GLYCINEBETAINE INDUCED MODULATION IN OXIDATIVE DEFENSE SYSTEM AND MINERAL NUTRIENTS SESAME (SESAMUM INDICUM L.) UNDER SALINE REGIMES

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Abstract

Salinity is a wide spread environmental constraint that limits the yield and productivity of crops by altering physiological and biochemical processes at the cellular and whole plant level. Various advancements have been made to quench salinity, of which seed priming is one of the cost-effective strategy. Current research was designed to know the salinity effect on sesame seeds primed with glycinebetaine (GB). The sesame cultivars (TS-5 and TH-6) were primed with two levels of GB (0 mM and 25 mM) for 16 h before sowing in plastic pots pre-filled with sand. After three weeks of sowing, plants were salinized (0 mM and 70 mM NaCl) to evaluate the variation in morphological, biochemical and ionic attributes of both sesame cultivars. The trial was planned up in three-factor factorial completely randomized design (CRD) and data were analyzed by co-stat software. Data of both sesame cultivars were collected after four weeks of establishment of saline regimes. Length and fresh/dry weight of root and shoot, total soluble proteins (TSP), GB and root K+ were decreased while catalase (CAT), peroxidase (POD), malondialdehyde (MDA), shoot Na+ and K+ were increased under saline conditions. Hydrogen peroxide (H2O2), POD, CAT, SOD, Total soluble protein (TSP) and GB were increased in GB primed seeds under 70 mM NaCl in both cultivars while fresh/dry weight of root and shoot was increased only in TH-6. Root Na+, root and shoot Ca2+ contents were not affected. Overall, seed priming with GB imparts positive effect on growth, biochemical and ionic status of both sesame cultivars counteracting the salinity toxicity by balancing TSP, CAT, POD, GB, shoot and root K+, MDA and root Na+. It is suggested that TS-5 showed better response under salinity for most of the attributes studied.

Key words: Glycinebetaine, Salinity, Sesame, Malondialdehyde, Catalase.

Introduction

Sesame (Sesamum indicum L.) is the oldest known oil yielding crop typically cultivated in arid and semi-arid regions of the world (Bouremia et al., 2011). Its high nutritional value prevents oxidation, human age-related diseases like heart diseases and cancer; increases antioxidant capacity, decrease the cholesterol level and blood pressure (Elleuch et al., 2011). Its oil is mostly used as medicinal as well as for food purpose as it increases the level of alpha-tocopherol by enhancing vitamin E activity (Amoo et al., 2017). Sesame yield, cultivation area and production have been decreased up to 20% due to different ecological constraints including salinity during last two to three decades (FAO, 2013).

Salinity is a major ecological constraint in agricultural production but its effects are much drastic in arid and semi-arid areas due to low rainfall and high evapotranspiration (Azevedo et al., 2006). According to Food and Agriculture Organization (FAO) survey, 4 million km² world area and almost 6% of all land in Asian sub-continent is affected by salinity. According to world ranking for most severely affected salt stress areas, Pakistan ranks at eight. Salinity may be either due to the destruction of soil texture or long-term malpractices of irrigation by humans (FAO, 2013). Salinity leads to poverty through reduced economic costs by decreasing crop production and by halting the agricultural practices on saline land. In Pakistan, the loss due to unproductivity of large area and decrease in crop yield due to salinity costs 15 billion and 55 billion rupees per annum respectively (Anon., 2006). Crop yield is decreased due to reduction in plant fresh and dry biomass, production of ROS, ionic imbalance, reduced water uptake and damage at the cellular and molecular level (Kalteh et al., 2018). Salinity has more deleterious effects on plants among all stresses (Aazipour et al., 2010). In sesame various researchers reported osmotic and ionic stress as primary salinity effects (Zhang et al., 2019) while nutritional imbalance (Chowdhury et al., 2017), hormonal imbalance (Iqbal & Ashraf, 2013), accumulation of osmoprotectant and oxidative stress (Hartley et al., 2014) are documented as the secondary effects. Both these effects are reported injurious causing growth reduction and ultimately yield drops (Hamayun et al., 2010).

