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Presowing seed treatment with glycine betaine confers NaCl tolerance in quinoa by modulating some physiological processes and antioxidant machinery

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Abstract: Soil salinity threatens the yield and food security worldwide and use of halophytes might be a valid option to cope with devastating salinity effects. The present investigation was accomplished to evaluate the role of glycine betaine (GB) as presowing seed treatment on growth parameters, photosynthetic pigments, gas exchange attributes, enzymatic antioxidants, lipid peroxidation (MDA), hydrogen peroxide (H_2O_2), endogenous GB, total soluble proteins (TSP), and yield in quinoa (*Chenopodium quinoa*) under saline conditions. Ames-13737 (Q7) and PI-634919 (Q9) quinoa accessions were used for this trial. The plants were applied with two salt stress treatments (Control and 450 mM NaCl) after 45 days of sowing. Presowing seed treatments with GB (water, 10 and 20 mM) were given for 12 h. Each treatment replicated four times through completely randomized design. Imposition of salinity triggered a major decrease in growth, photosynthetic pigments, net CO_2 assimilation rate (A), transpiration rate (E), and stomatal conductance (g_s), while the level of lipid peroxidation and activities of superoxide dismutase (SOD) and catalase (CAT) were enhanced. Slight increment in total soluble proteins was observed along with higher endogenous GB in quinoa under salinity. Both levels of GB increased the shoot length, shoot fresh, and dry weights in accession PI-634919 under saline regime. Photosynthetic attributes, E and g_s were increased when 20 mM of GB was applied under saline conditions. Activities of antioxidant enzymes, TSP, endogenous glycine betaine and yield parameters were also increased when GB was applied. Presowing treatment with GB in quinoa plants prominently decreased the MDA and H_2O_2 concentration. The concentration of 10 mM GB enhanced the panicle length while 20 mM GB showed same results for 1000 seed weight and TSP under saline conditions. Overall, quinoa accession Ames-13737 was better as compared to PI-634919 in terms of growth rate, photosynthetic properties, gas exchange parameters, enzymatic antioxidants, and yield.

Key words: Quinoa, glycine betaine, salinity, enzymatic antioxidants, yield

1. Introduction

Global warming induced climatic changes are among the major causes for salinity and drought stresses which ultimately reduce plant growth and yield. Therefore, the study of different physiological processes in plants is of prime importance for improvement in food security (Abdallah et al., 2020). Salinization, as a serious problem, is increasing day by day worldwide and considerably affects the arable lands particularly in dryland environments. Up to the mid of 21st century, 50% arable land will be lost due to salinity as the salinity is increasing about 10% annually (Al-Dakheel and Hussain, 2016). Accumulation of salts changes different physiological mechanisms like gas exchange parameters and chlorophyll fluorescence in crop plants (Shahbaz et al., 2017). Salinity also disturbs the numerous metabolic processes especially CO_2 assimilation rate and reduces the growth in rice (Shahbaz and Zia, 2011) and sunflower (Lalarukh and Shahbaz, 2018). Salinity is a major factor that reduces plant development and yield (Shahbaz et al., 2017; Lalarukh and Shahbaz, 2018).

Oxidative stress generated due to excessive production of reactive oxygen species (ROS) leads to the upregulation of defensive mechanism in plants, i.e., enzymatic (catalase, superoxide dismutase and peroxidase) as well as nonenzymatic (ascorbate, tocopherol and phenolics) antioxidants (Shafiq et al., 2015). Enzymatic antioxidants support the steady development and reclamation of ROS, produced under stress condition. Being a halophyte, quinoa has a diversified range of different salt tolerant mechanisms (Ruiz et al., 2016).

Halophytes are used as a source of food even under high saline conditions. Because of its tolerance to different abiotic stresses and superior nutritional profile, quinoa has a great potential to meet human food resources (Ruiz et al., 2016). Quinoa has strong ability to survive in harsh environmental conditions like salinity and drought.

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Some cultivars can tolerate salinity up to 40 dS/m (Adolf et al., 2012). Quinoa (*Chenopodium quinoa* Willd.) is herbaceous, annual, dicot plant belongs to C_3 crops. It is a facultative halophyte (Razzaghi et al., 2012).

Amino acids and proteins act as catalyst for energy sources and different enzymatic reactions in human body and they are also vital structural components in a biological system (Morrison and Laeger, 2015). Quinoa protein level is comparable to discover in milk and greater than cereals including wheat, rice, and maize. Protein contents of quinoa vary from 12.9% to 16.5% containing all essential amino acids (Meneguetti et al., 2011). As salinity is increasing worldwide, quinoa can cope with food security in future scenario.

Tolerance against salinity can not only be achieved by using specific plant species but also by using shotgun approaches with different chemical compounds. Different chemicals are used for plants under harsh environmental conditions and most of them considerably enhance crop development (Farooq et al., 2009). Use of proline, trehalose, and glycine betaine (GB) as organic osmolytes has been found to be prominent in induction of tolerance against salinity in many crop plants (Athar et al., 2015). Glycine betaine as compatible osmolyte maintains osmotic adjustment in saline conditions (Ashraf and Foolad, 2007), protects different proteins, stabilizes the structure of membranes, helps to protect different organelles from sodium toxicity, and it also protects photosynthetic process by acting as ROS scavenger (Malekzadeh, 2015) in crop plants.

Exogenously applied glycine betaine enhances the chlorophyll contents in safflower (Alasvandyari and Mahdavi, 2018), cucumber and total chlorophyll, carotenoids contents, and growth parameters in maize (Yang and Lu, 2005) under salinity. Use of glycine betaine in saline conditions improved the growth parameters and stomatal conductance in lettuce (Yildirim et al., 2015) and gas exchange parameters in maize (Yang and Lu, 2005). As it has been documented in the literature that glycine betaine has positive role in growth, photosynthetic and gas exchange parameters, enzymatic antioxidants, and yield under salinity in crop plants. So, glycine betaine may effectively ameliorate salinity effects in quinoa plants under saline conditions. The main objective of the current study is to explore the modulation in photosynthetic pigments, gas exchange parameters, enzymatic antioxidants, and yield in quinoa under saline regimes when seeds were primed with glycine betaine for 12 h.

2. Materials and methods

2.1. Plant material

Seeds of two quinoa accessions (variable in salinity tolerance), i.e., Ames-13737 (Q7) and PI-634919 (Q9), were procured from Alternate Crops Lab, Department of Agronomy, University of Agriculture, Faisalabad.

2.2. Experimental layout

A pot trial was executed at Old Botanical Garden, University of Agriculture, Faisalabad (coordinates: 31°25'45" N, 73°04'18" E, altitude 1644 m) to assess the role of glycine betaine on quinoa under salinity. Seeds of both accessions were subjected to presowing seed treatment for 12 h with water (Control), 10 and 20 mM glycine betaine. Before sowing, the seeds were dry in shade area. Plastic pots filled with 9 kg of soil were used for sowing 15 seeds of each accession. The initial ECe of soil was 14 mM. The salinity was maintained in successive time intervals to avoid the sudden salt injury. In first watering, 150 mM salinity was established using NaCl and measured through ECe meter (STARTER 3100). After three days of first salinity application, 300 mM salinity was sustained through second watering by adding NaCl. Third watering was applied to maintain the final 450 mM salinity as the plants were 45 days old since the time of sowing. Four replicates of each treatment were used by following completely randomized design.

2.3. Plant sampling

After three weeks of salinity application, two plants from each pot were uprooted carefully and their shoot fresh weight, shoot dry weight, and shoot length were determined. In addition, fresh leaves were removed for chlorophyll analysis and some of them stored immediately at -20 °C to determine the enzymatic antioxidants activity, malondialdehyde and hydrogen peroxide level, total soluble proteins, and endogenous glycine betaine. Yield parameters were measured at crop maturity.

2.4. Plant analysis and measurements

2.4.1. Morphological parameters

Growth and yield attributes

The length of shoot and panicle was measured by using meter rod while shoot fresh weight, shoot dry weight, and 1000 seed weight were determined with the help of loading balance.

2.4.2. Physiological parameters

Gas exchange parameters

IRGA (Infra-red gas analyzer, Model LCA-4] was used to measure A, E, g_s and C_i from top 3rd fully expanded leaf of each plant. All readings were recorded for 11 h by maintaining the following settings of leaf chamber: atmospheric pressure 98.7 kPa, the flow of air 399.8 mmol m^{-2} s^{-1}, PAR was maximum at leaf surface of 1693 µmol m^{-2} s^{-1}, vapor pressure 6.9 to 8.7 mbar, leaf temperature 29.7 to 31.8 °C, ambient concentration of CO_2 was 346 µmol mol^{-1}, and ambient temperature was 23 to 26.8 °C.

