi M, Mohammadian M, Seif M, Strobel WR, Sierka E, Kalaji HM. Synergistic effects of melatonin and gamma-aminobutyric acid on protection of photosynthesis system in response to multiple abiotic stressors. Cells. 2021;10(7):1631.
51. Kalhor MS, Aliniaeifard S, Seif M, Asayesh EJ, Bernard F, Hassani B, Li T. Enhanced salt tolerance and photosynthetic performance: implication of ɤ-amino Butyric acid application in salt-exposed lettuce (*Lactuca sativa* L.) plants. Plant Physiol Biochem. 2018;130:157–72.
52. Kajla M, Roy A, Singh IK, Singh A. Regulation of the regulators: transcription factors controlling biosynthesis of plant secondary metabolites during biotic stresses and their regulation by MiRNAs. Front Plant Sci. 2023;14:1126567.

Publisher's note