Several approaches are being deployed to increase the salinity tolerance of plants. Pre-sowing treatment of seeds by different compatible solutes is a very safe and cost-effective strategy to encounter salinity problem (Paparella et al., 2015). Compatible solutes or osmoprotectants like glycinebetaine (GB), proline, trehalose, reducing and non-reducing sugars are normally present in plants but their concentration enhanced in stress conditions (Yousuf et al., 2015). Sesame is moderately salinity tolerant plant reported to accumulate high amounts of glycinebetaine under saline environment (Shulav et al., 2008) that protect the plants from stress through different mechanisms such as cellular osmotic adjustment, intact membrane integrity, scavenging of reactive oxygen species and stabilizing enzymes/proteins. Glycinebetaine is also a major source of nitrogen and carbon for plants and help to maintain the structure of membrane in stressed environment (Gilberti et al., 2014).

Therefore, the objective of this trial is to analyze the saline adverse effects on morphological and biochemical attributes of sesame and its mitigation by pre-sowing treatment of GB. Furthermore, comparative behavior of TS-5 and TH-6 cultivars was studied to choose the most...
suitable cultivar for saline agriculture. In this study, it is shown that salinity imposed drastic effects on both cultivars by decreasing plant biomass and increasing MDA and H_{2}O_{2} contents. Glycinebetaine act as efficient growth enhancer by increasing anti-oxidant enzymes in both cultivars. TS-5 cultivar has more capacity to resist saline stress than TH-6.

**Material and Methods**

Ten seeds of two sesame cultivars (TS-5 and TH-6) were taken from Ayub Agricultural Research Institute. The seeds were surface sterilized with Sodium Hypochlorite (NaOCl) for few minutes and then washed with deionized water thrice to remove any contaminations. Prior to sowing, seeds were also treated with GB (25 mM) for 16 h as growth elicitor and water treated seeds were used as control. Washed and dried river sand (10 kg) was filled in each 12 inch plastic buckets and potted with ten seeds of each cultivar under natural light conditions and 37±2°C (maximum/minimum) temperature in the wire house of University of Agriculture, Faisalabad. Seed containing pots were irrigated with 500 mL full strength Hoagland nutrient rich solution in a periodicity of one week. After one week of sowing, pots were thinned by leaving four plants per pot and salinized (non-saline and 70 mM NaCl) by dissolving NaCl in Hoagland solution after three weeks of sowing. Salinity level of 70 mM was maintained in a step-wise manner i.e. initially treated with 50 mM NaCl and then 70 mM NaCl.

**Morphological parameters:** After five weeks of sowing, plants were up-rooted and root/shoot length were measured separately by measuring tape. Fresh weight was taken instantly after up-rooting while dry weight was measured after placing in an oven at 65°C till the constant weight maintained.

**Biochemical parameters:** By following the method of Dhindsa (1981), malondialdehyde (MDA) contents were analyzed. Homogenized mixture of 0.1 g fresh material and 1% trichloroacetic acid (TCA) was centrifuged at 12000 x g for 15 minutes. Supernatant (1 mL) and 0.5% Thioarabutaric acid (1 mL) were mixed with 20% TCA and incubated at 95°C followed by cooling in an ice bath. Reading was taken at two wavelengths i.e., 532 nm and 600 nm on spectrophotometer.

To determine Hydrogen peroxide concentration, 0.1 g fresh leaf material was homogenized with 0.1% of TCA in pre-chilled pestle and mortar. After centrifugation of the mixture, equal amount (0.5 mL) of supernatant and potassium phosphate buffer was poured in 1 mL of potassium iodide. The absorbance of the mixture was taken at 390 nm using water as the blank.