2.4.3. Biochemical parameters

2.4.3.1. Photosynthetic pigments

Measurement of photosynthetic pigments was made using the protocol of Arnon (1949). Five mL acetone (80%) was used to grind the fresh leaf sample (0.1 g). This material was placed in the dark overnight. Spectrophotometer (IRMECO U2020, IRMECO GmbH & Co. KG, Lütjensee, Germany) was used to determine the optical density of extract at 480, 645, and 663 nm using 80% acetone as blank.

Chl. *a* (mg g^{-1} f.wt.) = [12.7(OD663) - 2.69(OD645)] x V/1000 x W

Chl. *b* (mg g^{-1} f.wt.) = [22.9(OD645) - 4.68(OD663)] x V/1000 x W

Total chl. (mg g^{-1} f.wt.) = [20.2(OD645) + 8.02 (OD663)] x V/1000 x W

Carotenoids (mg g^{-1} f.wt.) = [(OD480) + 0.114 (OD663) – 0.638(OD645)] / 2500

2.4.3.2. Total soluble proteins (TSP)

The assessment of TSP was made by pursuing the method of Bradford (1976). Potassium phosphate buffer of pH 7.8 (5 mL) was used to homogenize the leaf sample. A chilled pestle and mortar were used to crush 0.25 g leaf sample and recovered extract was centrifuged at 4 °C at 12,000 rpm. Five mL of Bradford reagent and 100 µL extract were mingled and vortexed for 10 s. Absorbance for each sample was observed at 595 nm through spectrophotometer (IRMECO U2020, Lütjensee, Germany).

2.4.3.3. Glycine betaine (GB)

Fresh (0.25 g) leaf material was macerated in distilled H_2O (5 mL) and extract was centrifuged at 12,000 rpm for 15 min. Five hundred µL of a mixture (1 mL sample + 1 mL 2 N H_2SO_4) was added to test tube and placed in ice for 90 min followed by the respective addition of potassium tri-iodide (200 µL), distilled H_2O (2.8 mL), and 1,2-dichloroethane (6 mL). Finally, absorbance of single layer out of two, was recorded at 365 nm by using spectrophotometer (IRMECO U2020, Lütjensee, Germany).

2.4.3.4. Enzymatic antioxidants activities

Fresh leaf material of 0.25 g was homogenized by using 5 mL potassium phosphate buffer (pH 7.8) in pestle and mortar at 4 °C. The homogenized extract was centrifuged for 15 min at 12,000 rpm. The extracted material was stored at -20 °C to find peroxidase, catalase and superoxide dismutase activities.

2.4.3.4.1. Superoxide dismutase (SOD)

Photoreduction inhibition of Nitroblue tetrazolium (NBT) was utilized to measure SOD activity by following Giannoplitis and Ries (1977). A mixture of sample extract (50 µL), L-methionine (0.1 mL), triton-X (0.1 mL), distilled water (400 µL), riboflavin (50 µL), NBT (50 µL), and potassium phosphate buffer of pH 7.0 (1 mL) were put down under light for 20 min and absorbance was examined by using a spectrophotometer (IRMECO U2020) at 560 nm.

2.4.3.4.2. Catalase (CAT)

Three mL reaction mixture consisting of 1.9 mL potassium phosphate buffer, along with H_2O_2 (1 mL) and sample extract (0.1 mL), was prepared to investigate catalase activity followed by Chance and Maehly (1955). Absorption of this reaction mixture was recorded through spectrophotometer (IRMECO U2020), after a short interval of each 30 s for 120 s at 240 nm.

2.4.3.4.3. Peroxidase (POD)

Chance and Maehly (1955) approach was adopted to find peroxidase activity. For each sample, 1 mL reaction mixture, consisting of H_2O_2 (0.1 mL), sample extract (0.05 mL), guaiacol (0.1 mL), and phosphate buffer (0.75 mL) was prepared. The absorbance of each sample was recorded through spectrophotometer (IRMECO U2020) at 470 nm with 20 s interval for 2 min.

2.4.3.5. Lipid peroxidation

Malondialdehyde (MDA)

Three mL, 1% w/v tri-carboxylic acid was used to mangle 0.25 g fresh leaf material at 4 °C followed by Carmak and Horst (1991). The extract was centrifuged for 15 min at 12,000 rpm. The preparation of 20% TCA was made to prepare 0.5% v/v thiobarbituric acid in it and 4 mL of this solution mixed up with 1 mL supernatant. This mixture was placed in water bath for 90 min followed by its cooling in ice. Tri-carboxylic acid (5%) used as blank to record the absorbance for each sample at 532 and 600 nm through spectrophotometer (IRMECO U2020).

2.4.3.6. Reactive oxygen species

Hydrogen peroxide (H_2O_2)

The protocol of Velikova et al. (2000) was followed to estimate H_2O_2 concentration. The chilled conditions were maintained to grind fresh leaf sample (0.25 g) in 5 mL of 0.1% w/v tri-carboxylic acid, and the resulted extract was centrifuged at 12,000 rpm for 15 min. The mixture of 500 µL supernatant, 500 µL KPO_4 buffer and 1 mL, 1 M potassium iodide were vortexed for 60 s, and absorbance was recorded at 390 nm by using spectrophotometer (IRMECO U2020), using distilled H_2O as blank.

2.5. Statistical analysis

Data were analyzed statistically by using Co-stat software (Steel et al., 1996). 5% probability level was applied for Tukey's HSD to compare significant treatments. Furthermore, data were implied to multivariate analysis (PCA) and correlation matrix by using an R statistical software (R Core Team, 2019) to determine the variability and correlation between studied traits.

3. Results and discussion

3.1. Morphological parameters

Salinity imposition considerably ($P \leq 0.001$) reduced the shoot fresh weight (SFW), shoot dry weight (SDW), and shoot length (SL) in both quinoa accessions i.e. Ames-13737 and PI-634919 (Table 1, Figure 1). These results follow the results of previous studies in many crops like wheat (Kausar and Shahbaz, 2017) and sunflower (Lalarukh and Shahbaz, 2018), and this reduction is credited to excessive ROS production and metabolic disorders produced under stress condition. Ion toxicity and higher osmotic stress is another reason which might be responsible for reduction in growth parameters (Hasanuzzaman et al., 2013). Alteration in biochemical and physiological mechanisms in salinity, leads to lower growth and yield in plants (Waqas et al., 2019). Application of GB as presowing seed treatment significantly ($P \leq 0.001$) increased the SFW, SDW, and SL of quinoa in current study (Table 1, Figure 1). Role of GB in osmotic adjustment might be the basic reason of this increase or/and enhanced rate of photosynthesis (Ashraf and Foolad, 2007). These findings regarding GB enhanced growth under salinity were in parallel to the results as described in safflower (Alasvandyari and Mahdavi, 2018) and okra (Saeed et al., 2016).

Table 1. Mean squares from analysis of variance of data for growth, gas exchange attributes, and chlorophyll contents of quinoa (*Chenopodium quinoa* Willd.) when plant seeds were treated with glycine betaine (10 and 20 mM) for 12 h under saline (450 mM NaCl) conditions.

SOV	df	SFW	SDW	SL	*A*
Accessions (Acs)	1	313.14*	1.90005ns	513.52***	5.406*
Salinity (S)	1	57063.02***	833.08***	73664.6***	37.083***
Glycine betaine (GB)	2	3908.49***	70.431***	766.86***	30.25***
Acs × S	1	997.36***	0.9492ns	168.75*	2.769ns
Acs × GB	2	802.76***	9.5130***	251.11***	20.92***
S × GB	2	860.47***	3.2742ns	699.91***	0.480ns
Acs × S × GB	2	32.565ns	0.08389ns	181.841**	0.029ns
Error	36	70.718	1.0079	23.445	1.106
SOV	df	*E*	*A*/*E*	g_s	C_i
Accessions (Acs)	1	0.00007ns	16.387ns	2.083ns	1805.65ns
Salinity (S)	1	0.023**	473.14***	11102.08***	0.6533ns
Glycine betaine (GB)	2	0.027***	71.471***	3731.25***	702.79ns
Acs × S	1	0.0014ns	17.621ns	1518.75ns	90.75ns
Acs × GB	2	0.0012ns	47.046**	2327.08**	3640.42**
S × GB	2	0.1806***	3.005ns	277.08ns	2060.32*
Acs × S × GB	2	0.0007ns	0.405ns	681.25ns	389.44ns
Error	36	0.0031	5.845	402.08	492.58
SOV	df	C_i/C_a	Chl. *a*	Chl. *b*	Total Chl.
Accessions (Acs)	1	0.0145ns	0.3821***	0.0229***	0.5922***
Salinity (S)	1	0.000005ns	8.5657***	1.0158***	15.481***
Glycine betaine (GB)	2	0.0056ns	0.2854***	0.0823***	0.6696***
Acs × S	1	0.0007ns	0.0898**	0.0292***	0.2216***
Acs × GB	2	0.0293**	0.0849***	0.0135***	0.1645***
S × GB	2	0.0166*	0.0404*	0.0099***	0.0901***
Acs × S × GB	2	0.0031ns	0.1217***	0.0049**	0.1563***
Error	36	0.0039	0.008	0.0009	0.0092

ns = nonsignificant; *, **, *** = significant at 0.05, 0.01 and 0.001, respectively; df = degrees of freedom; SFW = shoot fresh weight; SDW = shoot dry weight; SL = shoot length; Chl. = chlorophyll; *A* = net CO_2 assimilation rate; *E* = transpiration rate; *A*/*E* = water use efficiency; g_s = stomatal conductance; C_i = substomatal CO_2 concentration.

