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Research Report

Exogenously Applied Glycine Betaine Regulates Some Chemical Characteristics and Antioxidative Defence Systems in Lettuce under Salt Stress

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Abstract. We investigated the effects of exogenous glycine betaine (GB) applications on antioxidant enzyme activity, dry matter, and the contents of organic acids, amino acids, total antioxidants, and total phenolics in lettuce, *Lactuca sativa,* under salt stress. The treatments included four concentrations of GB (0, 5, 10, and 25 mM) and two levels of salinity (0 and 100 mM of NaCl). The 25 mM GB treatment increased dry matter and the content of total phenolics in lettuce plants compared to the non-GB-treated plants under salt stress. Salinity (100 mM NaCl without GB) significantly reduced dry matter, total phenolic content, and total antioxidant content in the plants. However, the lettuce plants grown under salt stress generally had higher amino acid and organic acid contents than those grown under non-salinity conditions. GB treatments had different effect on amino acid and organic acid contents under salinity conditions. Superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX) activities were elevated under the 100 mM NaCl and 0 mM GB treatments, whereas higher concentrations of GB decreased them under salt stress. The 25 mM GB treatment mitigated the negative effect of salt stress and increased the dry matter by 44% compared to the plants that were treated with 100 mM NaCl and 0 mM GB. The results suggested that exogenous GB treatments could ameliorate the tolerance of lettuce to salt stress by increasing the total antioxidants and total phenolics, and regulating antioxidant enzyme activity, and altering the contents of organic acids and amino acids.

***Additional key words*:** amino acid, antioxidant enzymes, dry matter, organic acid, salinity

Introduction

Salinity is one of the major environmental factors that can impact crop production and reduce yields. Worldwide, 100 million ha or 5% of the world's arable land is adversely affected by high salt concentrations (FAO, 2008) and this percentage is increasing, which has attracted attention in scientific and political agendas. Plants that are grown under high salinity conditions show changes at both the macro and cellular levels, helping the plants to tolerate salt stress (Jamali et al., 2015). High saline environments cause osmotic stress due to the difficulties in absorbing water from the soil and ion toxicity that can negatively affect the growth of many plants. Osmotic stress and ionic toxicity cause changes in plant physiological processes, mineral distribution, enzyme activities, stomatal behavior, protein synthesis, and photosynthetic efficiency (Türkan and Demiral, 2009). These conditions increase the H_2O_2 content in cells and the activity of antioxidant enzymes such as SOD, CAT, and APX, and can also lead to lipid peroxidation and the destruction of cell membranes in plants (Eraslan et al., 2007).

The response of plants to salinity stress and other environmental conditions is complex and differs among species (Rayatpanah et al., 2012; Undurraga et al., 2009; Shams et al., 2016). Antioxidants are substances that prevent the formation of free radicals in cells (Esringü et al., 2011). Plants that are able to induce higher amounts of antioxidant enzymes in response to salinity stress show greater resistance to oxidative damage (Ashraf and Foolad, 2007).

Exposure to different stress conditions can result in oxidative stress, which causes damage to cell components, disrupting plant growth. Different types of osmoprotectants have been shown to accumulate in plant cells under stress conditions. These solutes have low molecular weight and are usually

non-toxic at high concentrations. It has been shown that osmoprotectants, like glycine betaine, can protect plants from the adverse effects of abiotic stresses by detoxification of reactive oxygen species (ROS), adjusting cellular osmotica, and protecting membrane integrity (Ashraf and Foolad, 2007; Hayat et al., 2012).

Researchers have generated transgenic plants with increased tolerance to salt stress, but the potential risks of consuming transgenic plants makes this an undesirable approach for potato, tomato, rice and carrot (Key et al., 2008). Much research has aimed to minimize the effects of salt stress in plants, using exogenous applications of substances such as osmoprotectants, phytohormones, antioxidants, and trace elements. One of these promising substances is glycine betaine (GB), a quaternary amine (Giri, 2011).

