

# Gamma Aminobutyric Acid (GABA) and Salinity Impacts Physiological Response and Expression of Salinity-Related Genes in Strawberry cv. Aromas

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## Research Article

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# Abstract

Salinity is one of the most crucial abiotic stresses which is the consequence of increase in the concentration of NaCl ions influencing plant's growth, development, and yield. Gama-butyric acid gamma (GABA) is a non-protein amino acid involved in various metabolic processes that accumulates in many plant species during stress conditions. The present study was aimed to evaluate the effect of GABA (0 and 25 mM) and salinity (3 and 5 dS/m) on physiological characteristics and expression pattern of some salinity-related genes in strawberry cv. Aromas under soilless culture condition 12, 24, and 36 h after treatments' initiation. Based on the results, salinity increased the content of H<sub>2</sub>O<sub>2</sub>, MDA, and proline while it decreased the percentage of MSI and the activity of SOD and POD antioxidant enzymes. In contrast, the implementation of GABA not only decreased H<sub>2</sub>O<sub>2</sub> and MDA content, and maintained MSI percentage, but also it improved the activity of antioxidant enzymes and the transcription level of *DREB*, *cAPX*, *MnSOD*, and *GST* genes. Under non-stress conditions, GABA acted as a mild stressor by imposing effects similar to abiotic stress which could help plants adapt under the adverse environmental conditions. We concluded that strawberry plants represented a higher salinity tolerance by enhancing both enzymatic and non-enzymatic antioxidant physiological protection mechanisms and also by increasing the transcription of salinity-related genes upon GABA application.

## 1. Introduction

With more than 8.9 million tons annual production and over 390.000 ha area harvested, strawberry (*Fragaria × ananassa* Duch.) is an economically important small fruit (FAO 2019). Due to the presence of health-promoting phytochemicals including vitamins (mainly C and E), flavonoids, tannins, carotenoids, and other antioxidant compounds, strawberry is known as a nutritionally valuable fresh fruit in human diet (Mazzoni et al. 2020). The quality of strawberry fruit is characterized by the nature and level of these valuable phytochemicals that can reduce inflammation, cardiovascular diseases, and even the risk of some particular cancers (Nemzer et al. 2020).

Salinity is known as one of the most serious environmental abiotic stresses that hamper plant growth and yield. Strawberry has been classified as a salt-sensitive species with a lower tolerance threshold (1.0 mmho/cm) to root medium electrical conductivity (EC) compared to other horticultural species like *Solanum lycopersicum* (2.5 mmho/cm), *Asparagus officinalis* (6.1 mmho/cm), and *Beta vulgaris* subsp. *vulgaris* (7.0 mmho/cm) (Sandhu et al. 2019). Depend on the duration and degree of stress and phenological phases, plant species represent physiologically different responses under salinity (Munns and Tester 2008). Salinity restricts the overall growth of strawberry plants through inducing leaves necrosis and senescence, retarding the transpiration and photosynthesis, limiting minerals absorption, and depleting carbohydrate and protein resources (Rahimi et al. 2011; Mirfattahi and Eshghi 2020; Zahedi et al. 2020; Yaghubi et al. 2019).

Plant cells perceive salt stress *via* plasmalemma membrane's receptors that transmit salinity signals in a downstream signaling cascade leading to a rapid and sharp increase in the concentration of reactive oxygen species (ROS) (Munns and Tester 2008; Rao et al. 2016). ROS are bifunctional molecules in plant cells where they can act as plant hormones with signaling impacts in low to medium levels, while at high concentrations, they impose oxidative damages on vital cellular components and structures (Tanveer and Ahmed 2020). Plant cells exploit both enzymatic and non-enzymatic antioxidant protection systems to eliminate accumulated ROS to protect plants against adverse impacts of salinity. Enzymatic antioxidant protection system comprises the involvement of antioxidant enzymes such as peroxidase (POD), catalase (CAT), and superoxide dismutase (SOD) enzymes. On the other hand, in osmotic mitigation strategy, plant cells utilize compounds like ascorbic acid, proline, phenolic, and soluble alcoholic sugars to alleviate adverse effect of salt stress (Munns and Tester 2008; Tanveer et al. 2020).

Responses of plants to salinity is a multigenic process in which the expression pattern of genes mediating ROS-scavenging and osmotic-regulating enzymes are affected by complex signaling pathways (Rao et al. 2016; Gupta and Huang 2014). It is thus essential to investigate the regulation of salinity-related genes and also undergoing physio-biochemical responses to clarify the possible physio-molecular mechanisms of salinity tolerance. In this connection, the knowledge of gene expression can help to understand the transcriptional interaction of plants under environmental abiotic stresses (Franzoni et al. 2019). As a cost-effective technique with various advantages and a broad range of application in plant biology, semi-quantitative RT-PCR has been used to study gene expression under various abiotic stresses (Fahmideh and Fooladvand 2018).

Transcription factors (TFs) are important proteins that improve salinity tolerance by modulating key downstream genes through the signaling networks that coordinate the endogenous physiological and molecular programs (Kumar et al. 2017). As members of the *AP2/ERF* family, dehydration responsive element binding protein (DREB) transcription factors involve in the induction of tolerance under different abiotic stresses such as cold, drought, oxidation, heat, and salinity (Pandey et al. 2015). Depend on the identity extent of *AP2/ERF* domain, six main DREB families have been identified in Arabidopsis. DREB proteins bind to specific *cis* regulating DNA sequences and regulate the transcription of salinity-related genes (Franzoni et al. 2019; Pandey et al. 2015).

As a diverse group of multi-functional enzymes, glutathione S-transferases (GSTs) generally mediate the conjugation of reduced tripeptide glutathione (GSH) as a cofactor to xenobiotic substrates for detoxification purposes. *GST* genes are differentially expressed under different abiotic stresses, and in the case of salinity, the modulation of different *GST* isoenzymes to adapt plants under stress is a complex process. Compared to wild-type plants, transgenic tobacco lines expressing the *GsGST* transgene represented six-fold more GST activity and higher salinity resistance (Ji et al. 2010; Lin and Pu 2010).

Superoxide dismutases (SODs) are a group of metalloenzymes that mediate the dismutation of superoxide radicals ( $O_2^-$ ) to molecular oxygen ( $O_2$ ) and hydrogen peroxide ( $H_2O_2$ ). SOD catalyzes the first line of defense in response to the harmful effect of accumulated ROS. Depending on the nature of metal cofactors present in the structure plant SODs, they are classified into three main groups, including MnSOD, FeSOD, Cu/ZnSOD (Saibi and Brini 2018). *MnSOD* gene was differentially expressed in rose (Bayanati et al. 2019) and strawberry (Christou et al. 2013) in response to NaCl-induced salinity stress. The seedlings of a salt-tolerant pea cultivar represented higher transcript levels of *MnSOD*, *CuZnSOD*, phospholipid hydroperoxide glutathione peroxidase (*PHGPX*), and cytosolic *GR* and *APX* in comparison to seedlings of the salt-sensitive cultivar (Hernández et al. 2000).

Plant cells employ the glutathione ascorbate cycle (AsA-GSH) as a central part of tolerance mechanism to mitigate deleterious impacts imposed by oxidative stress (Hasegawa et al. 2000). Ascorbate peroxidase (APX) act as an electron source to detoxify  $H_2O_2$ . APX uses ascorbate as the electron donor to reduce  $H_2O_2$  and its subsequent conversion to water and oxygen in plant cells (Auobi Amirabad et al. 2020; Foyer et al. 2003). The isoforms of ascorbate peroxidase are identified in the cytosol (cAPX), peroxisomes (pAPX), mitochondria (thylakoid membrane) (tAPX), chloroplasts (chAPX), and apoplasts (Apel and Hirt 2004). The transcript level of *APX* isoforms is controlled in response to abiotic stresses and also during plant growth and development (Caverzan et al. 2012). The transgenic Arabidopsis lines carrying cytosolic ascorbate peroxidases cloned from *Oriza sativa* L. (*OsAPXa* or *OsAPXb*) represented improved salinity tolerance than wild-type lines (Lu et al. 2007). Moreover, in the absence of the cytosolic ascorbate peroxidase, the chloroplast  $H_2O_2$ -scavenging system of Arabidopsis was completely collapsed resulted in  $H_2O_2$  accumulation and protein oxidation (Davletova et al. 2005).