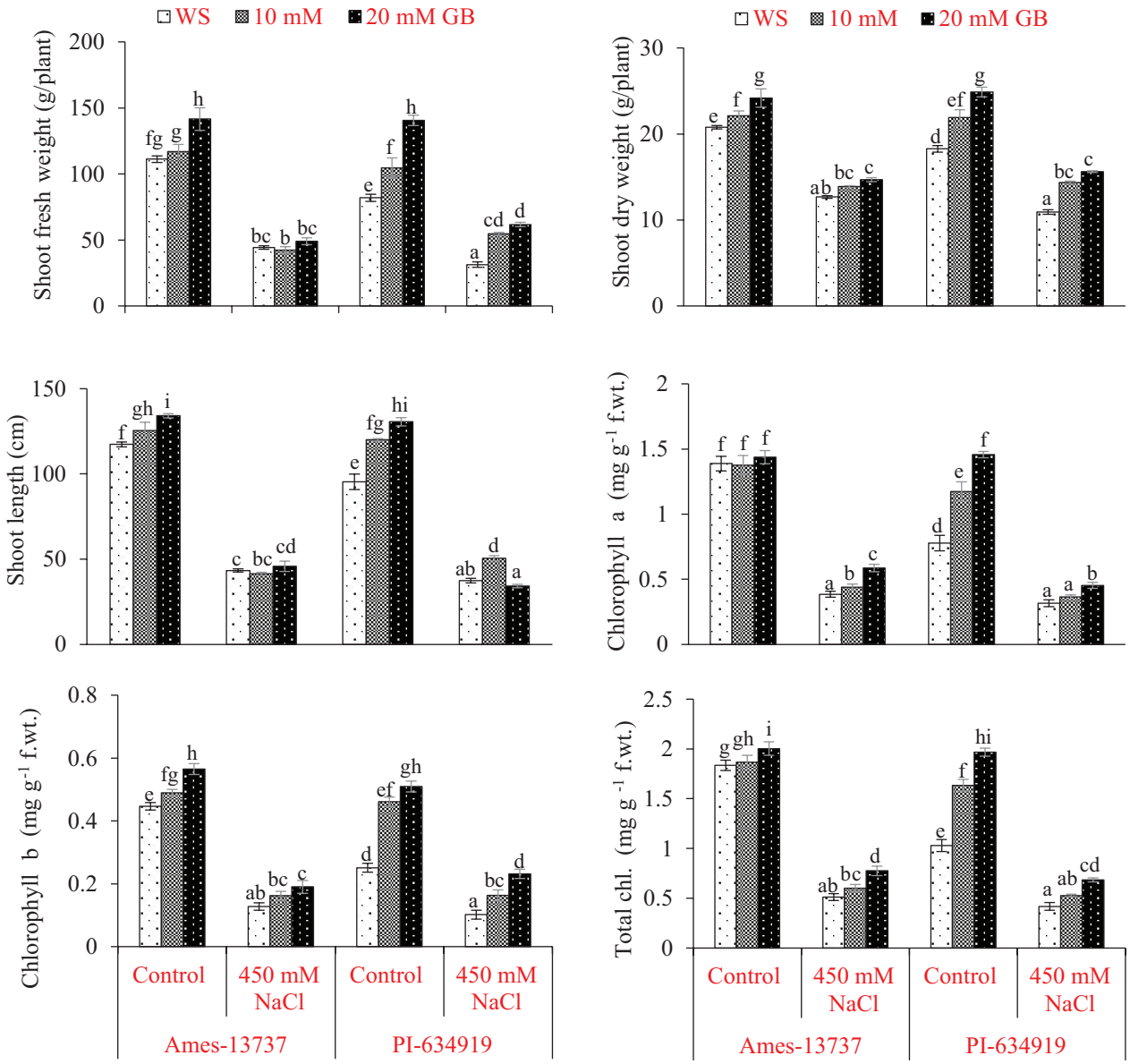

Figure 1. Growth parameters and chlorophyll contents of quinoa (*Chenopodium quinoa* Willd.) when seeds were treated with glycine betaine (10 and 20 mM) for 12 h under salinity (450 mM NaCl). (Mean ± S.E; n = 4). Letters (a–i) showing least significant difference between mean values.

A considerable growth and yield reduction occurred in quinoa at 40 dS m^{-1}, as it acquired its optimal developmental growth at 10-20 dS m^{-1} (Iqbal et al., 2017). Application of 450 mM NaCl showed a remarkable ($P \leq 0.001$) decrease in both panicle length and 1000 seed weight of both quinoa accessions. This reduction in yield parameters was more prominent in accession PI-634919 than Ames-13737 (Table 2, Figure 4). These results favor the past studies in quinoa (Iqbal et al., 2017; Waqas et al., 2019). The major causes of this reduction might be the osmotic and/or ionic stress, interruption in enzymatic antioxidant system, and variation in nutrient uptake (Hussain et al., 2018). The induction of oxidative and ionic stresses lead to reduced photosynthesis which eventually declined the length of panicle and seed yield in quinoa (Waqas et al., 2019). Current study showed a considerable ($P \leq 0.001$) increase in yield when seeds of quinoa plants treated with GB through presowing treatment (Table 2; Figure 4). These finding correlates to the past study in wheat (Kotb and Elhamahmy, 2014) and squash plant (Abdel-Mawgoud, 2017). This increase is attributed to GB application, as GB enhanced the osmotic potential in the cell that ensured the

Table 2. Mean squares from analysis of variance of data for carotenoids, total soluble proteins, enzymatic antioxidants, glycine betaine, malondialdehyde, hydrogen peroxide, and yield of quinoa (*Chenopodium quinoa* Willd.) when plant seeds were treated with glycine betaine (10 and 20 mM) for 12 h under saline (450 mM NaCl) conditions.

SOV	df	Carotenoids	TSP	SOD	POD
Accessions (Acs)	1	0.0017***	0.856***	66.035***	0.005*
Salinity (S)	1	0.0189***	1.393***	86.135***	0.025***
Glycine betaine (GB)	2	0.0013***	1.587***	197.38***	0.069***
Acs × S	1	0.00048***	0.288*	0.016ns	3.911ns
Acs × GB	2	0.0004***	0.005ns	2.666ns	4.335ns
S × GB	2	0.00008ns	0.072ns	0.016ns	0.002ns
Acs × S × GB	2	0.0002***	0.041ns	0.148ns	0.0005ns
Error	36	0.00002	0.062	3.774	0.0007
SOV	df	CAT	GB	MDA	H_2O_2
Accessions (Acs)	1	232.76***	64.091***	1.0086*	0.00004***
Salinity (S)	1	716.88***	61.724***	32.557***	0.0003***
Glycine betaine (GB)	2	844.02***	197.09***	5.5120***	0.0001***
Acs × S	1	5.135ns	2.0101ns	3.295***	0.00001**
Acs × GB	2	32.728*	2.0803ns	0.0133ns	0.000001ns
S × GB	2	65.958***	5.6933**	1.546**	0.00005***
Acs × S × GB	2	3.203ns	3.1058ns	0.0966ns	0.000003ns
Error	36	6.577	0.9564	0.1949	0.000002
SOV	df	Panicle length	1000 seed weight		
Accessions (Acs)	1	4.381ns	20.137***		
Salinity (S)	1	435.01***	44.525***		
Glycine betaine (GB)	2	19.975***	7.848***		
Acs × S	1	5.266ns	1.916*		
Acs × GB	2	5.583ns	0.061ns		
S × GB	2	12.451**	0.181ns		
Acs × S × GB	2	1.121ns	0.021ns		
Error	36	2.179	0.334		

ns = nonsignificant; *, **, *** = significant at 0.05, 0.01 and 0.001, respectively; df = degrees of freedom; TSP = total soluble proteins; SOD = superoxide dismutase; POD = peroxidase and CAT = catalase; GB = glycine betaine; MDA = malondialdehyde; H_2O_2 = hydrogen peroxide.

higher photosynthesis and enhanced plant yield (Estaji et al., 2019). So, application of GB upregulated the growth and yield under salinity.