Some plants can produce GB under abiotic stresses such as salinity (Ashraf and Foolad, 2007), but lettuce has been shown to be unable to produce GB (Roy and Basu, 2009). Salt stress can reduce the growth and yield of lettuce (Ekinci et al., 2012) and applications of GB have been shown to improve the plant's tolerance to salinity (Chen and Murata, 2011). As we know, minimal research has been conducted on the effects of GB on the organic acid, amino acid, antioxidant, and total phenolic contents in lettuce under salt stress. Therefore, the purpose of this study was to evaluate the effects of GB on amino acids, organic acids, antioxidant activity, total phenolic and total antioxidant contents in lettuce plants exposed to salt stress.

Material and Method

Chemicals and Plant Materials

Acetonitrile, methanol (HPLC grade, Merck, Darmstadt, Germany), and betaine hydrochloride (source of glycine betaine, Merck) were used. All other reagents were of analytical grade, without further refinement. Lettuce (*Lactuca sativa* var. longium cv. Yedikule) seeds were acquired from the Seed and Plant Improvement Institute of Istanbul, Turkey.

Greenhouse Experiment

Greenhouse experiments were conducted at the Faculty of Agriculture, University of Ataturk, Erzurum, Turkey, from April 5th, 2014 to October 7th, 2014. A completely randomized experimental design with four replications was used in this study. Lettuce seeds were sown in 216-celled styrofoam trays filled with peat. The mean temperature of the greenhouse was 24.5°C and the relative humidity was 78% during the experiment. Thirty-day-old seedlings were transplanted into 2 L pots filled with a mixture of peat: perlite (1:1, v: v). Each treatment had 4 replications with 5 plants for each replication, totaling 160 pots.

The treatments included four concentrations of glycine betaine (0, 5, 10, and 25 mM betaine hydrochloride) and two levels of salinity (0 and 100 mM of NaCl). The solutions of GB were prepared with distilled water containing 0.02% Tween-20 as a surfactant, and 100 mL of the GB solution was applied to the plant foliage using a handheld sprayer during late afternoon the day following transplanting. The control plants were sprayed with distilled water containing 0.02% Tween-20. The GB treatments were repeated at 10 and 20 days after transplanting.

For the salinity treatments, 0 or 100 mM NaCl was added to a base complete nutrient solution (Hoagland solution) three days after transplanting. After addition of the NaCl to the nutrient solution, the electrical conductivity of the solutions was measured at 1.76 and 11.82 $dS \cdot m^{-1}$ for the 0 and 100 mM NaCl, respectively. The electrical conductivity of the potting mixture was 4 $dS \cdot m^{-1}$, at the end of the experiment the electrical conductivities of control and 100 mM NaCl treatment were 10 and 70 $dS \cdot m^{-1}$ respectively. Thirty-five days after transplanting, 5 plants from each replicate were harvested, plant materials were dried at 70°C for 48 h, and dry weights (DW) per plant were determined.

Data Collection and Data Analysis

For analysis of the contents of amino acids, organic acids, total phenolics, and total antioxidants, and antioxidant enzyme activity, 3 plants from each replication were randomly selected 35 days after transplanting. Approximately 20 g of fresh leaves selected from the middle section of the plants were frozen in liquid nitrogen and then stored at -70ºC for analysis. Four laboratory replicates were used. SPSS 15 was used to performed data analysis and the data were subjected to analysis of variance (ANOVA) followed by Duncan's multiple range test at a significance level of 0.05.