Plants display a diverse range of defense interactions under abiotic environmental stresses from synthesis of hormones to programmed death of cells. Plant hormones are essentially required in numerous physiological processes of plant growth and development (Munns and Tester 2008; Gupta and Huang 2014). In this regard, GABA ( $\gamma$ -aminobutyric acid), a four-carbon free non-protein amino acid, comprises a significant fraction of free amino acids in plant cells that is synthesized from l-glutamate during the GABA shunt of tricarboxylic acid (TCA) cycle (Fait et al. 2008; Ji et al. 2018). Despite the protective function of GABA as an osmolyte in osmosis adjustment, recent evidence show that it also act as a signaling molecule with mitigation effects under abiotic stresses in particular salinity. GABA quickly accumulates in plant cells in response to stresses that can provide further insights into the importance of the GABA metabolic pathway (Fait et al. 2008; Xu et al. 2017). The mitigative impact of GABA under abiotic stresses is mainly mediated by the activation of GABA bypass pathway and TCA cycle *via* scavenging free radicals and regulating antioxidant activity (Li et al. 2021a). The interaction of GABA with other stress-related plant hormones such as ethylene and ABA has also been reported (Ji et al. 2018).

Based on above-mentioned facts and explanations, the present research framed to investigate the impact of GABA (2 mM) and salinity (3 and 5 dS/m) on some physiological characteristics and expression pattern of several salinity-related important genes in strawberry cv. Aromas under soilless culture condition 12, 24, and 36 h after treatments' initiation.

## 2. Material And Methods

### 2.1. Plant material, growth condition, and treatments

Well-grown and uniform strawberry plantlets cv. Aromas were individually sown in 7-liter plastic pots containing mixed coco peat and perlite at a ratio of 1:1 volume/volume. The containers were kept in the research greenhouse at the Department of Horticultural Sciences and Engineering, University of Kurdistan (temperature 20–23°C, RH 65–75% and PAR 700–1200 mol m<sup>-2</sup> s<sup>-1</sup>) (35°16'51.4"N 46°59'46.5"E). At the beginning of the experiment, six pots per replication and treatment were used, and the plants were manually fed with 500 ml half-strength Hoagland nutrition solution (Hoagland and Arnon 1950) for one week. The experiment was performed in triplicate. The established strawberry plants were then irrigated three-times a week (2 liters per plant). Salinity and GABA treatments were initiated after 20 days when strawberry plants had 5–6 fully grown leaves. The treatments were as follow: M, control; MG, 25 mM GABA; S3, 3 dS m<sup>-1</sup> EC; S5, 5 dS m<sup>-1</sup> EC; S3G, 25 mM GABA + 3 dS m<sup>-1</sup> EC, S5G, 25 mM GABA + 5 dS m<sup>-1</sup> EC. To obtain the considered EC values, NaCl was included into the nutrient solution. The treatments were started at 08:00 AM, and leaf materials were harvested 12, 24, and 36 h after treatment initiation, flash-frozen in liquid nitrogen and kept at – 80°C till the physiological and molecular analyses. The same conditions were applied for non-stressed (control) plants, and their leaf tissues were sampled at similar time intervals to exclude variation mediated by diurnal and circadian rhythms.

## 2.2. Physiological parameters

The method of Sairam (1994) was used to measure leaf membrane stability index (MSI). First, 100 mg leaf disks were soaked in 10 ml distilled water at 40°C for 30 min. The EC of samples' water (A1) was then determined by an INESA EC-meter (INESA, Shanghai, China). The leaf segments were then treated at 100°C for 10 min and the second EC values was recorded (C2). MSI was then calculated using  $MSI\% = [1 - (C1/C2)] \times 100$  equation. To quantify total soluble proteins (TSP), 200 mg leaf tissue was powdered in liquid nitrogen and resuspended in 2 ml extraction buffer containing 50 mM Tris-HCl, 0.5 M NaCl, 2 mM EDTA, and 4% polyvinylpyrrolidone. The resulted mixture was then centrifuged (10000× g for 10 min) at 4°C. The TSP content was then determined using Bradford (1976) assay. The concentration of proline was determined based on the procedure of Bates et al. (1973) using disks prepared from fully grown leaves. The content of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) was spectrophotometrically measured by the reaction with potassium iodate (KI) and recording the solution absorbance at 390 nm wavelength (Alexieva et al. 2001). The reads fitted in a standard curve drawn with given concentrations of H<sub>2</sub>O<sub>2</sub>. Superoxide dismutase (SOD) activity was determined by nitro blue tetrazolium (NBT) procedure (Beauchamp and Fridovich 1971). This procedure is based on the SOD ability to prevent NBT reduction and formation of formazan by superoxide radicals. All photochemical reactions were done by an UV-DU 520 Beckman spectrophotometer (Beckman Coulter, Inc., CA, USA). The concentration of malondialdehyde (MDA) was determined as a measure of lipid peroxidation based on the Dhindsa et al. (1981) method. Briefly, 300 mg leaf tissue was homogenated with 5 ml 0.1% TCA solution. The resulted mixture was then centrifuged for 10 min (10,000 rpm) at 4°C. Thereafter, 300 µl supernatant was mixed with 1.2 ml 0.5% thiobarbituric acid, and the mixture was treated at 95°C for 30 min, and the reaction was cooled on ice. The supernatant's absorbance was determined at 532 nm. The reads were corrected by subtracting the non-specific absorbance at 600 nm and the MDA concentration was finally measured using the extinction coefficient 155 mM<sup>-1</sup> cm<sup>-1</sup>. Ascorbate peroxidase (APX) activity was determined based on the Hemeda and Klein (1990) method. This procedure monitors the rate of ascorbate oxidation. The reaction sample (1 ml) comprised 40 µL of the crude protein extract, 90 µL 0.3% hydrogen peroxide, 90 µL 1% guaiacol as substrate, and 780 µL 50 mmol KH<sub>2</sub>PO<sub>4</sub> buffer. The activity of APX was finally determined by the increase in absorbance at 470 nm as a result of guaiacol oxidation ( $E = 26.6 \text{ mM}^{-1} \text{ cm}^{-1}$ ).

## 2.3. RNA purification and synthesis of cDNA

Total RNA was purified from leaf tissue based on the Mazzara and James (2000) method. The DNA contamination was degraded using DNase (Fermentas, St. Leon-Rot, Germany) by incubation at 37°C for 30 min. The RNA pellets were then dissolved in DEPC-treated water and stored at – 70°C. The quantity and quality of purified RNA samples analyzed by both spectrophotometry at 260 nm and 280 nm wavelengths and agarose gel electrophoresis. The synthesis of first-strand cDNA was performed by Sinaclon first-strand cDNA synthesis kit (CinaClone, Tehran, Iran) using 1 µg RNA sample as template based on the protocol provided by the manufacturer.

## 2.4. Semi-quantitative RT-PCR Analysis

The sequences of *DREB*, *cAPX*, *MnSOD*, and *GST* genes for *Fragaria × ananassa* Duch. were obtained from the NCBI database. The gene-specific primer pairs (Table 1) were designed using the online tool Primer3plus (<http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi>). Actin was employed as reference (housekeeping) gene to analyze the relative gene expression.

The size of PCR amplicons was 606 bp for Actin, 159 bp for *cAPX*, 164 bp for *DREB*, 194 bp for *MnSOD*, and 180 bp for *GST*. PCR reactions carried out in a final volume of 10  $\mu$ l, comprising 20 ng pure DNA, 200  $\mu$ M dNTPs, 0.4 U Taq DNA polymerase (SinaClone, Tehran, Iran), 1  $\mu$ l 10X PCR buffer, 2 mM MgCl<sub>2</sub>, and 10 pmol/ $\mu$ l forward and reverse primers. PCR reactions performed in a BioRad thermocycler (Bio-Rad, Hercules, CA, USA) based on the following program: 5 min initial denaturation at 94°C followed by 28 cycles of 30 s denaturation at 94°C, 30 s primer annealing at 56–57°C (56°C for *GTS* and 57°C for other gene sequences) and 45 s extension at 72°C, with a final extension of 7 min at 72°C. The amplicons were then separated in 1.5% agarose gel and stained with ethidium bromide. The density of visualized bands was quantified using Gelquant.Net software (<http://biochemlabsolutions.com/GelQuantNET.html>). The intensity of interest and reference genes (*Actin*) was used to estimate relative expression.

Table 1  
Oligonucleotides used as primers for Semi-quantitative RT-PCR analysis

Primer	GeneBank accession	Sequence (5' → 3')	T <sub>m</sub> (°C)	Product size
<i>Actin</i> -F	LC017712.1	TGTGCTGGACTCTGGTGATG	57	606
<i>Actin</i> -R		TGGAATGTACTCAGGGAAGCC		
<i>DREB</i> -F	TA10928_57918	CGGAGTTGGTTTTCCAAGAA TCCGGGTACTCGTTCAAATC	57	164
<i>DREB</i> -R				
<i>cAPX</i> -F	TA478_3747	CACAAGGAACGGTCTGGATT CGCAGCGTATTTCTCAACAA	57	154
<i>cAPX</i> -R				
<i>MnSOD</i> -F	CO381280	TGTGGCTGGCTTTAGACAAA	57	194
<i>MnSOD</i> -R		CTTCCAATTGATGACCTTCCA		
<i>GST</i> -F	AY902368.1	TTCTCATTACAACGGCAAA	56	180
<i>GST</i> -R		TCCTGCTCATCTCCTTTCGT		

## 2.5. Data analysis

Statistical analysis was performed using SAS ver. 9.4 (SAS Institute Inc., Cary, NC, USA). A factorial experiment based on the completely randomized design (CRD) was employed. Mean comparison was conducted based on the analysis of variance (ANOVA) and Least Significant Difference (LSD) Test at a significance level of 5% ( $P \leq 0.05$ ). All analyses were performed with three biological replications. The “ggplot” library implemented in the Agricola package was employed to generate a heatmap of gene expression data using R software (Version 3.2.3).