3.2. Physiological parameters

A considerable reduction in net CO_2 assimilation rate (A), transpiration rate (E), stomatal conductance (g_s), and water use efficiency (A/E) was recorded under 450 mM NaCl stress in both quinoa accessions (Table 1, Figure 2). These findings were in accordance with results obtained from okra (Saeed et al., 2016) and wheat (Kausar and Shahbaz, 2017) etc. Decreased stomatal conductance was also observed by Negi et al. (2014) under salinity. Substomatal CO_2 concentration (C_i) and C_i/C_a ratio remained unchanged under both salinity and glycine betaine application in current investigation (Table 1, Figure 2). These observations were in parallel to the findings in rice (Shahbaz et al. 2017) and sunflower (Lalarukh and Shahbaz, 2018). In wheat, imposition of salinity showed nonsignificant effect regarding C_i (Arfan et al., 2007). Salt stress decreased A as it lowered the K^+ uptake from the soil (Sudhir and Murthy, 2004) or this decrease was because of lower stomatal conductance or toxicity of ions. Transpiration rate reduction under salinity might be due to reduction in guard cell turgidity (Tezara

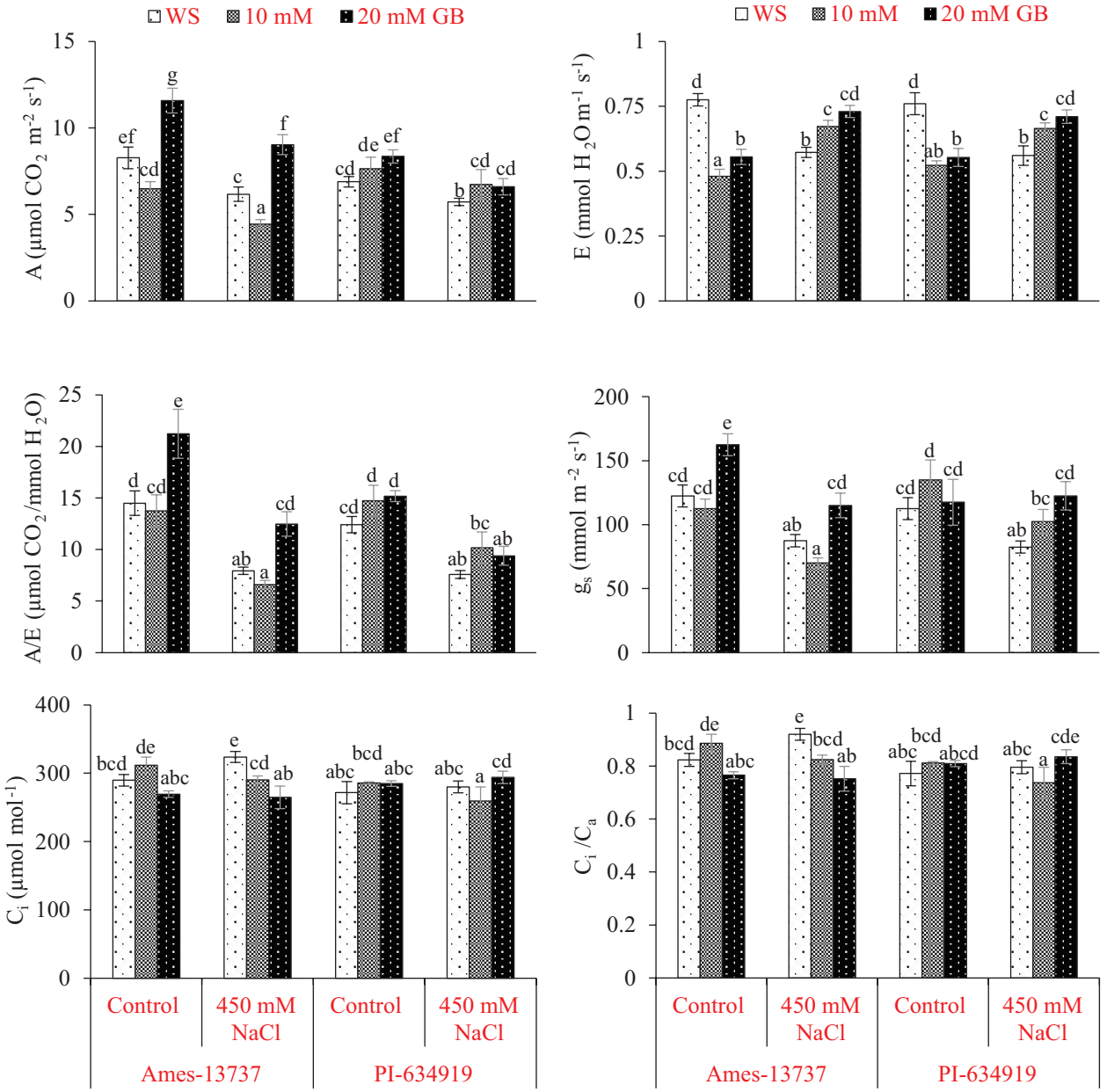

Figure 2. Gas exchange parameters of quinoa (*Chenopodium quinoa* Willd.) when seeds were treated with glycine betaine (10 and 20 mM) for 12 h under salinity (450 mM NaCl). (Mean ± S.E; n = 4). Letters (a-g) showing least significant difference between mean values.

et al., 2002) because of unavailability of mineral nutrients such as K^+, necessary for guard cells turgidity. Reduction in stomatal conduction under salinity is due to imbalance in ion absorption from soil. As K^+ is involved in regulation of the stomatal movement but it is unavailable in saline environment (Sudhir and Murthy, 2004). Application of GB as presowing seed treatment, increased the *A*, *E* and g_s in current study under salinity (Table 1, Figure 2). Increase in *A* might be due to increase in chlorophyll content when GB is applied (Saeed et al., 2016). This increase in *A* has also been associated with increased rate of photosynthesis (Kausar et al., 2014) and stomatal conductance. Enhanced transpiration rate after GB application might be due to organic osmolytes accumulation or increase in uptake of beneficial ions (Saeed et al., 2016) while increased stomatal conductance after glycine betaine application might be due to better osmotic adjustment in plant cells (Saeed et al., 2016).

3.3. Biochemical attributes

Rate of photosynthetic pigments played a vital role in plant growth. In current study, application of 450 mM NaCl considerably ($P \leq 0.001$) decreased the chlorophyll

pigments, i.e. chlorophyll *a* and *b*, and total chlorophyll in both quinoa accessions (Table 1, Figure 1). A clear ($P \leq 0.001$) decrease in carotenoid contents were also observed under salinity (Table 2; Figure 3). Decreased rate of these photosynthetic pigments are in harmony with various crops like maize (Ashraf et al., 2018), tomato (Ahanger et al., 2019), and quinoa (Abdallah et al., 2020). This reduction in photosynthetic pigments occurs because chlorophyll synthesis was affected by Na^+ and Cl^- aggregation, as these ions disturb the different enzymes which are involved in chlorophyll synthesis (Silva et al., 2014). Another reason of this reduction is the enhanced chlorophyllase activity which degrade the chlorophyll molecules (Hasanuzzaman et al., 2014). Reduction in carotenoids content was due to their antioxidant activity as these help in alleviating oxidative stress (Ashraf and Harris, 2013). Application of glycine betaine improved the chlorophyll contents in leaves (Saeed et al., 2016). In our study, glycine betaine (GB) imposition through seed priming increased all the photosynthetic pigments prominently ($P \leq 0.001$) under salinity (Table 1, Figure 1). Previous study for GB application showed the same results of higher chlorophyll