Amino Acid Analysis

For analysis of amino acids, HCl (0.1 N) was added to 1 g of fresh leaf sample, homogenized with ultraturraks, and incubated at 4°C for 12 h. Samples were then vortexed and centrifuged at 1,200 rpm for 50 min and the supernatants were filtered through a 0.22 μm (Millex Millipore) filter. The supernatants were then transferred to vials for amino acid analysis by HPLC as described (Ashraf and Foolad, 2007; Siddiqui et al., 2015). Briefly, a Zorbax Eclipse-AAA 4.6 × 150 mm, 3.5 μm column (Agilent 1200 HPLC) was used and readings were recorded at 254 nm. The amino acids were identified by comparison with the following standards: O-phthaldialdehyde (OPA), fluorenylmethyl-chloroformate (FMOC), and 0.4 N borate. The following was used as the mobile phase for the chromatography system: mobile phase A: 40 mM NaH_2PO_4 (pH 7.8) and mobile phase B: aceto-

nitrile/methanol/water (45/45/10, v/v/v) solutions. The flow rate of the mobile phase was 2 mL·min^{-1} and the column temperature was 40°C. Quantities of aspartate, glutamate, asparagine, serine, glutamine, histidine, glycine, threonine, arginine, alanine, tyrosine, cysteine, valine, methionine, tryptophan, phenylalanine, isoleucine, leucine, lysine, hydroxyproline, sarcosine, and proline were determined as pmol·ng·mg^{-1} after a 26-minute derivation process in HPLC.

Organic Acid Analysis

Deionized water (10 mL) was added to 1 g of fresh leaf sample and the solution was homogenized with ultraturraks. After centrifugation at 1,200 rpm for 50 minutes, supernatants were filtered through 0.22 μm filters (Millex Millipore). In vials, supernatants were subjected to HPLC using a Zorbax Eclipse-AAA 4.6 × 250 mm, 5-μm column (Agilent 1200 HPLC) and recorded at an absorbance of 220 nm in a UV detector. The flow speed was 1 mL·min^{-1} and the column temperature was 25°C. Oxalic, propionic, tartaric, butyric, malonic, malic, lactic, citric, maleic, fumaric, and succinic acids were determined using 25 mM potassium phosphate (pH 2.5) as the mobile phase (Siddiqui et al., 2015).

Antioxidant Enzyme Activity Analysis

CAT activity was measured based on the rate of hydrogen peroxide decomposition as described (Abedi and Pakniyat, 2010). POD activity was measured at 436 nm with a UV/VIS spectrophotometer (Perkin Elmer Lambda 25, USA) according to its capability to turn guaiacol into tetraguaiacol and SOD activity was measured at 560 nm based on the inhibition in the photochemical diminution of nitroblue tetrazolium as described (Abedi and Pakniyat, 2010). APX was measured as described (Ouhibi et al., 2014). Briefly, for SOD, POD, and CAT activities, the frozen lettuce leaves were homogenized in 5 mL of 100 mM phosphate buffer (pH 7.0) containing 1% (w/v) PVPP at 4°C. The homogenate was centrifuged at 15,000×g for 15 min and the supernatant was directly examined for enzyme activities. For APX activity, the frozen lettuce leaves were homogenized in 5 mL of 50 mM Tris-HCl buffer (pH 7.0) containing 1 mM sodium ascorbate, 1 mM DTT, 1 mM EDTA, 1 mM reduced glutathione, 5 mM $MgCl_2$, and 1% PVPP (w/v) at 4°C.

CAT activity was determined by a decrease in absorbance at 240 nm that was caused by hydrogen peroxide decomposition. The reaction mixture contained 50 mM phosphate buffer (pH 7.0), 10 mM H_2O_2, and 100 μL of the plant extract. The oxidation extinction coefficient for H_2O_2 was 39.4 mM cm^{-1}. The activity of APX was calculated by measuring the decline in absorbance at 290 nm due to the oxidation of ascorbic acid. The reaction buffer contained 0.5 mM ascorbate, 0.1 mM H_2O_2, 0.1 mM EDTA, and 100 μL of enzyme extract. The concentration of oxidized ascorbate was calculated with the extinction coefficient £ = 2.8 mM·cm^{-1}; one unit of APX was defined as 1 μmol·mL^{-1} ascorbate oxidized per minute.