## 3. Results

### 3.1. Physiological parameters

As shown in Table 2, the lowest H<sub>2</sub>O<sub>2</sub> content ( $21.35 \pm 0.39$  nM g<sup>-1</sup> FW) was recorded in control plants (M) 24 h after treatment initiation. In contrast, the content of H<sub>2</sub>O<sub>2</sub> was increased in salt-stressed strawberry plants where higher salinity intensity (5 dS m<sup>-1</sup> EC) led to higher generation of H<sub>2</sub>O<sub>2</sub> molecules. On the other side, GABA foliar application could reduce the H<sub>2</sub>O<sub>2</sub> content of strawberry plants under salinity stress, while under non-saline condition, GABA acted as a weak stressor and its implementation slightly increased the H<sub>2</sub>O<sub>2</sub> concentration. Except for salt-stressed plants treated with GABA, the content of H<sub>2</sub>O<sub>2</sub> represented an increasing/decreasing/increasing trend at 12, 24 and 36 h sampling time intervals, respectively (Table 2). A similar effect of salinity and GABA was also observed for MDA content with the difference that MDA accumulated in leaf tissues over time. In this regard, the lowest ( $6.89 \pm 0.19$  nM g<sup>-1</sup> FW) and the highest ( $19.40 \pm 0.36$  nM g<sup>-1</sup> FW) MDA content was obtained in control and S5 treatments, 24 h and 36 h after treatment initiation, respectively (Table 2).

Table 2

Influence of gamma aminobutyric acid (GABA) on H<sub>2</sub>O<sub>2</sub> and Malondialdehyde (MDA) content and membrane stability index (MSI) in strawberry under NaCl-induced stress and non-stress conditions at 12 h, 24 h and 36 h time intervals.

Treatments	H <sub>2</sub> O <sub>2</sub> (nM g <sup>-1</sup> FW)			MDA (nM g <sup>-1</sup> FW)			MSI (%)		
	12h	24h	36h	12h	24h	36h	12h	24h	36h
<b>M</b>	22.51 ± 0.30 <sup>m</sup>	21.35 ± 0.39 <sup>lm</sup>	24.09 ± 0.30 <sup>l</sup>	6.81 ± 0.25 <sup>bc</sup>	6.38 ± 0.19 <sup>c</sup>	6.89 ± 0.19 <sup>bc</sup>	87.33 ± 2.23 <sup>bc</sup>	93.14 ± 1.98 <sup>bc</sup>	88.02 ± 1.37 <sup>bc</sup>
<b>MG</b>	36.59 ± 0.46 <sup>k</sup>	28.30 ± 0.32 <sup>b</sup>	39.15 ± 4.10 <sup>k</sup>	6.67 ± 0.76 <sup>g</sup>	7.82 ± 0.41 <sup>f</sup>	8.72 ± 0.28 <sup>f</sup>	91.09 ± 2.33 <sup>g</sup>	86.76 ± 1.69 <sup>g</sup>	86.47 ± 1.28 <sup>g</sup>
<b>S3</b>	52.08 ± 0.35 <sup>lm</sup>	37.69 ± 0.19 <sup>lm</sup>	54.44 ± 0.30 <sup>k</sup>	12.83 ± 0.20 <sup>b</sup>	14.10 ± 0.39 <sup>b</sup>	14.83 ± 0.42 <sup>bc</sup>	74.03 ± 0.59 <sup>b</sup>	70.70 ± 2.32 <sup>b</sup>	70.51 ± 0.94 <sup>b</sup>
<b>S3G</b>	24.92 ± 0.26 <sup>k</sup>	35.25 ± 0.46 <sup>k</sup>	45.13 ± 0.36 <sup>j</sup>	10.71 ± 0.58 <sup>c</sup>	12.09 ± 0.57 <sup>d</sup>	10.63 ± 0.57 <sup>d</sup>	76.38 ± 1.33 <sup>c</sup>	74.72 ± 1.60 <sup>bc</sup>	76.02 ± 2.43 <sup>bc</sup>
<b>S5</b>	69.40 ± 0.56 <sup>d</sup>	61.58 ± 0.36 <sup>i</sup>	71.05 ± 0.91 <sup>h</sup>	16.36 ± 0.23 <sup>a</sup>	17.42 ± 0.27 <sup>a</sup>	19.40 ± 0.36 <sup>b</sup>	69.48 ± 0.98 <sup>d</sup>	66.97 ± 0.88 <sup>d</sup>	62.24 ± 1.01 <sup>d</sup>
<b>S5G</b>	47.19 ± 0.60 <sup>gf</sup>	51.14 ± 0.19 <sup>ef</sup>	57.60 ± 0.91 <sup>e</sup>	12.04 ± 0.35 <sup>d</sup>	13.19 ± 0.43 <sup>de</sup>	11.99 ± 0.22 <sup>de</sup>	74.30 ± 1.25 <sup>de</sup>	71.15 ± 1.72 <sup>de</sup>	75.83 ± 1.45 <sup>de</sup>

Different letters in each column indicate significances by LSD tests at  $P < 0.05$ . M, control; MG, 25 mM GABA; S3, 3 dS m<sup>-1</sup> EC; S5, 5 dS m<sup>-1</sup> EC; S3G, 25 mM GABA + 3 dS m<sup>-1</sup> EC, S5G, 25 mM GABA + 5 dS m<sup>-1</sup> EC.

Membrane stability index (MSI) was also significantly affected by salinity and GABA treatments. In this connection, the non-stressed strawberry plants represented the highest MSI percentage (93.14 ± 1.98) after 24 h, while the lowest MSI percentage (62.24 ± 1.01) was observed in salinized strawberry plants without GABA application 36 h after treatment imposition. GABA foliar application generally increased the percentage of MSI in salt-stressed plants, but it decreased the MSI percentage of control plants. On the other side, MSI steadily decreased under salinity stress over time, both with or without GABA application (Table 2).

The content of proline was increased in salt-stressed and GABA-treated plants in a time-dependent manner. Under NaCl-induced salt stress, the GABA application increased the content of proline and this increase was more profound under lower salinity level (3 dS m<sup>-1</sup> EC). In this connection, the highest and the lowest content of proline was recorded in S3G treatment after 36 h (Table 3). The application of GABA was also led to an increase in proline concentration under non-stress condition.

Table 3

Influence of gamma aminobutyric acid (GABA) on the content of proline, total soluble protein (TSP), and the activity of superoxidase dismutase (SOD) in strawberry under NaCl-induced stress and non-stress conditions at 12 h, 24 h and 36 h time intervals.