For measuring glycinebetaine, 0.5 g leaf dry material was extracted in 10 mL of 0.5% toluene and kept overnight at 4°C. By following Grieve & Grattan (1983) procedure, a mixture of dry extract and 2 N H_{2}SO_{4} was mixed with 200 μL of potassium tri-iodide solution followed by constant shaking and cooling on ice bath. Then 2.8 mL of pre-chilled distilled H_{2}O and 5 mL of 1-2 di-chloroethane were added in each sample. The resultant mixture was vortexed for 30 s and left the test tubes at room temperature which forms two layers. The organic layer was pipetted for recording its absorbance at 365 nm by spectrophotometer.

To estimate total soluble proteins (TSP), fresh (0.5g) leaf sample was ground in potassium phosphate buffer (pH 7.8), then centrifuged at 12000 x g for 15 minutes and extracted the upper layer in another appendorf (Bradford, 1976). In a test tube 0.1 mL extract was added in 2 mL Bradford reagent and left on the bench for 10 minutes. Optical density was taken with UV-visible spectrophotometer at 595 nm (IRMESCO U2020).

Peroxidase (POD) activity was measured by the method of Chance & Maehly (1955) in which equal amount (100 μL) of 20 mM guaiacol, H_{2}O_{2} and enzyme extract was taken in 2.7 mL of potassium phosphate buffer. Increase in absorbance was monitored by the time lapse of 20 seconds on spectrophotometer.

The catalase (CAT) reaction solution [1.9 mL potassium phosphate buffer and 1 mL H_{2}O_{2}] was dissolved in aliquot (100 μL) of the enzyme extract and decrease in absorbance was noted spectrophotometrically at 240 nm for two minutes by the gap of 30 seconds.

For determining Superoxide dismutase (SOD) activity, Giannopolitis & Ries (1977) protocol was followed. In a test tube 400 μL distilled water, 250 μL Potassium phosphate buffer, 100 μL L-Methionine, 100 μL triton- X, 50 μL of NBT, 50 μL Riboflavin and 50 μL of enzyme extract was taken. Resultant mixture was kept under white light for 15 minutes and absorbance was recorded at 560 nm.

**Ion analysis:** Oven dried samples were dipped in concentrated sulphuric acid by Allen et al., (1976) method and digested on hot plate at 250°C till transparency. This solution was further diluted in distilled water and Na^{+}, K^{+} and Ca^{2+} concentration was assessed by flame photometer.

**Statistical analysis:** An experiment was planned according to 3-factor factorial design. Analysis of variance of data for all parameters with LSD 0.05 was calculated using the Co-STAT software (Steel & Torrie, 1976).

**Results**

**Morphological parameters:** A remarkable (p≤0.001) decrease was recorded in shoot/root fresh weight under salinity stress in both sesame cultivars. TH-6 cultivar responded best both under 70 mM salinity and control conditions. A substantial (p≤0.001) differential response was recorded in response to GB in both cultivars as TH-6 cultivar root fresh biomass was remarkably (p≤0.001) increased by GB seed priming under both salinity levels (Table 1, Fig. 1).

Salinity showed drastic (p≤0.05) decrease in shoot/root dry weight in both cultivars. Plant dry biomass was decreased adversely in TH-6 as compared to TS-5 cultivar. Data for shoot dry weight represented the substantial (p≤0.05) differential response in two cultivars while no difference was found in two cultivars for root dry biomass. Glycinebetaine did not affect root and shoot dry weight (Table 1; Fig. 1).
Shoot length of both sesame cultivars exhibited no change to salinity but pre-sowing seed treatment with GB enhances the shoot length effectively. However, a striking ($p \leq 0.01$) difference in response to GB of both sesame cultivars was observed as TH-6 showed more reduction in shoot length as compared to TS-5 under salt stress. Toxicification with salt showed substantial ($p \leq 0.05$) decline in root length in both sesame cultivars. Combination of salinity with GB was also substantial ($p \leq 0.01$). TS-5 exhibited reduction in 70 mM NaCl which was lessened by 25 mM GB. TH-6 showed decrease in root length however, 25 mM GB increased the root length than non-primed plants under 70 mM NaCl (Table 1; Fig. 1).