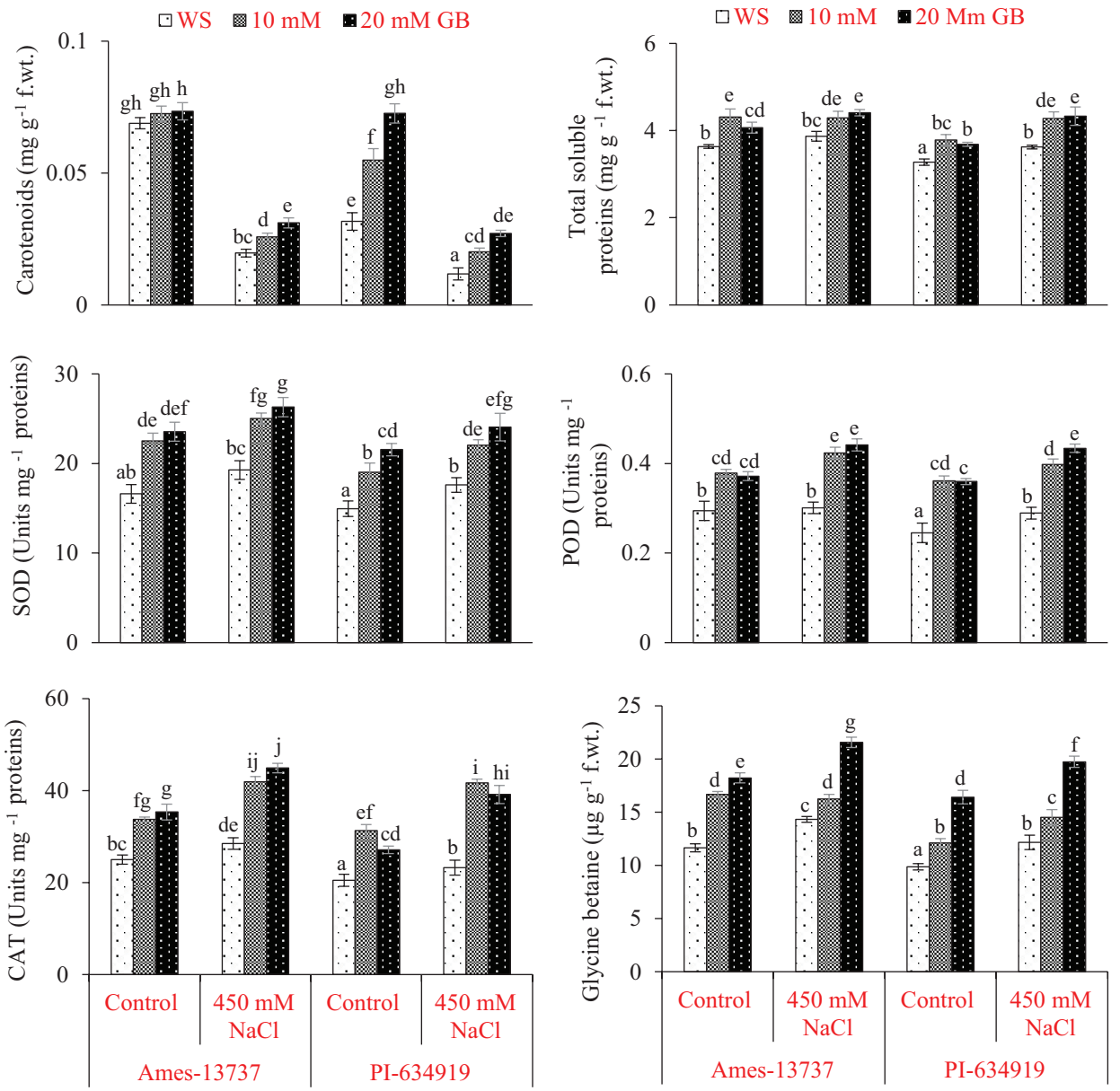

Figure 3. Carotenoids, total soluble proteins, enzymatic antioxidants, and glycine betaine of quinoa (*Chenopodium quinoa* Willd.) when seeds were treated with glycine betaine (10 and 20 mM) for 12 h under salinity (450 mM NaCl). (Mean ± S.E; n = 4). Letters (a-j) showing least significant difference between mean values.

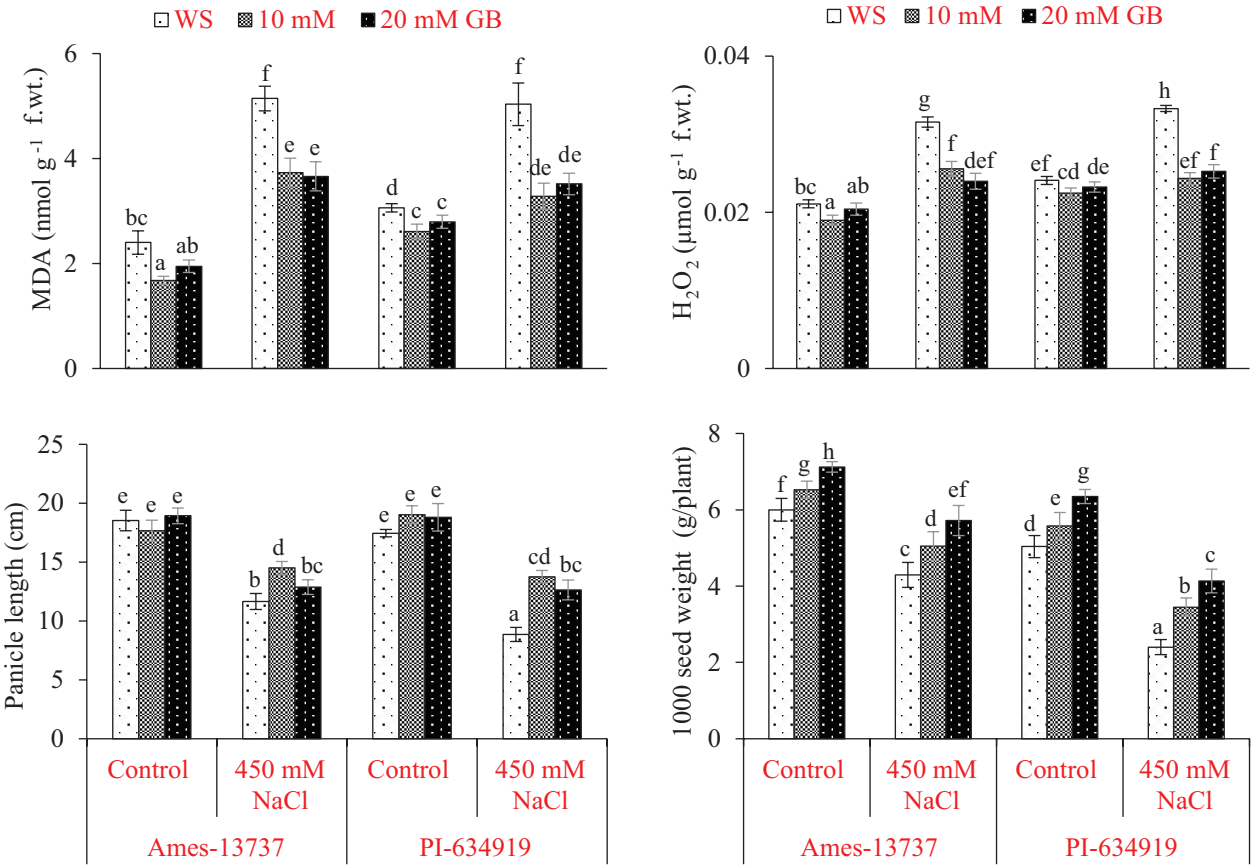

Figure 4. Malondialdehyde, hydrogen peroxide, and yield attributes of quinoa (*Chenopodium quinoa* Willd.) when seeds were treated with glycine betaine (10 and 20 mM) for 12 h under salinity (450 mM NaCl). (Mean ± S.E; n = 4). Letters (a-h) showing least significant difference between mean values.

contents in wheat and total chlorophyll in *Lolium perenne* (Hu et al., 2012). Glycine betaine confers the protection of outer polypeptides in oxygen evolving photosystem II complex against dissociation under higher salt concentration (Chen and Murata, 2008). It looks like that glycine betaine protects the cell membrane integrity and photosynthetic machinery under saline conditions (Alasvandyari and Mahdavi, 2018).

Reactive oxygen species (ROS) production during salinity stress, leads to the oxidative damage in lipids, proteins and DNA, disturbing the cellular metabolism of plants (Gupta and Huang, 2014). Lipid peroxidation is directly associated with MDA accumulation. Oxidative stress under salinity leads to lipid peroxidation and measured as a sign of increased damage (Campo et al., 2014). Malondialdehyde and hydrogen peroxide (H_2O_2) level enhanced significantly ($P \leq 0.001$) under saline conditions in our study (Table 2, Figure 4). Our findings are in accordance with the past studies in rice (Shahbaz et al., 2017) and quinoa (Derbali et al., 2020). Presowing seed treatment with GB prominently ($P \leq 0.001$) reduced the both MDA and H_2O_2 both under nonsaline and saline conditions. Accession Ames-13737 showed lower lipid peroxidation than PI-634919 (Table 2; Figure 4). These observations were previously studied in lettuce (Yildirim et al., 2015) and safflower (Alasvandyari et al., 2017). Glycine betaine application enhanced the lipid stability in different cellular membranes by scavenging ROS (Ashraf and Foolad, 2007). It was also observed the GB reduced the salinity induced lipid peroxidation through stabilizing antioxidant defense system (Hoque et al., 2007).