Treatments	Proline (mg/g FW)			Total soluble protein (mg/g FW)			SOD (U mg <sup>-1</sup> protein)			POD (U mg <sup>-1</sup> protein)		
	12h	24h	36h	12h	24h	36h	12h	24h	36h	12h	24h	36h
<b>M</b>	0.23 ± 0.01 <sup>j</sup>	0.24 ± 0.02 <sup>j</sup>	0.23 ± 0.03 <sup>i</sup>	0.79 ± 0.004 <sup>b</sup>	0.58 ± 0.003 <sup>e</sup>	0.77 ± 0.005 <sup>b</sup>	2.03 ± 0.04 <sup>gh</sup>	3.05 ± 0.14 <sup>e</sup>	1.99 ± 0.0 <sup>h</sup>	7.22 ± 0.17 <sup>h</sup>	15.53 ± 0.79 <sup>f</sup>	7.34 ± 0.28 <sup>h</sup>
<b>MG</b>	0.34 ± 0.01 <sup>h</sup>	0.36 ± 0.02 <sup>h</sup>	0.44 ± 0.02 <sup>h</sup>	0.66 ± 0.010 <sup>cd</sup>	0.67 ± 0.008 <sup>cd</sup>	0.59 ± 0.006 <sup>e</sup>	2.27 ± 0.07 <sup>f</sup> -h	6.16 ± 0.21 <sup>b</sup>	4.56 ± 0.05 <sup>d</sup>	8.98 ± 0.48 <sup>h</sup>	15.06 ± 0.27 <sup>f</sup>	20.68 ± 0.60 <sup>e</sup>
<b>S3</b>	0.30 ± 0.02 <sup>h</sup>	0.33 ± 0.01 <sup>g</sup>	0.37 ± 0.02 <sup>f</sup>	0.60 ± 0.007 <sup>e</sup>	0.78 ± 0.007 <sup>b</sup>	0.39 ± 0.004 <sup>g</sup>	2.64 ± 0.06 <sup>e</sup> -g	6.21 ± 0.14 <sup>b</sup>	5.33 ± 0.09 <sup>c</sup>	26.09 ± 0.57 <sup>c</sup>	12.87 ± 0.44 <sup>g</sup>	14.86 ± 0.90 <sup>f</sup>
<b>S3G</b>	0.52 ± 0.03 <sup>d</sup>	0.57 ± 0.04 <sup>b</sup>	0.59 ± 0.02 <sup>a</sup>	0.63 ± 0.005 <sup>de</sup>	0.81 ± 0.012 <sup>b</sup>	0.70 ± 0.012 <sup>c</sup>	2.82 ± 0.07 <sup>d</sup> -g	4.87 ± 0.09 <sup>ef</sup>	2.20 ± 0.07 <sup>cd</sup>	7.86 ± 0.12 <sup>f</sup> -h	20.06 ± 0.16 <sup>e</sup>	21.28 ± 1.02 <sup>e</sup>
<b>S5</b>	0.30 ± 0.01 <sup>g</sup>	0.32 ± 0.01 <sup>f</sup>	0.40 ± 0.01 <sup>f</sup>	0.50 ± 0.011 <sup>b</sup>	0.81 ± 0.006 <sup>g</sup>	0.36 ± 0.010 <sup>cd</sup>	3.90 ± 0.07 <sup>cd</sup>	8.01 ± 0.13 <sup>a</sup>	6.16 ± 0.08 <sup>b</sup>	33.90 ± 0.07 <sup>a</sup>	23.71 ± 0.06 <sup>d</sup>	32.14 ± 0.08 <sup>a</sup>
<b>S5G</b>	0.37 ± 0.01 <sup>e</sup>	0.38 ± 0.02 <sup>e</sup>	0.43 ± 0.04 <sup>c</sup>	0.67 ± 0.007 <sup>cd</sup>	1.02 ± 0.004 <sup>a</sup>	0.59 ± 0.008 <sup>g</sup>	1.99 ± 0.06 <sup>h</sup>	4.65 ± 0.12 <sup>d</sup>	2.49 ± 0.09 <sup>eh</sup>	20.66 ± 0.81 <sup>e</sup>	29.06 ± 1.02 <sup>b</sup>	21.63 ± 1.05 <sup>e</sup>

Different letters in each column indicate significances by LSD tests at  $P < 0.05$ . M, control; MG, 25 mM GABA; S3, 3 dS m<sup>-1</sup> EC; S5, 5 dS m<sup>-1</sup> EC; S3G, 25 mM GABA + 3 dS m<sup>-1</sup> EC, S5G, 25 mM GABA + 5 dS m<sup>-1</sup> EC.

Under non-stress condition, TPS content was affected by sampling time in which it decreased at the second sampling time (24 h) compared to the first (12 h) and third (36 h) sampling times. At the first sampling time, both salinity levels caused a significant decrease in TSP content compared to the control strawberry plants. On the other hand, GABA foliar application recovered TPS content under salt stress. This mitigative impact of GABA was more profound 36 h after treatment initiation under higher salinity level (5 dS m<sup>-1</sup> EC).

The activity of the SOD enzyme was increased by the effect of both GABA and salinity treatments and this increase was more evident 24 h after treatment initiation compared to 12 and 36 h sampling times. In this regard, the highest SOD activity (8.01 U mg<sup>-1</sup> protein) was obtained in S5 treatment after 24 h. SOD was also significantly increased in strawberry plants treated with GABA under non-stress condition. In the case of POD, both salinity and GABA increased its activity in a time-dependent manner. In this regard, the control plants at first sampling time (12 h) represented the lowest POD activity (7.22 ± 0.17 U mg<sup>-1</sup> protein). On the contrary, the application of nutrient solution with EC of 5 dS m<sup>-1</sup> resulted in the highest activity of POD (33.90 ± 0.07 U mg<sup>-1</sup> protein). Under saline condition, GABA decreased POD activity, while under non-saline condition, it led to an increase in POD activity.

### 3.2. Gene expression pattern analysis

The expression analysis of salinity-related genes can provide more details of early activated processes of salinity response in plants and also the mitigation mechanisms mediated by GABA application. In this regard, the transcript level of several important stress-related genes, including *DREB*, *cAPX*, *MnSOD*, and *GST* was analyzed in strawberry cv. Aromas fed with GABA under stress and non-stress conditions at 12, 24, and 36 h after treatment implementation.

Based on the semi-quantitative RT-PCR results, *DREB*, *cAPX*, *MnSOD*, and *GST* reacted sensitively to salt stress, GABA application, and sampling time. In this term, the lowest *DREB* transcript level was obtained with S5 treatment (nutrient solution with 5 dS m<sup>-1</sup> EC) at 12 h sampling time, while the highest *DREB* expression was found in S3 treatment (nutrient solution with 5 dS m<sup>-1</sup> EC) 24 h after salt stress imposition (Fig. 1 and Fig. 5A). *DREB* expression shown an increasing and decreasing trend in most treatments. At the third sampling time (36 h), *DREB* expression was decreased in MG, S3, S3G, and S5 treatments in comparison to the second sampling time (24 h). GABA application led to an increase of *DREB* transcript level in stressed and non-stressed strawberry plants. This pattern of expression has also shown by the heatmap graph (Fig. 5A).

In the case of *cAPX* gene, the control plants represented the lowest transcript level, while the highest relative expression was observed in strawberry plants treated with nutrient solution having 5 dS m<sup>-1</sup> EC. Regarding the sampling time, there was no difference between 12, 24, and 36 h except for MG and S5 treatments where *cAPX* expression at 12 and 36 h sampling times was lower and higher than 24 h, respectively. On the other hand, the *cAPX* expression increased in response to salt stress and higher salinity level led to higher *cAPX* transcript levels. Under non-stress condition, GABA application led to a slight increase in *cAPX* expression 36 h after treatment. In contrast, GABA increased *cAPX* transcript level particularly under higher salinity intensity (Fig. 2 and Fig. 5B).

Similar to *cAPX*, the lowest *MnSOD* gene expression was obtained in the control plants and there was no significant difference between sampling times. The expression of *MnSOD* was improved by the effect of both salinity and GABA. In this term, GABA application induced *MnSOD* expression in a time-dependent manner. Compared to control plants, simultaneous implementation of salinity (3 dS m<sup>-1</sup> EC) and GABA after 36h, resulted in the highest *MnSOD* transcript level. On the other hand, *MnSOD* expression decreased under more severe salinity (5 dS m<sup>-1</sup> EC) alone or in combination with GABA (Fig. 3 and Fig. 5C).

*GST* represented an increasing pattern of expression in response to salinity alone or in combination with GABA application. There was no given effect of sampling time. For example, *GST* expression 36 h after the implementation of treatments was lower than 12 and 24 sampling times, while in S5G treatment (5 dS m<sup>-1</sup> salinity + 1mM GABA), the highest *GST* expression was recorded at 36 h sampling time (Fig. 4 and Fig. 5D). Compared to other genes including *cAPX*, *DREB*, and *MnSOD*, there were slight differences in the expression of *GST* between the studied treatments.

## 4. Discussion

The receptors of plant cells' plasmalemma receive the salinity signals and transmit these signals through a downstream signaling cascade leading to unique physiological and molecular events. GABA ( $\gamma$ -aminobutyric acid), a four-carbon free non-protein amino acid, comprises a significant fraction of free amino acids in plant cells. In the present research, salinity increased the content of H<sub>2</sub>O<sub>2</sub>, MDA, and proline and decreased the percentage of MSI and the activity of SOD and POD antioxidant enzymes. In contrast, the implementation of GABA not only decreased H<sub>2</sub>O<sub>2</sub> and MDA content, and maintained MSI percentage under salt stress, but also it improved the activity of antioxidant enzymes and the transcription level of *DREB*, *cAPX*, *MnSOD*, and *GST* genes.

Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) is one of the most important reactive oxygen species that is produced as a byproduct of cellular metabolism processes such as photorespiration in the peroxisomes or is generated as a product of superoxide degradation mediated by the SOD enzyme (Apel and Hirt 2004). ROS are not dangerous for plant cells as long as their generation and neutralization in plant cells are balanced. However, abiotic stresses such as salinity upset this balance and increase ROS accumulation. The increasing H<sub>2</sub>O<sub>2</sub> content under salt stress condition has been reported in various strawberry cultivars (Yaghubi et al. 2016; Yaghubi et al. 2019; Sandhu et al. 2019; Zahedi et al. 2020; Mirfattahi and Eshghi 2020).