**Biochemical parameters:** Malondialdehyde (MDA) concentration was considerably ($p \leq 0.05$) enhanced under salinity in both cultivars. Seed priming with GB decreased the MDA content substantially ($p \leq 0.05$) in both media i.e 0 mM and 70 mM NaCl. A non-significant difference in response had been observed in both cultivars. Combination of cultivar, salinity and GB proved considerable ($p \leq 0.05$) Activity of hydrogen peroxide ($H_2O_2$) remained independent of GB, cultivar and salinity (Table 1, Fig. 2).

Data regarding GB content showed the efficient ($p \leq 0.001$) rise under salinity as compared to control conditions in both cultivars but TH-6 showed more prominent increase under 70 mM salinity. Seed priming with GB remarkably ($p \leq 0.01$) enhanced GB content. Interactions of GB with cultivars and salinity was substantial ($p \leq 0.001$) and ($p \leq 0.05$) respectively. Both cultivars showed substantial ($p \leq 0.001$) differential response to salinity and GB (Table 1; Fig. 2).

Salinity expressively ($p \leq 0.001$) enhanced total soluble proteins in both cultivars. Seed primed with GB represented substantial ($p \leq 0.01$) increase of TSP in salinity stress. Combination of salinity and GB also proved significant ($p \leq 0.05$) (Table 1, Fig. 2).

Enzymatic anti-oxidants such as the activity of catalase (CAT) exhibited efficient ($p \leq 0.001$) rise under salt stress and seed priming with GB in both cultivars. The non-significant differential response was recorded in TS-5 and TH-6. The interaction between GB and salinity also proved significant ($p \leq 0.001$) (Table 1; Fig. 2).

Pre-sowing seed treatment with GB and salinity considerably ($p \leq 0.05$) enhanced peroxidase activity in both cultivars but the effect was more evident in cultivar TH-6 which showed more significant ($p \leq 0.01$) increase. The interaction between cultivar and salinity also proved significant ($p \leq 0.05$) (Table 1; Fig. 2).

Impostion of salinity and seed priming with GB presented the unremarkable effect on superoxide dismutase activity. There was no significant difference in response of two cultiivars to salinity. The combination of salinity with GB presented the significant ($p \leq 0.05$) response (Table 1, Fig. 2).

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**Fig. 1.** Modulation of morphological attributes with and without salt stress in GB pre-treated sesame plants.
Table 1. Mean squares from analyses of variance of data for different growth, biochemical and minerals attributes of sesame plants (grown from non-primed and GB primed seeds) under control and salt stress conditions.