Total SOD activity was assayed by following the superradical-induced reduction of nitro blue tetrazolium. Briefly, 200 μL of the reaction mixtures (50 mM phosphate buffer (pH 7.8), 0.1 mM EDTA, 63 μM NBT, 50 μM riboflavin, 13 mM methionine, and 50 μL of plant extract) were placed in the wells of a 96-well microplate under a 40 W fluorescent lamp. After 10 min, the light was turned off and the absorbance was read at 560 nm. A non-illuminated reaction mixture, conducted in the same manner, was used as the blank. One unit of SOD was used, which produced a 50% inhibition of NBT reduction.

Analysis of Total Phenolic and Antioxidant Content

Total phenols were determined as described (Siddiqui et al., 2015). All samples were analyzed in three replicates. An aliquot (0.125 mL) of a suitable diluted methanolic solution was added to 0.125 mL of Folin-Ciocalteu reagent and 0.5 mL of distilled water. The mixture was shaken and incubated for 6 min before the addition of 1.25 mL of 7% Na_2CO_3. The solution was brought to a final volume of 3 mL with distilled water and mixed thoroughly. The absorbance was read at 760 nm after incubation in the dark for 90 min at 23°C. The total phenolic content was recorded as mg of gallic acid equivalents per g of fresh weight (mg GAE·g^{-1} FW) from an adjusted curve with gallic acid.

Total antioxidants in the lettuce samples were measured using the DPPH and ABTS β-carotene-linoleic acid coupled oxidation model system. a-Tocopherol was used for the antioxidant reference. DPPH and ABTS radical scavenging assays results were reported as IC_{50} value and percentage discoloration, respectively. The dose-dependence behavior of the extracts towards DPPH and ABTS radicals was also assayed by applying differing quantities (0.1-0.5 g) of the extract. The antioxidant activity as measured by the β-carotene/linoleate model system and was calculated as percentage discoloration (County, 2006; Surender Reddy et al., 2015).

Results

Dry Weight

As shown in Fig. 1., salt stress (100 mM NaCl) decreased the DW by as much as 75% in lettuce ($p < 0.05$) compared to the control (without NaCl and GB application). However, exogenous GB treatments increased the DW of the plants treated with 100 mM NaCl compared to the control (without GB application). The application of higher levels of GB had more significant effects on DW than the lower levels of GB. On average, the 25 mM GB application increased DW by

Table 1. Effect of NaCl and glycine betaine (GB) on organic acid contents in lettuce leaves ($ng \cdot mg^{-1}$). Data followed by a different letter were significantly different ($p \leq 0.05$) according to Duncan's Multiple Range Test. Data are represented as means ± SD from four replicates

NaCl mM	GB mM	Oxalic	Propionic	Tartaric	Butyric	Malonic	Malic	Lactic	Citric	Maleic	Fumaric	Succinic
0	0	0.43±0.05 e	1.49±0.07e	5.73±0.33 c	12.23 ±0.4c	20.25±1.1b	4.45±0.5bc	35.30±2.5b	11.0±0.2d	5.39±0.5a	3.850±0.1c	23.13±1.3c
	5	0.53±0.03de	1.70±0.02d	6.70±0.4 c	15.37±0.6 a	19.98±1.2b	5.49±0.5a	38.70±2.2b	13.15±0.4b	5.91±0.5a	6.04±0.5ab	28.74±1.1ab
	10	0.56±0.05 d	1.48±0.04 e	6.92±0.2bc	12.01±0.4 c	16.82±1.1c	3.68±0.3d	36.81±2.4b	12.44±0.2bc	5.01±0.4a	4.36±0.4c	23.70±1.2c
	25	0.45±0.06 e	1.56±0.1 de	6.93±0.1bc	12.91±0.4 c	20.00±1.2b	4.06±0.4cd	35.29±1.9b	11.33±0.1d	5.11±0.4a	4.01±0.2c	26.33±1.5bc
100	0	1.0±0.09 b	2.07±0.05 c	6.60±0.2c	12.11±0.9 c	16.66±0.9c	4.33±0.3bc	40.11±2.2b	11.96±0.2cd	6.51±0.6a	5.42±0.3abc	26.37±1.3bc
	5	1.46±0.09 a	2.63±0.04 a	7.64±0.1a	14.72±0.8ab	18.40±1.2bc	4.78±0.4b	40.10±2.1b	12.82±0.3bc	6.92±0.6a	6.44±0.2a	28.54±1.6ab
	10	0.96±0.03 b	2.38±0.09b	7.32±0.1ab	15.59±0.6 a	23.51±1.3a	5.84±0.4a	46.82±2.6a	14.36±0.6a	6.47±0.3a	5.42±0.2abc	30.94±0.9a
	25	0.85±0.05 c	2.33±0.05b	7.28±0.2 ab	13.34±0.7bc	16.75±0.9c	4.45±0.2bc	35.36±1.8b	12.72±0.4bc	5.75±0.4a	4.88±0.1bc	28.86±1.2ab