In the present study, GABA foliar application reduced the content of H<sub>2</sub>O<sub>2</sub> molecules generated and accumulated under salinity stress. This alleviation effect has been attributed to the signaling role of GABA in the up-regulation of key genes encoding enzymes

that are involved in ROS metabolism, including NADPH oxidase, peroxidase, and amino oxidase (Shi et al. 2010). Furthermore, Arabidopsis plants mutated in the succinic semialdehyde dehydrogenase (SSADH) gene were more susceptible to abiotic stresses as they could not scavenge accumulated ROS. The succinic acid and NADH can enter the TCA cycle from the GABA biosynthesis pathway (Kinnersley and Turano 2010). It was thus postulated that the degradation of GABA may limit ROS accumulation under environmental stresses. Decrease in the endogenous GABA concentration could increase glutamate level (Bouche and Fromm 2004; Fait et al. 2008). Considering the effective role of glutamate in photorespiration reactions (Teiz and Zyger, 2006), it could be concluded that the slight increase in H<sub>2</sub>O<sub>2</sub> content under non-saline condition observed in the present research may be due to the increase in the rate of photorespiration. This effect has also been attributed to the up-regulation of the RBOHD genes encoding the NADPH oxidases (Liu et al. 2018). These H<sub>2</sub>O<sub>2</sub> molecules present in the apoplast and probably function as signals to induce the antioxidant system to protect plant cells from deleterious impact of salinity (Jin et al. 2019).

The cell membrane damage and electrolyte leakage that manifested as increased MDA content under salinity is closely associated with the excessive accumulated ROS. The present findings demonstrated that cellular injuries imposed by salinity is mitigated by GABA as shown by lower MDA content and higher MSI percentage. The alleviative impact of GABA under salinity stress has been also reported in rice (Sheteiwy et al. 2019), poplar (Ji et al. 2018), wheat (Wang et al. 2019), and muskmelon (Jin et al. 2019).

Proline is an important amino acid and its accumulation in the plant cells is a typical physiological response to a variety of stresses, including salinity. Proline accumulation during stress is due to its increased synthesis and decreased degradation (Tanveer et al. 2020). Increasing proline biosynthesis under salinity is partially regulated by ABA and H<sub>2</sub>O<sub>2</sub> through inducing the expression of P5CS gene encoding pyrroline-5-carboxylate synthase enzyme (Kubala et al. 2015). In the present study, GABA application increased the concentration of proline, both under stress and non-stress conditions. These results were in line with the findings obtained in corn (Li et al., 2016) and black pepper (Vijayakumari and Puthur, 2016) where GABA application increased proline content. Glutamate is common precursor of proline and GABA that regulate the biosynthesis of these amino acids in two different ways. It has been reported that the exogenous application of GABA reduces GAD and therefore increases glutamate accumulation (Soleimani Aghdam et al., 2016). On the other hand, salinity increases the activity of P5CS enzyme. In this condition, more glutamate flux enters the proline biosynthesis pathway causing proline accumulation (Kubala et al. 2015). Recent findings show that the accumulated GABA under salinity can also function as a carbon skeleton source for the TCA cycle after the relieving stress (Kinnersley and Turano 2010).

Salinity induces the activity of proteases and thus reduces the protein concentration in plant cells. Furthermore, the ROS accumulated during salt stress can damage and degrade proteins. On the other hand, the lower K<sup>+</sup>/Na<sup>+</sup> ratio under salinity stress pressure disrupts translation process within the cells because K ions act as the binders of tRNAs to ribosomes (Munns and Tester 2008). Chaperones are a group of salinity-induced proteins that are synthesized to prevent the degradation of structural and functional proteins thereby counteracting the salinity-induced oxidative stress (Witzel et al. 2009). Some of these proteins degrade or convert to other biological forms in the first few hours of stress causing a decrease in the total soluble protein content over time (Kosová et al. 2013; Hasegawa et al. 2000). Regarding the decrease-increase-decrease pattern of TSP content observed in MG, S3, S3MG, S5, and S5MG treatments, it can be assumed that salt-stressed strawberry plants need about 24 hours to implement the mechanisms related to protein accumulation. GABA recovered the glutamate content in muskmelon plants under salinity stress (Jin et al. 2019). Glutamate is the precursor of arginine that is a high N:C ratio amino acid and acts as an important storage of nitrogen in higher plants (Ramesh et al. 2017). Since nitrogen is the major component of amino acid structure, the GABA-induced glutamate accumulation can increase the biosynthesis of arginine and thus keep protein apparatus active under salinity stress (Forde and Lea 2007). In contrast, decreasing TSP content in GABA-treated plants under non-saline condition could be due to H<sub>2</sub>O<sub>2</sub> accumulation imposed by GABA.

Plants activate enzymatic antioxidant protection system in response to accumulated ROS during various abiotic stress like salinity. As a crucial step of cell protection, SOD provides the first line of defense against the damages induced by ROS through dismutation of O<sub>2</sub><sup>•-</sup> to hydrogen peroxide and molecular oxygen (Apel and Hirt 2004). POD is another important antioxidant enzyme that is mainly located in the apoplast space and vacuole, where it mediates the breaking down of H<sub>2</sub>O<sub>2</sub> and its conversion to water and oxygen (Gupta and Huang 2014; Apel and Hirt 2004). Therefore, both enzymes help plant cells to maintain an optimum redox balance under stress conditions. The role of SOD and POD in the mitigation of oxidative damages induced by salinity has been reported in other strawberry cultivars (Zahedi et al. 2020; Yaghubi et al. 2016; Tanou et al. 2009; Saidimoradi et al. 2019). In line

with the present results, the higher activity of antioxidative enzymes induced by GABA implementation in non-stressed plants has also been reported in maize (Li et al. 2016). Under non-saline condition, GABA acts as a mild stressor by imposing effects similar to abiotic stress which can help plants adapt under adverse environmental conditions. Although the improving effect of GABA on the activity of antioxidant enzymes under salinity has been documented (Malekzadeh et al., 2012; Li et al., 2015), in the present study, GABA foliar application reduced SOD and POD activity in salt-stressed strawberry plants. The possible reasons for this effect can be explained in two ways. First, GABA may act mainly as an osmo-protectant in a non-enzymatic manner to cope with the adverse effects of salinity, which decrease need to the antioxidative protection system. On the other hand, it has been hypothesized that GABA induces salt-tolerance through the regulation of ion channels with high selectivity for  $K^+$  over  $Na^+$  to keep a modest accumulation of  $Na^+$  in plant tissues and to keep a high  $K^+/Na^+$  ratio (Wang et al. 2019). The lower  $Na^+$  accumulation in leaves decreases ROS generation and in turn, reduces the activity of antioxidant enzymes. Salinity tolerance is not always dependent on the higher activity of antioxidant enzymes, and in some cases, high antioxidative activity is considered as a sign of salinity sensitivity (Abogadallah 2010). Moreover, increasing the SOD activity 24 h after the treatments initiation is probably due to more activity of chloroplasts and mitochondria during day, which also reflected by lower  $H_2O_2$  at this sampling time.

Plants regulate their transcriptional status in reaction to multiple abiotic stresses, which is an extremely important mechanism for stress adaptation. The interaction of plants to salinity is a multigenic process in which the expression pattern of genes mediating ROS-scavenging and osmotic-regulating enzymes are affected by complex signaling pathways. Here, the expression of studied salinity-related genes including *DREB*, *cAPX*, *MnSOD*, and *GST* was affected by salinity, GABA and sampling time.

Transcription factors, particularly dehydration responsive element binding (DREB) proteins, are essential elements in the regulation of salinity-related genes, in an ABA-independent pathway. DREBs involve in plant adaptation under different environmental stresses through the upregulation during stress (Hasegawa et al. 2000). They attach to cis-elements of promoters within stress-related genes and therefore enhance the expression of these downstream genes. This activate the adaptive physiological events such as osmotic and enzymatic antioxidant protection mechanisms that enable plants to cope with adverse impact of abiotic stresses (Shavrukov et al. 2016; Dubouzet et al. 2003). In accordance with our study, the enhanced *DREB* transcript level under salinity has also been observed in other plant species. For example, *DREB* was up-regulated in grape varieties under the increasing level of salinity (Pandey et al. 2015). It has also been reported that overexpression of *DREB1A* improves drought stress tolerance in rice and maize plants simultaneously with increase in endogenous GABA and glutamine content (Kudo et al. 2017). The calcium-dependent protein kinases (CDPKs) are key downstream signaling elements of  $Ca^{2+}$  messenger that contribute in plant adaptation under abiotic environmental stresses by the modulation of corresponding transcription factors such as DREB, WRKY, and other functional genes (Li et al. 2021b). Li et al. (2021b) found that GABA application in Creeping bentgrass (*Agrostis stolonifera* L.) improves *DREB1/2* and *WRKY1/24/41* expression by triggering CDPK signaling and therefore inducing the expression of stress-related functional genes mainly responsible for enhanced stress tolerance (Singh et al. 2002; Asano et al. 2012).