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>df</th>
<th>Shoot fresh wt.</th>
<th>Root fresh wt.</th>
<th>Shoot length</th>
<th>Root length</th>
<th>Shoot dry wt.</th>
<th>Root dry wt.</th>
<th>MDA</th>
<th>H$_2$O$_2$</th>
<th>GB</th>
<th>TSP</th>
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<tr>
<td>Cultivars (Cvs)</td>
<td>1</td>
<td>551.95***</td>
<td>41.06***</td>
<td>2295.0**</td>
<td>0.13ns</td>
<td>12.58*</td>
<td>0.76ns</td>
<td>1.39ns</td>
<td>13.10ns</td>
<td>15297.14***</td>
<td>0.000611ns</td>
<td></td>
</tr>
<tr>
<td>Salinity (S)</td>
<td>1</td>
<td>2180.97***</td>
<td>7.53***</td>
<td>790.03ns</td>
<td>84.5*</td>
<td>2.55ns*</td>
<td>0.34*</td>
<td>36.34*</td>
<td>124.35ns</td>
<td>13823.33***</td>
<td>6.68***</td>
<td></td>
</tr>
<tr>
<td>GB</td>
<td>1</td>
<td>39.87ns</td>
<td>27.47***</td>
<td>140.28*</td>
<td>2ns</td>
<td>0.518ns</td>
<td>0.34ns</td>
<td>28.61*</td>
<td>16.29ns</td>
<td>8502.98**</td>
<td>0.81**</td>
<td></td>
</tr>
<tr>
<td>Cvs × S</td>
<td>1</td>
<td>101.17*</td>
<td>0.087ns</td>
<td>11.28ns</td>
<td>50ns</td>
<td>2.48ns</td>
<td>0.22ns</td>
<td>4.16ns</td>
<td>23.74ns</td>
<td>1029.06ns</td>
<td>0.04ns</td>
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</tr>
<tr>
<td>Cvs × GB</td>
<td>1</td>
<td>530.72***</td>
<td>31.58***</td>
<td>57.78ns</td>
<td>24.5ns</td>
<td>2.83ns</td>
<td>0.30ns</td>
<td>12.5ns</td>
<td>18.59ns</td>
<td>27928.15***</td>
<td>0.0000848ns</td>
<td></td>
</tr>
<tr>
<td>S × GB</td>
<td>1</td>
<td>57.88ns</td>
<td>7.13**</td>
<td>19.53ns</td>
<td>120.12**</td>
<td>0.69ns</td>
<td>0.30ns</td>
<td>0.00ns</td>
<td>101.20ns</td>
<td>4923.20*</td>
<td>0.39*</td>
<td></td>
</tr>
<tr>
<td>Cvs × S × GB</td>
<td>1</td>
<td>65.66*</td>
<td>0.97ns</td>
<td>81.28ns</td>
<td>1.13ns</td>
<td>0.09ns</td>
<td>0.33ns</td>
<td>40.85*</td>
<td>0.021ns</td>
<td>195.68ns</td>
<td>0.00ns</td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>24</td>
<td>347.0</td>
<td>12.23ns</td>
<td>195.36</td>
<td>15.23</td>
<td>66.89</td>
<td>0.43</td>
<td>6.47</td>
<td>61.69</td>
<td>705.10</td>
<td>0.062</td>
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<table>
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<th>SOV</th>
<th>df</th>
<th>df</th>
<th>CAT</th>
<th>POD</th>
<th>SOD</th>
<th>Shoot Na*</th>
<th>Shoot K*</th>
<th>Shoot Ca$^{2+}$</th>
<th>Root Na*</th>
<th>Root K*</th>
<th>Root Ca$^{2+}$</th>
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<td>47.03**</td>
<td>0.70ns</td>
<td>8.71*</td>
<td>12.75ns</td>
<td>5.69ns</td>
<td>187.59***</td>
<td>89.41*</td>
<td>2.25ns</td>
<td></td>
</tr>
<tr>
<td>Salinity (S)</td>
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<td>4.07*</td>
<td>3.68ns</td>
<td>4.42**</td>
<td>117.045**</td>
<td>8.50ns</td>
<td>30.96ns</td>
<td>661.47***</td>
<td>5.69ns</td>
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</tr>
<tr>
<td>GB</td>
<td>1</td>
<td>55.81***</td>
<td>20.70*</td>
<td>0.36ns</td>
<td>0.195ns</td>
<td>124.03**</td>
<td>11.88ns</td>
<td>85.08*</td>
<td>66.61*</td>
<td>0.94ns</td>
<td></td>
</tr>
<tr>
<td>Cvs × S</td>
<td>1</td>
<td>0.05ns</td>
<td>24.43*</td>
<td>2.88ns</td>
<td>3.18ns</td>
<td>38.72ns</td>
<td>122.07**</td>
<td>0.068</td>
<td>125.96**</td>
<td>13.13ns</td>
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</tr>
<tr>
<td>Cvs × GB</td>
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<td>0.21ns</td>
<td>8.50ns</td>
<td>3.05ns</td>
<td>16.10**</td>
<td>30.045ns</td>
<td>41.63ns</td>
<td>0.45ns</td>
<td>17.74ns</td>
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<tr>
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<td>52.07***</td>
<td>0.15ns</td>
<td>6.53*</td>
<td>17.85**</td>
<td>162***</td>
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<td>4.36ns</td>
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<td>0.40ns</td>
<td>0.36ns</td>
<td>4.71ns</td>
<td>7.31*</td>
<td>15.12ns</td>
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<td>5.96ns</td>
<td>71.49*</td>
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<tr>
<td>Error</td>
<td>24</td>
<td>0.57</td>
<td>3.49</td>
<td>1.13</td>
<td>35.64</td>
<td>244.16</td>
<td>11.52</td>
<td>11.83</td>
<td>12</td>
<td>5.044</td>
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*, **, *** = Significant at 0.05, 0.01 and 0.001 levels, respectively
ns = Non-significant
GLYCINEBETAIN INDUCED MODULATION IN SESAME UNDER SALINE REGIMES