50% compared to the plants that were treated with 100 mM NaCl and 0 mM GB.

Organic Acids

The organic acid content in the lettuce plants was affected by salinity stress and GB treatments. As shown in Table 1, salinity stress had no significant effect on butyric, malic, maleic, and succinic acid contents. However, the 100 mM NaCl treatment increased the contents of oxalic, propionic, tartaric, lactic, citric, and fumaric acids in the lettuce plants. GB increased the contents of some organic acids in lettuce compared to the non-treated plants. In the lettuce plants grown under salt stress, the 10 mM GB treatment resulted in the greatest increase in the contents of the organic acids malonic, malic, butyric, lactic, and citric acids.

Amino Acids

The effect of salinity and GB treatment on different amino acids of lettuce is shown in Table 2.1 and 2.2. In general, the 100 mM NaCl application did not affect the amino acid content in lettuce except for serine, glycine, and proline. The lettuce plants grown under salt stress had higher serine, glycine, and proline values than the plants grown under non-salinity conditions. Exogenous GB treatments decreased the contents of most amino acids in lettuce grown under salt stress with the exception of serine, glycine, and proline, which were elevated by GB treatment.

CAT, POD, SOD, APX, Total Phenolic, and Total Antioxidant Content

The changes in CAT, POD, APX, SOD, total phenolics, and total antioxidant contents in the lettuce plants grown under salt stress are shown in Table 3. The application of 100 mM NaCl increased the content of CAT, POD, SOD, and APX by about 38, 44, 65, 91, and 33%, respectively, compared to the plants grown in non-saline conditions. GB applications significantly decreased the contents of APX, SOD, POD, and CAT in the plants grown in the salinity conditions compared to the plants treated with100 mM NaCl and 0 mM GB.

The phenolic content and total antioxidant content decreased in the plants treated with 100 mM NaCl. The highest concentration of GB (25 mM) elevated the total antioxidant and total phenolic contents in the plants exposed to saline conditions compared to the lower GB concentrations (Table 3). The 25 mM GB treatment increased the total phenolic content by about 40% compared to the 0 mM GB control.

Discussion

Many studies showed that GB could play an important role as an osmoprotectant in plants grown under salinity conditions (Abbas et al., 2010; Ashraf and Foolad, 2007; Sakamoto and Murata, 2002). Exogenous applications of GB may increase the salt tolerance of crops and enhance crop productivity under high salinity conditions. Therefore, we studied the effects of various concentrations of GB on amino acids, organic acids, and antioxidant activity in lettuce plants grown under salt stress.