The increased *cAPX* activity is a part of the enzymatic antioxidant protection system, which occurs in response to accumulated ROS under salinity. The activation and preservation of a strong enzymatic antioxidant protection system usually associate with improved salinity tolerance. Lin and Pu (2010) reported an increase in the expression of *cAPX*, *pAPX*, and *chAPX* genes in leaves of *Ipomea batatas* 24 and 48 h after the NaCl implementation. Similarly, the transcript level of genes encoding ascorbate peroxidases increased in Arabidopsis under NaCl-induced salinity, and the higher expression was recorded after the prolonged stress course (Attia et al. 2008).

It has been reported that individual SOD isoforms play unique or synergistic functions in plant adaptation under abiotic stresses. Drought-stressed clover plants (*Trifolium sativum*) represented an increased *MnSOD* expression compared to *FeSOD* and *Cu/ZnSOD* genes (Zhang et al. 2015). It has been stated that more SOD activity, even 0.15-fold, can significantly enhance the salt tolerance in plants, confirming the important role of SOD as a part of antioxidant protection system of plant cells (Hu et al. 2012).

GABA-induced tolerance to abiotic stress has been attributed to the enhancement of antioxidant defense in plants. For example, exogenous GABA effectively improved heat and drought tolerance of creeping bentgrass which was associated with the upregulation of POD and APX ascorbate peroxidase genes. The application of GABA induced heat tolerance in rice seedling by maintaining higher SOD, CAT, and APX activity. GABA treatment significantly enhanced POD activity and alleviated chilling-induced oxidative damage in banana (*Musa* spp.). It has been currently found that increasing the GABA content in plants significantly

upregulates several POD genes (POD2, POD5, POD19, POD24, POD31, and POD51) under water stress resulting in higher POD enzyme activity (Li et al. 2021b). Furthermore, the level of endogenous GABA has been increased under strong salt stress in rice (Sheteiwy et al. 2019) and tomato (Wu et al. 2020), suggesting that the accumulation of GABA could also protect plants under salt stress. In this connection, the extensive studies have provided the evidence that salt stress activates the GABA signaling pathway with a broad effect on various developmental and physiological processes of plants (Kinnersley and Turano 2010; Ramesh et al. 2017).

The role of GSTs in the alleviation of deleterious impact of salt stress has been reported in soybean (Dinler et al. 2014) and barley (Walia et al. 2006). Moreover, the transformation of Arabidopsis plants with a *GST* transgene cloned from tamarisk (*Tamarix hispida*) resulted in salinity tolerance improvement (Yang et al. 2014), and it recommended that GST regulates the programmed cell death (Csiszár et al. 2014). GST class members have GSH-conjugating activity with various xenobiotics and they possibly involve in the conjugation of toxic metabolites produced during salinity to GSH (Hu et al. 2011). The concentration of glutathione in muskmelon seedlings increased in response to GABA application by increasing the activity of GR, DHAR, APX, and MDAR enzymes. It has been concluded that AsA-GSH cycle, but not CAT, plays a major function in GABA-regulated reduction of H<sub>2</sub>O<sub>2</sub> (Jin et al. 2019). The increasing GSH content in GABA treated-plants may modulate ROS molecules generated by GABA application. This could be the reason why GST expression increased in GABA-treated strawberry plants under non-stress condition in the present study.

## Conclusion

In summary, the oxidative stress induced by salt stress adversely impacted the strawberry plants cv. Aromas. In contrast, the implementation of GABA not only decreased the content of H<sub>2</sub>O<sub>2</sub> and MDA, and maintained the percentage of MSI, but also it improved the activity of antioxidant enzymes and the transcription level of *DREB*, *cAPX*, *MnSOD*, and *GST* genes. Under non-saline condition, GABA acts as a mild stressor by imposing effects similar to abiotic stress which can help plants adapt under adverse environmental conditions. Our study can aim to characterize optimum approaches to mitigate the deleterious impacts imposed by salt stress and the development of a functional relationship between GABA application and temporal physiological and molecular response of salt-stressed strawberry plants. We concluded that strawberry plants represent higher salinity tolerance by transcription activation of salinity-related genes and physiological response upon GABA application.

## Abbreviations

GST, Glutathione S-transferases; MDA, Malondialdehyde; MSI, Membrane Stability Index; SOD, superoxide dismutase; ROS, Reactive Oxygen Species; SOD, Superoxide Dismutase; cAPX, Cytosolic Ascorbate Peroxidase

## Declarations

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### Authors' contributions:

YV and FN participated in the experiment design and coordination, and guaranteed of integrity of the entire study. SG carried out the experiment. YV and NG performed the statistical analysis. YV, FN and NG was involved in drafting the manuscript, evaluating the statistical analysis and critical revising the manuscript. All authors read and approved the final manuscript.

### Conflict of interest

The authors declare that they have no conflict of interest.

### Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

## Consent to participate

Not applicable.

## Consent for publication

Not applicable.