Fig. 2. Modulation of biochemical attributes with and without salt stress in GB pre-treated sesame plants.

Fig. 3. Modulation of ionic relations with and without salt stress in GB pre-treated sesame plants.
Ion analysis: Shoot Na⁺ in sesame cultivars were markedly (p≤0.01) enhanced in saline environment while Na⁺ ion concentration did not change in roots under saline regimes. Cultivars TH-6 significantly accumulated more Na⁺ than TS-5. Seed priming with GB exhibited the unremarkable effect on Na⁺ ion accumulation in shoots under saline conditions but significantly (p≤0.05) hampered Na⁺ ion accumulation in roots (Table 1, Fig. 3).

Shoot and root K⁺ ions decreased significantly under salinity stress in both cultivars but the pre-sowing treatment of GB efficiently (p≤0.01 and p≤0.05) enhanced K⁺ ion accumulation. Combination of salinity and GB proved significant (p≤0.001) in shoots. Salinity stress and pre-sowing treatment of GB showed the non-significant response in Ca²⁺ ion accumulation in roots and shoots in sesame cultivars. No remarkable differential response of two cultivars has been recorded to salinity and GB (Table 1, Fig. 3).

Discussion

Root and shoot growths are important physiological parameters to determine stress effects (Bazrafshan & Ehsanzadeh, 2016). In most studies, root and shoot dry biomass showed decreasing trend in response to salinity because of decrease in the overall uptake of water and suite of metabolic activities (Munns & Tester, 2008). Retardation of major physiological, molecular, biochemical processes and ionic and osmotic imbalance are responsible factors for reduction in shoot fresh weight under saline regimes (Kahrizi et al., 2013). In current study, root and shoot dry weight impaired under saline environment in both cultivars. TH-6 showed more decrease as compared to TS-5. Similar results have been obtained by El-nasharty et al., (2019) in wheat, Bazrafshan & Ehsanzadeh, (2016) on sesame, Farissi et al., (2014) on alfalfa and Manaf (2016) in Vigna unguiculata. The non-significant result of GB was recorded in salinity and control conditions on both cultivars except root fresh weight which was contradictory to Khadouri (2015) who reported marked rise in root/shoot dry weight by application of GB. No GB effect on sesame has been studied yet. Shoot length remained uniform under salinity conditions while root length was severely retarded by 70 mM salinity stress in both cultivars which supported the previous study of Celik & Atak (2012) on Nicotiana tabacum and Bekele et al., (2017) on Sesamum indicum. Seed priming with GB mitigated the salinity harmful effects by increasing the root length in both cultivars. These findings are similar to Khadouri (2015) on cowpea (Vigna unguiculata).