Survival of plants under salinity, needs a defensive role of enzymatic antioxidants. As the action of these enzymes plays a protective role against ROS, which is produced under various abiotic stresses (Ashraf et al., 2018). Enzymatic as well as nonenzymatic antioxidants both hinder the generation and assembly of hydrogen peroxide (H_2O_2) and different radicals, i.e. superoxide and hydroxyl (Tariq and Shahbaz, 2020). The activities of SOD and CAT significantly ($P \leq 0.001$) increased in current study when quinoa plants were provided with 450 mM NaCl (Table 2, Figure 3) and these results were in agreement with the study of Ahanger et al. (2019) on tomato. Application of GB nullify the shattering effects of salinity through ROS

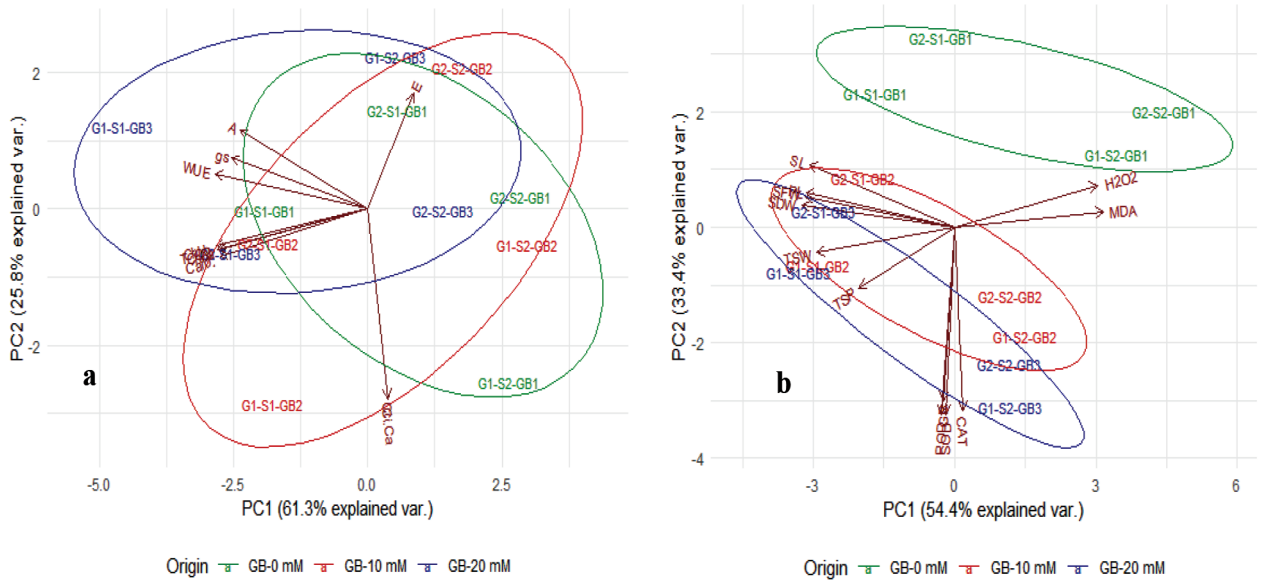

Figure 5. PCA biplot analysis for a) chlorophyll contents and gas exchange attributes b) growth, yield, antioxidant enzymes, reactive oxygen species (H_2O_2), MDA and osmolytes (TSP and GB) of *Chenopodium quinoa* when seeds were treated with glycine betaine (10 and 20 mM) for 12 h under salinity (450 mM NaCl). **Figures legend:** G1 showing Ames-13737, G2. PI-634919, S1- 0 mM NaCl, S2- 450 mM NaCl, GB- 0 mM, GB- 10 mM, GB- 20 mM, *A*- CO_2 assimilation rate, g_s- stomatal conductance, *E*- transpiration rate, C_i- sub stomatal CO_2 concentration, Chl. *a*- chlorophyll *a*, Chl. *b*- chlorophyll *b*, T. Chl- total chlorophyll, Caro- carotenoids, TSP- total soluble proteins, SFW- shoot fresh weight, SDW- shoot dry weight, SL- shoot length, SOD- superoxide dismutase, POD- peroxidase, CAT- catalase, MDA- malondialdehyde, H_2O_2- hydrogen peroxide, TSW- thousand seed weight, PL- panicle length, GB- glycine betaine.

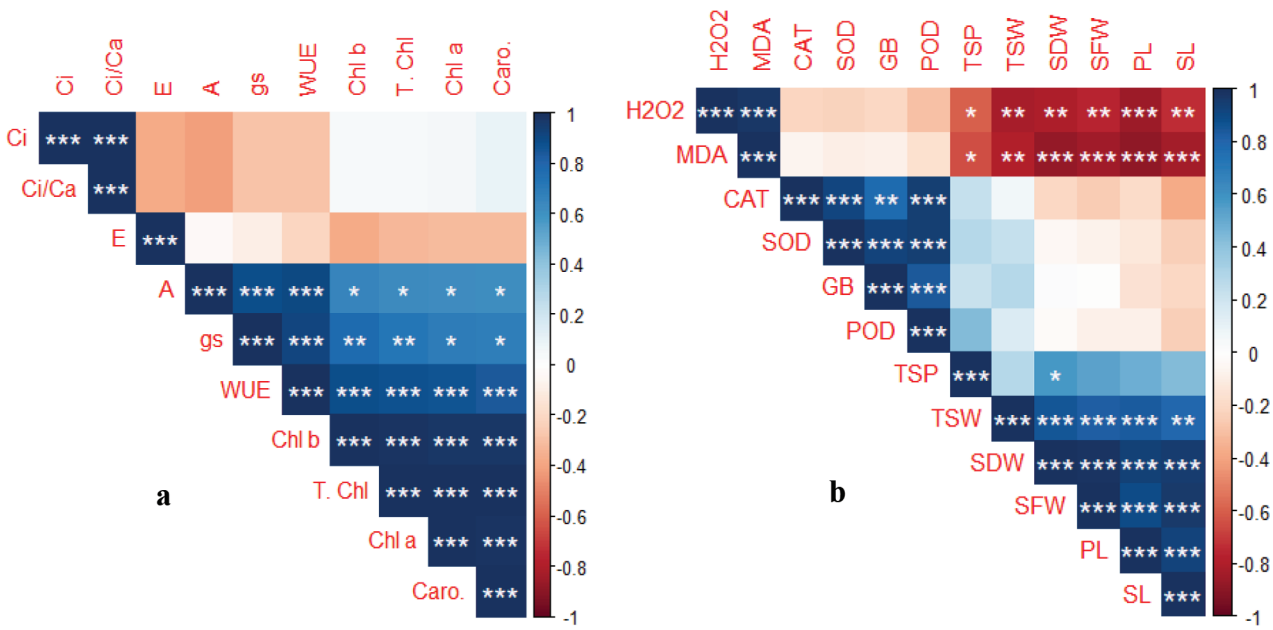

Figure 6. Correlation matrix for a) chlorophyll contents and gas exchange attributes b) growth, yield, antioxidant enzymes, reactive oxygen species (H_2O_2), MDA and osmolytes (TSP and GB) of *Chenopodium quinoa* when seeds were treated with glycine betaine (10 and 20 mM) for 12 h under salinity (450 mM NaCl). **Figures legend:** *A*- CO_2 assimilation rate, g_s- stomatal conductance, *E*- transpiration rate, C_i- sub stomatal CO_2 concentration, Chl. *a*- chlorophyll *a*, Chl. *b*- chlorophyll *b*, T. Chl- total chlorophyll, Caro- carotenoids, TSP- total soluble proteins, SFW- shoot fresh weight, SDW- shoot dry weight, SL- shoot length, SOD- superoxide dismutase, POD- peroxidase, CAT- catalase, MDA- malondialdehyde, H_2O_2- hydrogen peroxide, TSW- thousand seed weight, PL- panicle length, GB- glycine betaine.

scavenging and by shielding the activities of various enzymatic antioxidants (Hoque et al., 2007). In present study, presowing seed treatment with GB increased the activities of SOD, CAT and POD (Table 2, Figure 3). Sofy et al. (2020) had shown that exogenous GB supplementation increase the activities of above-mentioned enzymes in common bean under saline conditions. Glycine betaine invalidates the adversative impacts of oxidative stress produced under salinity through triggering/maintaining enzymatic antioxidants that scavenge the ROS and/or quenching the ROS assembly through an unknown mechanism (Chen and Murata, 2008). They stated that GB probably improved the tolerance against abiotic stresses by triggering the various genes having reactive oxygen species hunting antioxidant enzymes.