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## Figures

### *DREB*

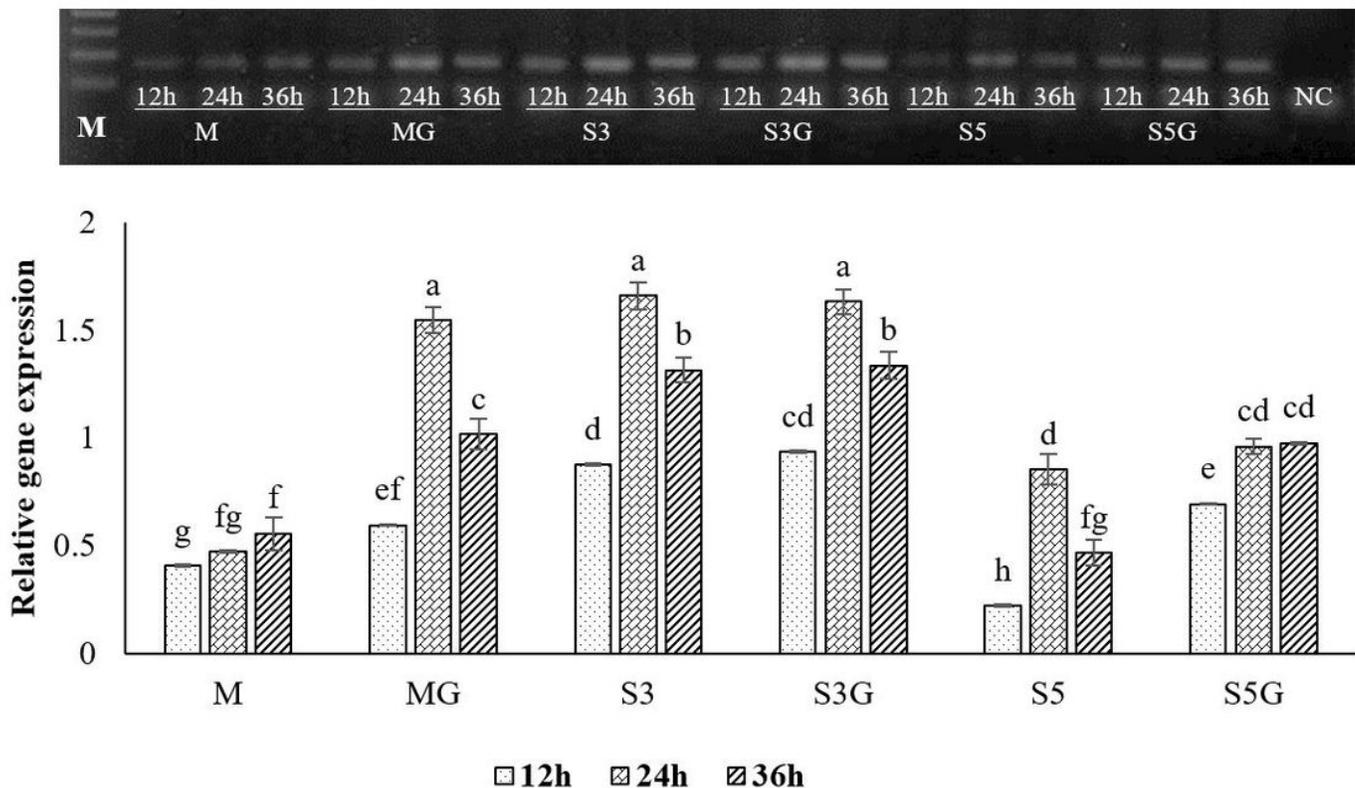
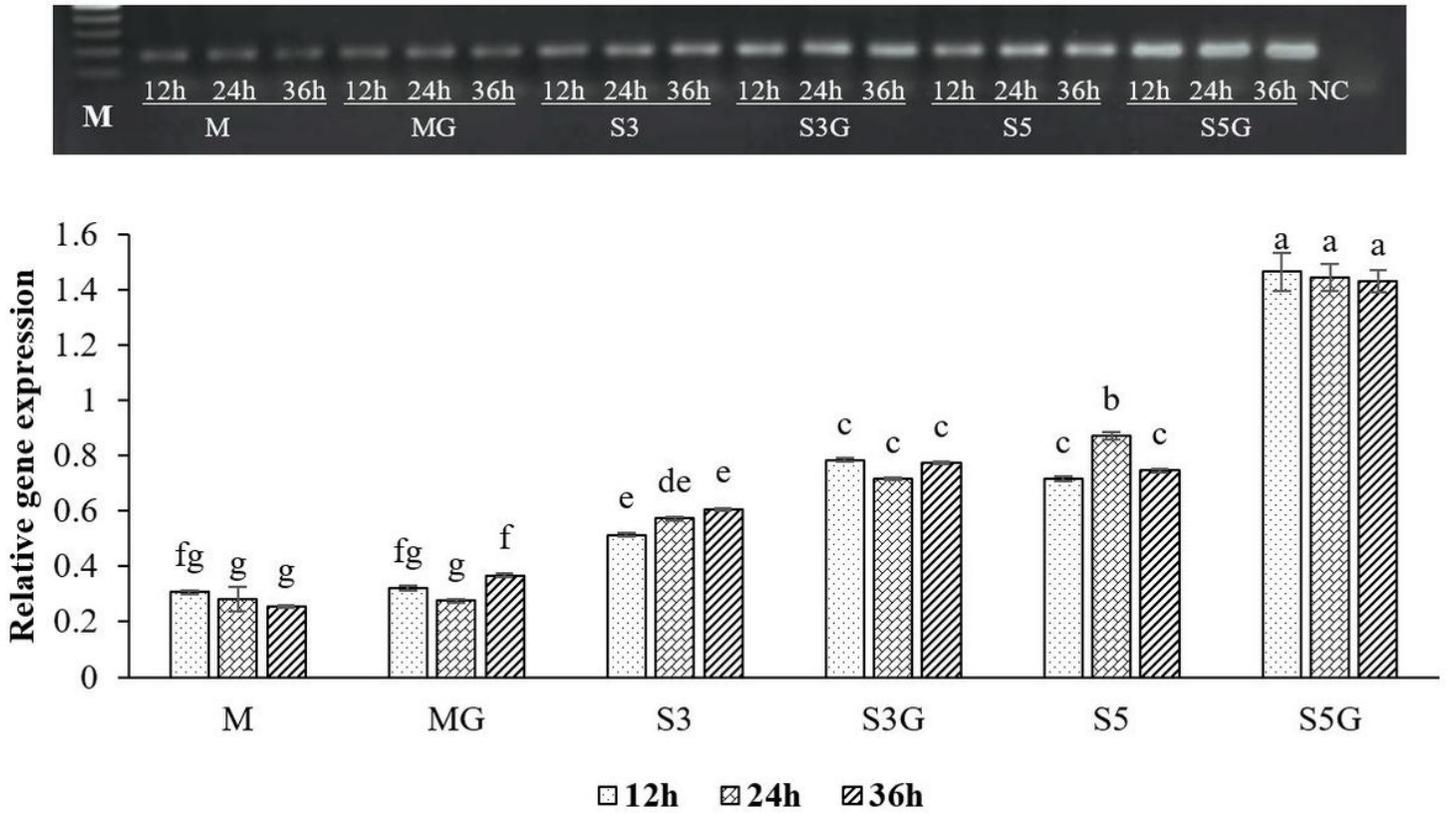


Figure 1

Expression of GST gene in strawberry leaf tissue following GABA treatment under NaCl-salt stress and non-stress conditions at 1h, 24 h and 48 h time intervals. Different letters indicate significances by LSD tests at  $P < 0.05$ . M, control; MG, 25 mM GABA; S3, 3 dS  $m^{-1}$  EC; S5, 5 dS  $m^{-1}$  EC; S3G, 25 mM GABA + 3 dS  $m^{-1}$  EC, S5G, 25 mM GABA + 5 dS  $m^{-1}$  EC.

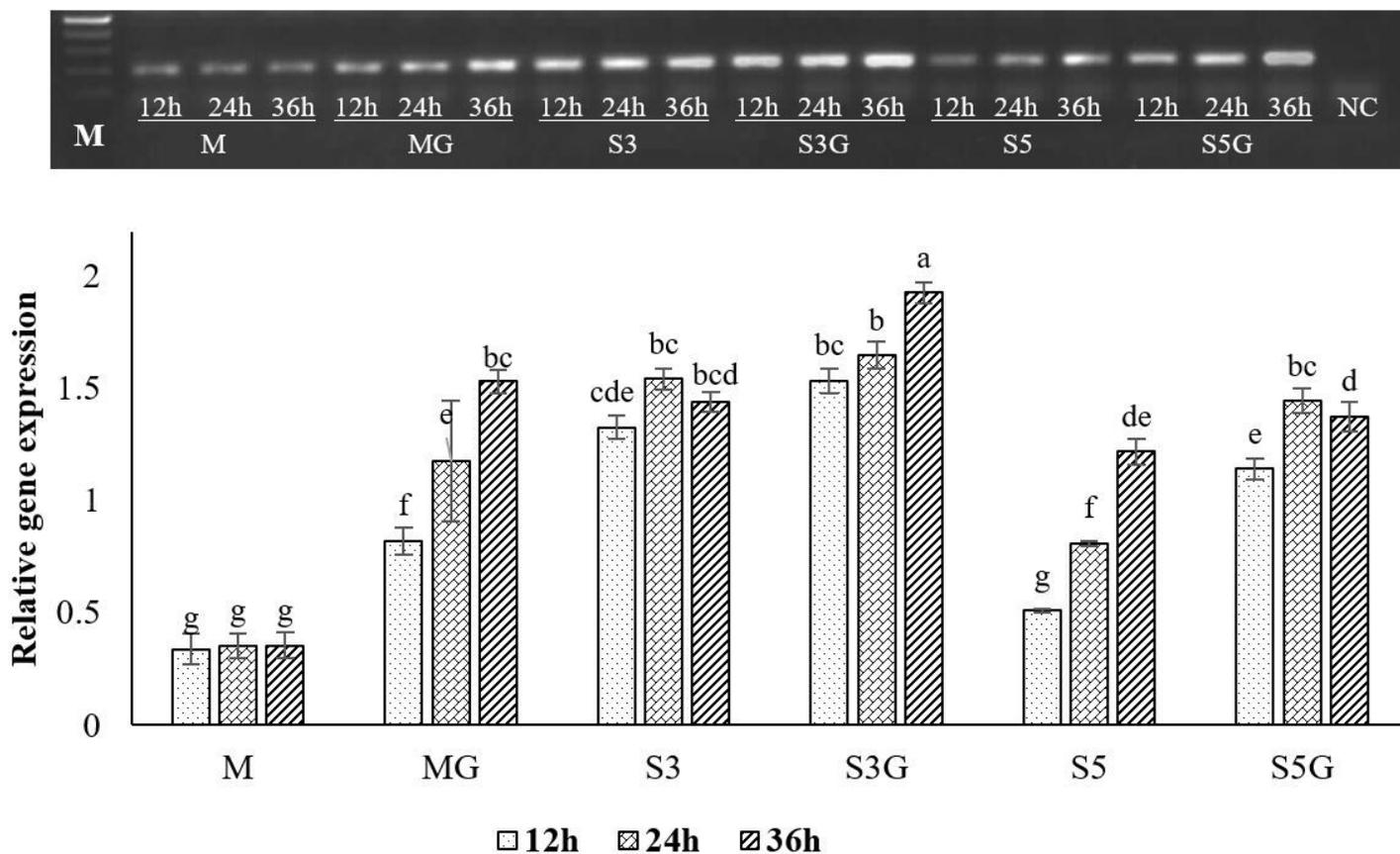
### cAPX



**Figure 2**

Expression of GST gene in strawberry leaf tissue following GABA treatment under NaCl-salt stress and non-stress conditions at 1h, 24 h and 48 h time intervals. Different letters indicate significances by LSD tests at  $P < 0.05$ . M, control; MG, 25 mM GABA; S3, 3 dS  $m^{-1}$  EC; S5, 5 dS  $m^{-1}$  EC; S3G, 25 mM GABA + 3 dS  $m^{-1}$  EC, S5G, 25 mM GABA + 5 dS  $m^{-1}$  EC.