Salt stress negatively affected dry matter in the lettuce plants regardless of the GB treatments (Fig. 1). Similarly, a study by Yildirim et al. (2011) showed that salt stress could adversely affect biomass accumulation in lettuce; however, foliar GB treatments decreased the extent of the growth suppression due to salt stress. Our findings are in accordance with Abbas et al. (2010) who found that GB ameliorated the adverse effect on the growth of eggplant. Moreover, some researchers have shown that GB applications increased dry matter under non-stress condition as well (Armin and Miri, 2014). The effect that GB has on plant growth could be due to neutralizing the toxicity induced by salt stress. Earlier studies showed that exogenous GB treatments ameliorated the negative effects of various abiotic stresses on plant growth (Abbas et al., 2010; Ashraf and Foolad, 2007; Hu et

Table 2.1. Effect of NaCl and glycine betaine (GB) on amino acid contents in lettuce leaves (ng·μg^{-1}). Data followed by a different letter were significantly different ($p \leq 0.05$) according to Duncan's Multiple Range Test. Data are represented as means ± SD from four replicates

NaCl mM	GB mM	Aspartate	Glutamate	Asparagine	Serine	Glutamine	Histidine	Glycine	Threonine	Cysteine	Tyrosine
0	0	1.84± 0.07a	1.76±0.06bc	3.38±0.3bc	4.24±0.6bc	2.92±0.07bc	0.86±0.02bc	0.71±0.04c	2.27±0.08bc	0.92±0.06abc	0.49±0.01a
	5	1.49±0.06b	1.24±0.06d	3.89±0.4b	5.86±0.4bc	2.89±0.05bc	0.83±0.02bc	0.69±0.03c	3.19±0.04a	0.55±0.04d	0.33±0.01c
	10	1.37±0.06b	1.26±0.03d	2.77±0.4bc	5.11±0.5bc	2.73±0.05bc	0.80±0.01bc	0.83±0.03b	2.34±.05bc	0.69±0.04bcd	0.39±0.02bc
	25	1.34±0.04b	1.17±0.03d	2.53±0.2bc	4.90±0.3bc	2.57±0.03c	0.79±0.03bc	0.78±0.05bc	2.27±0.05bc	0.62±0.03cd	0.39±0.02c
100	0	3.04±0.1a	3.31±0.15a	5.23±0.3a	8.40±0.3a	4.06±0.1a	1.50±0.06a	0.77±0.06c	2.62±0.06ab	0.98±0.04ab	0.45±0.03ab
	5	1.93±0.08b	1.82±0.06bc	3.58±0.5bc	6.79±0.5b	3.19±0.06b	0.98±0.03b	0.78±0.04bc	2.10±0.07bc	0.84±0.01bcd	0.39±0.02bc
	10	1.92±0.06b	2.13±0.1b	3.80±0.4bc	8.64±0.6a	2.94±0.4bc	0.97±0.02b	1.01±0.05a	2.52±0.06b	1.22±0.02a	0.50±0.01a
	25	1.54±0.05b	1.57±0.07cd	2.46±0.2c	8.43±0.6a	2.92±0.03bc	0.74±0.03c	0.97±0.03b	1.86±0.07c	0.80±0.02bcd	0.40±0.01 bc

Table 2.2. Effect of NaCl and glycine betaine (GB) on amino acid contents in lettuce leaves (ng·μg^{-1}). Data followed by a different letter were significantly different ($p \leq 0.05$) according to Duncan's Multiple Range Test. Data are represented as means ± SD from four replicates