Plants improve the bad impacts of salinity induced oxidative and osmotic stresses by stimulating their self-resistant mechanism through compatible osmolytes accumulation in cytosol (El-Esawi et al., 2018). Total soluble proteins (TSP) in leaf exhibited a slight increase under salinity in our study (Table 2, Figure 3), and this increase in TSP followed the previous results in sesame (Tariq and Shahbaz, 2020). This increase in TSP is linked to increased *de novo* synthesis of stress related proteins (Tariq and Shahbaz, 2020). Proteins help in osmotic adjustment under salinity. Further increase in TSP was observed in quinoa when GB was applied as presowing seed treatment in this experiment (Table 2, Figure 3). Enhanced level of total soluble proteins was observed as GB increases the hydration of membranes which protects the protein macromolecules under salinity (Wahid and Close, 2007). Glycine betaine mediated increase in TSP was already observed in stress conditions (Nawaz and Wang, 2020).

A prominent ($P \leq 0.001$) increase was observed in endogenous glycine betaine level when 450 mM NaCl was applied in both quinoa accessions (Table 2, Figure 3). These results meet the findings observed by Estaji et al. (2019). Presowing seed treatment with GB enhanced the endogenous GB level considerably ($P \leq 0.001$) in current investigations. Application of 20 mM GB more consistently increased the endogenous GB level in both accessions under both nonsaline and saline conditions as the highest level was observed in Ames-13737 under saline conditions (Table 2, Figure 3). Endogenous increase in GB level was already observed in wheat (Raza et al., 2007) and eggplant (Abbas et al., 2010). Application of GB quickly enhanced the plant tolerance under stress conditions by increasing the production of osmoregulators including endogenous GB (Estaji et al., 2019). Plants use this osmoregulatory pool to reduce the drastic salinity effects. A large GB production under salinity, protects thylakoid membranes and enhances the stability of mitochondrial enzymes (Genard et al., 1991).

4. Multivariate analysis

Principal component analysis (PCA) results showed variability among genotypes, chlorophyll contents, and gas exchange attributes of *Chenopodium quinoa* when seeds were treated with glycine betaine for 12 h under salinity stress (Figure 5a). The PCA showed total variability of 61.3% and 25.8% (87.1%). The principal components of GB- 0mM were *E* with higher positive eigenvalues more than 1.5 with a significant increase under higher salinity level S2- 450 mM. The major contributors to GB-10 mM were chlorophyll pigments (Chl *a*, Chl *b*, and total chl.) and C_i with higher negative eigenvalues. The major contributor to GB-20 mM were gas exchange traits with higher positive eigenvalues. The explained variations among growth, yield, antioxidants enzymes, reactive oxygen species (H_2O_2), MDA, and osmolytes (TSP and GB) of *Chenopodium quinoa* were 54.4 % and 33.4 % (87.8 %). There was no major contributor assessed in GB- 0 mM. The principal contributors to GB-10 mM were SFW, SDW, SL, TSW, and TSP with higher increase in plant growth traits like biomass and SL. The principal components of GB-20 mM were TSW, GB, and enzymatic antioxidants (Figure 5b).

5. Correlation matrix

Among studied traits and genotypes under saline stress and GB application had shown a significant correlation ($P < 0.05$). Among chlorophyll contents and gas exchange attributes, Chl. *a*, Chl. *b* and total chlorophyll were significantly positively correlated; however, WUE and E were highly negatively correlated ($P < 0.05$) as shown in Figure 6a. Among growth, yield, antioxidants enzymes, reactive oxygen species (H_2O_2), MDA, and osmolytes (TSP and GB) of *Chenopodium quinoa*, POD, SOD, CAT, and GB, as well as SFW and SDW, were significantly positively correlated, while H_2O_2, MDA, and SL were negatively correlated (Figure 6b).

6. Conclusion

Salinity stress decreased the SFW, SDW, SL, chlorophyll pigments, yield, and various gas exchange parameters like *A*, *E*, and g_s, while increased the ROS along with TSP and endogenous GB. Seed priming with GB proved to be very helpful in mitigating salinity effects. Glycine betaine application increased the growth parameters, chlorophyll contents, gas exchange parameters, enzymatic antioxidants, TSP, endogenous GB, and yield in quinoa plant along with a considerable decrease in MDA and H_2O_2. Among both quinoa accessions, Ames-13737 performed better as compared to PI-634919 while in glycine betaine levels, 20 mM performed better as compared to 10 mM GB under saline regimes with respect to growth, gas exchange parameters, enzymatic antioxidants, and yield.

References

Abbas W, Ashraf M, Akram NA (2010). Alleviation of salt-induced adverse effects in eggplant (*Solanum melongena* L.) by glycinebetaine and sugarbeet extracts. Scientia Horticulturae 125: 188-195.

Abdallah MM, El-Sebai TN, Ramadan AAE, El-Bassiouny HMS (2020). Physiological and biochemical role of proline, trehalose, and compost on enhancing salinity tolerance of quinoa plant. Bulletin of the Natural Research Centre 44: 1-13.

Abdel-Mawgoud AMR (2017). Soil and foliar applications of glycine betaine ameliorate salinity effects on squash plants grown under Bahraini conditions. Middle East Journal 6: 315-322.

Adolf VI, Shabala S, Andersen MN, Razzaghi F, Jacobsen SE (2012). Varietal difference of quinoa's tolerance to saline conditions. Plant Soil 357: 117-129.

Ahanger MA, Qin C, Maodong Q, Dong XX, Ahmad P et al. (2019). Spermine application alleviates salinity induced growth and photosynthetic inhibition in *Solanum lycopersicum* by modulating osmolyte and secondary metabolite accumulation and differentially regulating antioxidant metabolism. Plant Physiology and Biochemistry 144: 1-13.

Alasvandyari F, Mahdavi B (2018). Effect of glycine betaine and salinity on photosynthetic pigments and ion concentration of safflower. Desert 23: 265-271.

Alasvandyari, F., B. Mahdavi and S. M. Hosseini. 2017. Glycine betaine affects the antioxidant system and ion accumulation and reduces salinity-induced damage in safflower seedlings. Archives of Biological Sciences 69: 139-147.

Al-Dakheel AJ, Hussain MI (2016). Genotypic variation for salinity tolerance in *Cenchrus ciliaris* L. Frontier in Plant Science 7: 1-12.

Arfan M, Habib RA, Ashraf M (2007). Does exogenous application of salicylic acid through the rooting medium modulate growth and photosynthetic capacity in two differentially adapted spring wheat cultivars under salt stress? Journal of Plant Physiology 164: 685-694.

Arnon DT (1949). Copper enzyme in isolated chloroplasts polyphenoloxidase in *Beta vulgaris*. Plant Physiology 24: 1-15.

Ashraf M, Foolad MR (2007). Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environmental and Experimental Botany 59: 206-216.

Ashraf M, Harris PJC (2013). Photosynthesis under stressful environments: an overview. Photosynthetica 51: 163-190.

Ashraf MA, Akbar A, Parveen A, Rasheed R, Hussain I et al. (2018). Phenological application of selenium differentially improves growth, oxidative defense and ion homeostasis in maize under salinity stress. Plant Physiology and Biochemistry 123: 268-280.

Athar H, Zafar ZU, Ashraf M (2015). Glycinebetaine improved photosynthesis in canola under salt stress: evaluation of chlorophyll fluorescence parameters as potential indicators. Journal of Agronomy and Crop Science 201: 428-442.

Bradford MM (1976). A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72: 248-254.

Carmak I, Horst JH. 1991. Effects of aluminum on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (*Glycine max*). Physiologia Plantarum 83: 463-468.

Campo S, Baldrich P, Messeguer J, Lalanne E, Coca M et al. (2014). Overexpression of a calcium-dependent protein kinase confers salt and drought tolerance in rice by preventing membrane lipid peroxidation. Plant Physiology 165: 688-704.

Chance B, Maehly AC (1955). Assay of catalase and peroxidases. Methods in Enzymology 2: 764-775.

Chen TH, Murata N (2008). Glycinebetaine: an effective protectant against abiotic stress in plants. Trends in Plant Science 13: 499-505.

Derbali W, Goussi R, Koyro HW, Abdelly C, Manaa A (2020). Physiological and biochemical markers for screening salt tolerant quinoa genotypes at early seedling stage. Journal of Plant Interactions 15: 27-38.

El-Esawi MA, Alaraidh IA, Alsahli AA, Alamri SA, Ali HM et al. (2018). *Bacillus firmus* (SW5) augments salt tolerance in soybean (*Glycine max* L.) by modulating root system architecture, antioxidant defense systems and stress-responsive genes expression. Plant Physiology and Biochemistry 132: 375-384.