Plants have an effective machinery to quench reactive oxygen species produced under different environmental stresses which otherwise lead to disruption of photosynthetic pigments, lipids and other important biomolecules (Farhoudi et al., 2011). Enzymatic and non-enzymatic antioxidant both hamper the production and accumulation of hydrogen peroxide H₂O₂, superoxide•O²⁻ and hydroxyl radicals (OH•) (Abogadallah, 2010). Results regarding POD and CAT showed increase in salinity which was further boosted up by pre-sowing treatment with GB. These antioxidants made the plant tolerant and able to cope up the alarming effects of various biotic and abiotic stresses. These results were comparable to the previous study of Billah et al., (2017) in maize and Koca et al., (2007) in sesame. The concentration of SOD and H₂O₂ did not change in saline regimes. Increased level of MDA content was recorded under salinity as compared to non-saline plants. High MDA contents indicate a high level of membrane damage by lipid peroxidation which is accelerated in stress conditions (Qureshi et al., 2014). Upon application of GB, a sharp reduction was observed in MDA showing boosting of scavenging mechanism. TS-5 showed a great increase in MDA content as compared to TH-6 suggesting it more salt sensitive. MDA contents are an indicator of stress and their increased level is directly linked to lipid peroxidation. Current findings are reinforced by the previous study of Sundaram & Rathinasabapathi (2010) on Arabidopsis thaliana and Bazrafshan & Ehsanzadeh (2016) on sesame.

Leaf total soluble proteins also showed the marked increase in salinity as recorded by Dutta et al., (2015) on wheat and Porcel et al., (2015) on rice and on maize. TSP increase can be correlated with the increased expression of some stress proteins accelerated by the increased solute potential in the saline environment (Dunbar et al., 1997).

Glycinebetaine is an organic osmolyte that mitigates the detrimental effects of salt stress (Farhoudi et al., 2015). A marked decrease in GB contents of sesame was observed upon exposure to salinity in both cultivars. Pre-sowing GB treatment increased GB contents and TS-5 accumulated more in saline regimes as compared to control conditions.

To cope with the salinity-induced water stress, accumulation of inorganic solutes is the most suitable strategy to maintain its turgor which ultimately balances the cell water potential by enhancing water uptake (Taiz & Zeiger, 2010). In this way the ionic imbalance of plant deteriorates (Aktindal, 2019). Study about Na⁺ content showed increased uptake in shoots under salinity stress and it led to ionic imbalance and toxicity. This response was cultivar specific as TH-6 accumulated more Na⁺ than TS-5. Root Na⁺ contents remained independent in saline regimes but showed decreasing trend upon pre-sowing GB treatment. Current results are in accordance to the findings of Zhani et al., (2013) and Chaparzadeh et al., (2014). Ca²⁺ is an important inorganic essential nutrient which also acts as the secondary messenger and initiates the cascade of cellular signalling. Conc. of Ca²⁺ under stress depends upon the sensitivity of plant species and also an organ of the plant where it is measured (Kader & Lindberg, 2010). In current experiment Ca²⁺ level remained independent under salinity and GB treatment in both root and shoot samples. These results are in accordance to Sekmen et al., (2012) in Gypsophila ob lanceolata in which independent trend in Ca²⁺ was observed at 150 mM but concentration was increased at 300 mM. Root K⁺ decreased under salinity conditions in both cultivars but in GB treated plants, its concentration was increased. The same comparative trend was observed in sesame (Bekele et al., 2017).
Conclusion

Salinity stress adversely modulated the plant morpho-physiological pattern by decreasing dry biomass and enhancing of certain stress-related enzymatic compounds. GB is an effective osmoprotectant that further enhance enzymatic antioxidants and K⁺ influx and slower the rate of cellular membrane damage by decreasing MDA. Cultivar TS-5 is more salinity tolerant in most of the attributes studied.

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