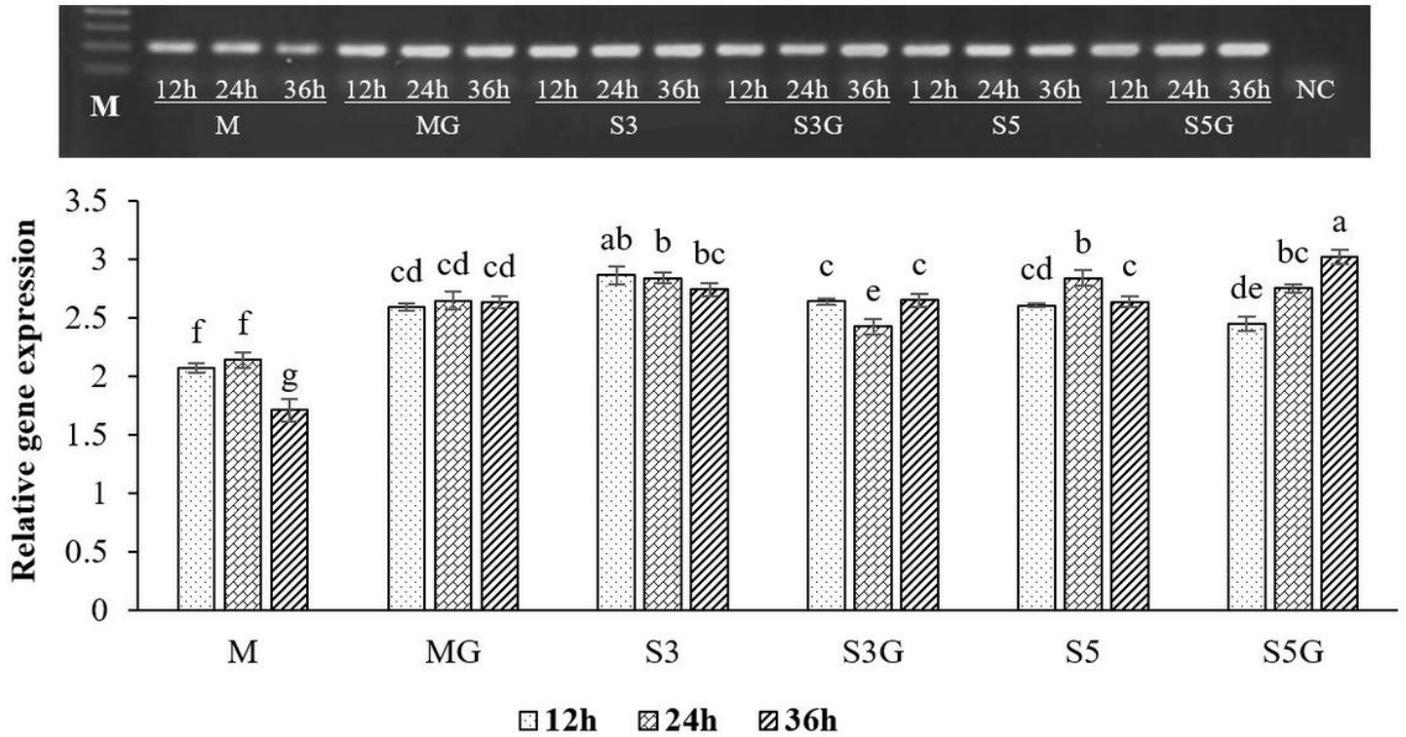
## *MnSOD*



**Figure 3**

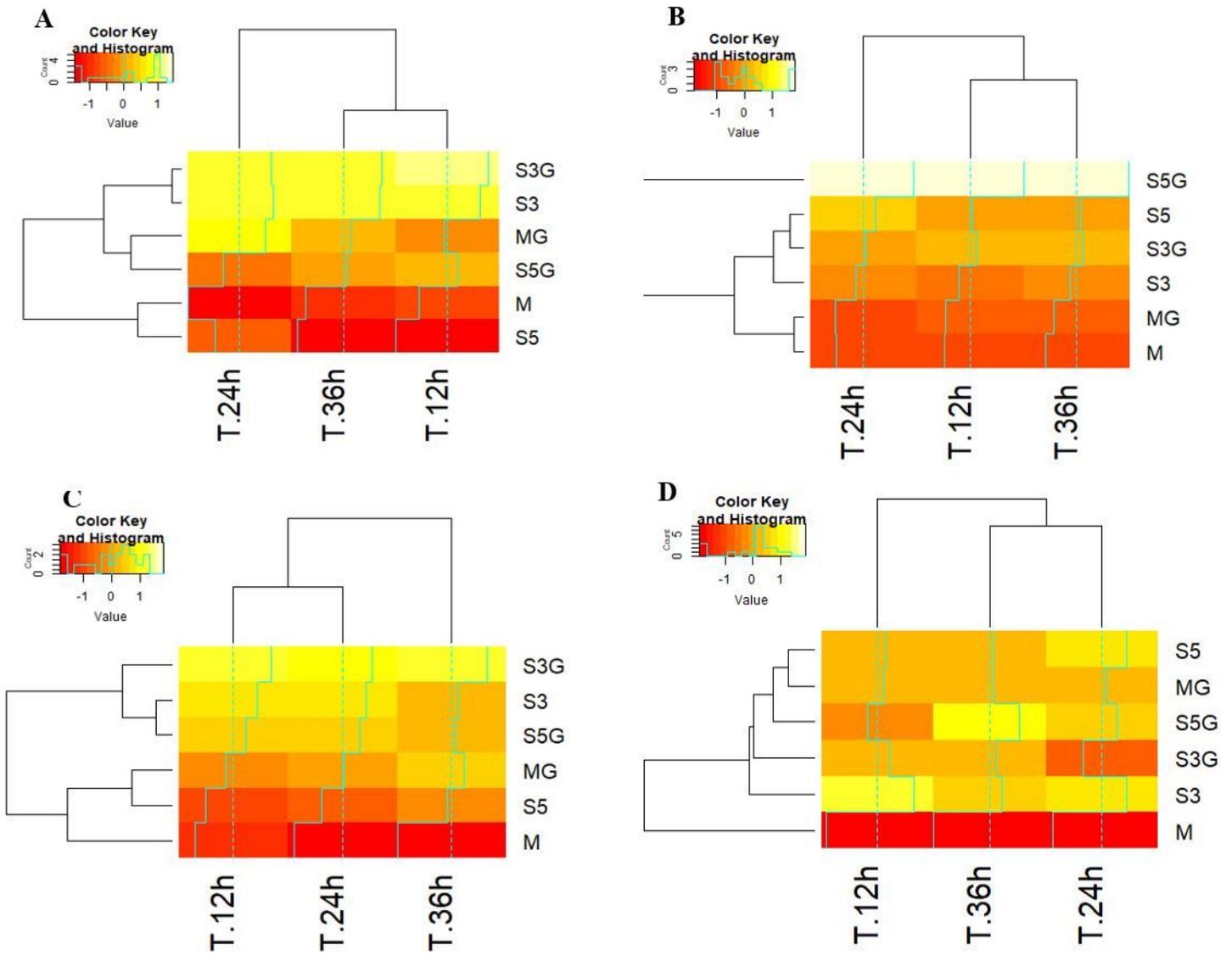
Expression of MnSOD gene in strawberry leaf tissue following GABA treatment under NaCl-salt stress and non-stress conditions at 12 h, 24 h and 36 h time intervals. Different letters indicate significances by LSD tests at  $P < 0.05$ . M, control; MG, 25 mM GABA; S3, 3 dS m<sup>-1</sup> EC; S5, 5 dS m<sup>-1</sup> EC; S3G, 25 mM GABA + 3 dS m<sup>-1</sup> EC, S5G, 25 mM GABA + 5 dS m<sup>-1</sup> EC.

# *GST*



**Figure 4**

Expression of GST gene in strawberry leaf tissue following GABA treatments under NaCl-salt stress and non-stress conditions at 12 h, 24 h and 36 h time intervals. Different letters indicate significances by LSD tests at  $P < 0.05$ . M, control; MG, 25 mM GABA; S3, 3 dS  $m^{-1}$  EC; S5, 5 dS  $m^{-1}$  EC; S3G, 25 mM GABA + 3 dS  $m^{-1}$  EC, S5G, 25 mM GABA + 5 dS  $m^{-1}$  EC.



**Figure 5**

The heat map of gene expression data. (A) DREB, (B) cAPX, (C) MnSOD, and (D) GST. M, control; MG, 25 mM GABA; S3, 3 dS m<sup>-1</sup> EC; S5, 5 dS m<sup>-1</sup> EC; S3G, 25 mM GABA + 3 dS m<sup>-1</sup> EC, S5G, 25 mM GABA + 5 dS m<sup>-1</sup> EC.