Estaji A, Kalaji HM, Karimi HR, Roosta HR, Moosavi-Nezhad SM (2019). How glycine betaine induces tolerance of cucumber plants to salinity stress? Photosynthetica 57: 753-761.

Farooq M, Wahid A, Kobayashi N, Fujita D, Basra SMA (2009). Plant drought stress: effects, mechanisms and management. In: Lichtfouse E, Navarrete M, Debaeke P, Véronique S, Alberola C (editors). Sustainable Agriculture. Dordrechti Netherlands: Springer, pp. 153-188.

Genard H, Le-Saos J, Billard JP, Tremolieres A, Boucaud J (1991). Effect of salinity on lipid composition, glycine betaine content and photosynthetic activity in chloroplasts of *Suaeda maritima*. Plant Physiology and Biochemistry 29: 421-427.

Giannopolitis CN, Ries SK (1977). Superoxide dismutase: I. Occurrence in higher plants. Plant Physiology 59: 309-314.

Gupta B, Huang B (2014). Mechanism of salinity tolerance in plants: physiological, biochemical, and molecular characterization. International Journal of Genomics 2014: 1-18.

Hasanuzzaman M, Alam M, Rahman A, Hasanuzzaman M, Nahar K et al. (2014). Exogenous proline and glycine betaine mediated upregulation of antioxidant defense and glyoxalase systems provides better protection against salt-induced oxidative stress in two rice (*Oryza sativa* L.) varieties. BioMed Research International 2014: 1-17.

Hasanuzzaman M, Nahar K, Fujita M (2013). Plant response to salt stress and role of exogenous protectants to mitigate salt-induced damages. In: Ahmad P, Azooz MM, Prasad MNV (editors). Ecophysiology and Responses of Plants Under Salt Stress. New York, NY, USA:Springer, pp. 25-87.

Hoque MA, Banu MNA, Okuma E, Amako K, Nakamura Y et al. (2007). Exogenous proline and glycinebetaine increase NaCl-induced ascorbate glutathione cycle enzyme activities, and proline improves salt tolerance more than glycinebetaine in tobacco Bright Yellow-2 suspension-cultured cells. Journal of Plant Physiology 164: 1457-1468.

Hu LHT, Zhang X, Pang H, Fu J (2012). Exogenous glycine betaine ameliorates the adverse effect of salt stress on perennial ryegrass. Journal of the American Society for Horticultural Science 137: 38-46.

Hussain S, Khalid MF, Saqib M, Ahmad S, Zafar W et al. (2018). Drought tolerance in citrus rootstocks is associated with better antioxidant defense mechanism. Acta Physiologiae Plantarum 40: 1-10.

Iqbal S, Basra SMA, Afzal I, Wahid A (2017). Exploring potential of well adapted quinoa lines for salt tolerance. International Journal of Agriculture and Biology 19: 933-940.

Kausar F, Shahbaz M (2017). Influence of strigolactone (GR24) as a seed treatment on growth, gas exchange and chlorophyll fluorescence of wheat under saline conditions. International Journal of Agriculture and Biology 19: 321-327.

Kausar N, Nawaz K, Hussain K, Bhatti KH, Siddiqi EH et al. (2014). Effect of exogenous application of glycine betaine on growth and gaseous exchange attributes of two maize (*Zea mays* L.) cultivars under saline conditions. World Applied Sciences Journal 29: 1559-1565.

Kotb MA, Elhamahmy MA (2014). Improvement of wheat productivity and their salt tolerance by exogenous glycine betaine application under saline soil condition for long-term. Zagazig Journal of Agricultural Research 41: 1127-1143.

Lalarukh I, Shahbaz M (2018) Alpha-tocopherol induced modulations in morpho-physiological attributes of sunflower (*Helianthus annuus*) when grown under saline environment. International Journal of Agriculture and Biology 20: 661-668.

Malekzadeh P (2015). Influence of exogenous application of glycinebetaine on antioxidative system and growth of salt stressed soybean seedlings (*Glycine max* L.). Physiology and Molecular Biology of Plants 21: 225-232.

Meneguetti QA, Brenzan MA, Batista MR, Bazotte RB, Silva DR (2011). Biological effects of hydrolyzed quinoa extract from seeds of *Chenopodium quinoa* Willd. Journal of Medicinal Food 14: 653-657.

Morrison CD, Laeger T (2015). Protein-dependent regulation of feeding and metabolism. Trends in Endocrinology and Metabolism 26: 256-262.

Nawaz M, Wang Z (2020). Abscisic acid and glycine betaine mediated tolerance mechanisms under drought stress and recovery in *Axonopus compressus*: a new insight. Scientific Reports 10: 1-10.

Negi J, Hashimoto-Sugimoto M, Kusumi K, Iba K (2014). New approaches to the biology of stomatal guard cells. Plant and Cell Phyisology 55: 241-250.

Raza SH, Athar HR, Ashraf M, Hameed A (2007). Glycinebetaine-induced modulation of antioxidant enzymes activities and ion accumulation in two wheat cultivars differing in salt tolerance. Environmental and Experimental Botany 60: 368-376.

Razzaghi F, Ahmadi SH, Jacobsen SE, Jensen CR, Andersen MN (2012). Effects of salinity and soil–drying on radiation use efficiency, water productivity and yield of quinoa (*Chenopodium quinoa* Willd.). Journal of Agronomy and Crop Science 198: 173-184.

Ruiz KB, Biondi S, Martínez EA, Orsini F, Antognoni F et al. (2016). Quinoa-A model crop for understanding salt-tolerance mechanisms in halophytes. Plant Biosystems 150: 357-371.

Saeed HM, Mirza JI, Anjum MA (2016). Glycinebetaine-induced modulations in some biochemical and physiological attributes of okra under salt stress. Pakistan Journal of Botany 48: 2205-2210.

Shafiq S, Akram NA, Ashraf M (2015). Does exogenously-applied trehalose alter oxidative defense system in the edible part of radish (*Raphanus sativus* L.) under water-deficit conditions? Scientia Horticulturae 85: 68-75.

Shahbaz M, Abid A, Masood A, Waraich EA (2017). Foliar-applied trehalose modulates growth, mineral nutrition, photosynthetic ability and oxidative defense system of rice (*Oryza sativa* L.) under saline stress. Journal of Plant Nutrition 40: 585-599.

Shahbaz M, Zia B (2011). Does exogenous application of glycinebetaine through rooting medium alter rice (*Oryza sativa* L.) mineral nutrient status under saline conditions? Journal of Applied Botany and Food Quality 84: 54-60.

Silva CL, Benin G, Bornhofen E, Beche E, Todeschini MH et al. (2014). Nitrogen use efficiency is associated with chlorophyll content in Brazilian spring wheat. Australian Journal of Crop Science 8: 957-964.

Sofy MR, Elhawat N, Alshaal T (2020). Glycine betaine counters salinity stress by maintaining high K^+/Na^+ ratio and antioxidant defense via limiting Na^+ uptake in common bean (*Phaseolus vulgaris* L.). Ecotoxicology and Environmental Safety 200: 110732.

Steel RCD, Torrie JH, Deeky DA (1996). Principles and procedures of statistics a biometric approach. New York, NY, USA: McGraw-Hill Book Company.

Sudhir P, Murthy SDS (2004). Effects of salt stress on basic processes of photosynthesis. Photosynthetica 42: 481-486.

Tariq A, Shahbaz M (2020). Glycinebetaine induced modulation in oxidative defense system and mineral nutrients sesame (*Sesamum indicum* L.) under saline regimes. Pakistan Journal of Botany 52: 775-782.

Tezara W, Mitchell V, Driscoll SP, Lawlor DW (2002). Effects of water deficit and its interaction with CO_2 supply on the biochemistry and physiology of photosynthesis in sunflower. Journal of Experimental Botany 53: 1781-1791.

Velikova V, Yordano I, Edreva A (2000). Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective role of exogenous polyamines. Plant Science 151: 59-66.

Wahid A, Close TJ (2007). Expression of dehydrins under heat stress and their relationship with water relations of sugarcane leaves. Biologia Plantarum 51: 104-109.

Waqas M, Yaninga C, Iqbal H, Shareef M, Hafeez-ur-Rehman et al. (2019). Soil drenching of paclobutrazol: An efficient way to improve quinoa performance under salinity. Physiologia Plantarum 165: 219-231.

Yang X, Lu C (2005) Photosynthesis is improved by exogenous glycinebetaine in salt-stressed maize plants. Physiologia Plantarum 124: 343-352.

Yildirim E, Ekinci M, Turan M, Dursun A, Kul R et al. (2015). Role of glycine betaine in mitigating deleterious effect of salt stress on lettuce (*Lactuca sativa* L.). Archives of Agronomy and Soil Science 61: 1673-1689.