NaCl mM	GB mM	Valine	Methionine	Tryptophan	Phenylalanine	Isoleucine	Leucine	Lysine	Hydroxyproline	Sarcosine	Proline
0	0	0.26±0.01b	0.56±0.02bc	0.60±0.0bc	1.21±0.05ab	0.77±0.01ab	1.14±0.07 bc	1.21±0.07b	1.09±0.04bc	1.99±0.08bc	0.04±0.0c
	5	0.19±0.01d	0.47±0.02c	0.37±0.01d	0.99±0.05c	0.66±0.01bc	0.75±0.08 d	0.86±0.02c	0.62±0.02c	1.74±0.08d	0.03±0.0c
	10	0.23±0.01bc	0.55±0.02bc	0.45±0.01cd	1.20±0.07ab	0.72±0.01abc	0.99±0.07 c	1.05±0.02bc	0.69±0.03c	1.99±0.05bc	0.07±0.0b
	25	0.22±0.01bcd	0.56±0.01bc	0.45±0.01cd	1.19±0.07ab	0.65±0.02c	0.95±0.06 cd	1.00±0.04bc	0.76±0.04c	1.88±0.09cd	0.04±0.0c
100	0	0.30±0.02a	0.93±0.01a	0.80±0.02a	1.34±0.05a	0.82±0.02a	1.44±0.07 a	1.47±0.04a	1.99±0.05a	2.30±0.08a	0.08±0.0b
	5	0.24±0.01bc	0.68±0.03b	0.65±0.02ab	1.15±0.03b	0.69±0.02bc	1.16±0.06 bc	1.22±0.05b	1.52±0.05ab	2.06±0.08bc	0.11±0.0a
	10	0.29±0.02a	0.65±0.02b	0.52±0.01bcd	1.32±0.02a	0.82±0.03a	1.35±0.05 ab	1.22±0.05b	1.56±0.054ab	2.15±0.04ab	0.08±0.0ab
	25	0.21±0.01cd	0.49±0.01c	0.55±0.01bc	1.15±0.02 b	0.75±0.04abc	1.08±0.05 c	1.13±0.06b	1.08±0.04bc	1.89±0.07cd	0.11±0.0a

Table 3. Effect of NaCl and glycine betaine (GB) on superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX) activity, total phenolic, and total antioxidant contents in lettuce leaves. Data followed by a different letter were significantly different ($p \leq 0.05$) according to Duncan's Multiple Range Test. Data are represented as means ± SD from four replicates

NaCl mM	GB mM	CAT (EU·gr^{-1} leaf)	POD (EU·gr^{-1} leaf)	SOD (EU·gr^{-1}eaf)	APX (EU·gr^{-1} leaf)	Total Phenolic (mg GA·g^{-1} FW)	Total Antioxidant content (μmol TE·g^{-1} FW)
0	0	508.98 ± 10.50 c	80.75 ± 4.00 b	118.80 ± 15.00 g	4.92 ± 0.70 d	2.74 ± 1.50 b	3.947 ± 0.34 a
	5	498.25 ± 13.00 c	97.20 ± 6.00 b	228.79 ± 4.52 b	8.52 ± 0.65 d	3.34 ± 1.65 b	3.238 ± 0.24 b
	10	488.59 ± 8.00 c	68.41 ± 2.00 bc	189.79 ± 22.00cd	9.43 ± 0.73 d	3.36 ± 1.44 b	3.127 ± 0.28 bc
	25	501.81 ± 9.50 c	45.93 ± 1.50 c	161.58 ± 20.00de	9.28 ± 0.96 d	4.52 ± 2.04 a	3.860 ± 0.34 a
100	0	826.42 ± 24.00 a	144.42 ± 8.00 a	338.98 ± 23.00 a	53.16 ± 3.00 a	0.78 ± 0.08 d	2.414 ± 0.22 d
	5	533.00 ± 15.00bc	97.64 ± 6.00 b	214.82 ± 5.50 bc	37.24 ± 1.63 b	1.52 ± 0.33 c	2.639 ± 0.22 cd
	10	600.52 ± 17.00 b	97.38 ± 5.00 b	129.09 ± 16.00 ef	18.88 ± 4.73 c	1.37 ± 0.04 cd	2.441 ± 0.25 d
	25	456.84 ± 6.50 c	71.77 ± 3.00 bc	113.45 ± 7.05 g	9.17 ± 2.00 d	1.83 ± 0.14 c	2.832 ± 0.23 bc

al., 2012). Furthermore, exogenously applied GB easily penetrates leaves and is carried to other organs, which may contribute to improved stress tolerance (Ashraf and Foolad, 2007).

In this study, with 100 mM NaCl in the growth medium, the contents of APX, SOD, POD, and CAT increased. Reactive oxygen species cause oxidative damage to membrane lipids and proteins (Molassiotis et al., 2006). Under saline conditions, plants produce enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX). CAT and APX decrease the oxidative damage in plants. Under salinity conditions, CAT and POX complement the role of SOD in reducing toxicity caused by salinity (Esfandiari et al., 2007). SOD catalyses the dismutation of superoxide radical anions to hydrogen peroxide and oxygen (Ashraf and Foolad, 2007). Increases in the amount of proline, APOX, POD, SOD, and CAT in the lettuce plants

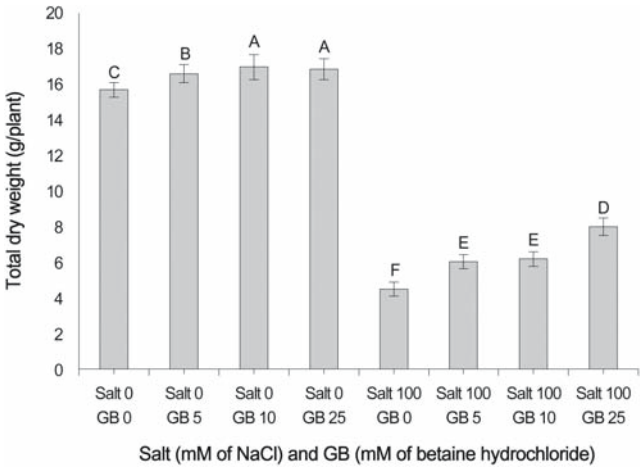

Fig. 1. The effect of NaCl and glycine betaine (GB) applications on total dry weight in lettuce. Vertical bars represent the standard deviation (n = 4). Dry weight contents followed by different letters were significantly different ($p \leq 0.05$) according to Duncan's Multiple Range Test.

in this study suggested that lettuce has some tolerance to salinity, though its tolerance is moderate because a decrease in DM was observed. The exogenous application of GB did not increase the amount of APX, SOD, POD, and CAT in the lettuce plants grown under salt stress. Contrary to our findings, Hu et al. (2012) found that exogenous applications of GB increased the amount of APX and CAT in ryegrass. These conflicting results could be due to different genotypes and environmental conditions.

Amino acids and organic acids stabilize cellular macromolecules, maintain the osmotic balance, and neutralize the free radicals produced under salt stress conditions (Sneha et al., 2013). Alternative respiration is a process that protects cells under environmental stress (Peters et al., 2012). Serine and glycine are the products of alternative respiration. In this study, serine and glycine contents increased with exogenous applications of GB. GB may improve the tolerance of lettuce plants to salinity stress by increasing the levels of serine, glycine, and proline. Some plants can produce GB under abiotic stress (Anjum et al., 2011), but previous studies have shown that lettuce could not produce GB (Roy and Basu, 2009). Our findings revealed that salinity stress caused the lettuce plants to produce some amino acids, such as sarcosine, serine, and glycine, in large amounts (Table 2.1), and these amino acids have a significant role in GB production (Niu et al., 2014).

Salt stress resulted in a decrease in total phenolic and total antioxidant contents in lettuce in compare to the plants that grown in non-salinity condition. However, exogenous GB treatments increased the total antioxidant and total phenolic contents of lettuce under salt stress compared to the non-GB-treated plants. Phenolic compounds in plants are generated via the phenylpropanoid cycle and can be induced by abiotic stresses (Yuan et al., 2010). Phenol production in plants under salt stress is dependent on the salt sensitivity of plants (Kim et al., 2008). The total phenolic content in the lettuce plants treated with 25 mM GB was significantly increased. So exogenous GB applications could increase lettuce tolerance under salt stress by increasing the total phenolic content.

Conclusion

The results of this study have shown that lettuce has a moderate level of tolerance to salinity. Exogenous applications of GB can improve the tolerance of lettuce to abiotic stress. Exogenous GB treatments increased dry matter in lettuce grown in salt-stress conditions compared to the non-GB-treated plants by altering the contents of amino acids, organic acids, total phenolics, and total antioxidant activity. Therefore, growers can increase the yield of lettuce with exogenous applications of glycine betaine under salinity conditions.

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