MITIGATION OF THE OXIDATIVE EFFECTS OF SALT STRESS ON GROWTH AND PHOTOSYNTHESIS BY THE EXOGENOUS TREATMENT OF TREHALOSE IN *TRIGONELLA FOENUM-GRACUM*

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(Received 17th Dec 2024; accepted 27th Feb 2025)

Abstract. Trehalose (Tre) is an important sugar and acting as osmolyte in stress mitigation. Experiments were performed to analyze the positive impact of Tre (1, 5, 10, and 20 mM) supplementation on growth, antioxidant system, osmolytes, secondary metabolites, and photosynthetic parameters of *Trigonella foenum-graecum* under salt stress. Treatment of 100 mM NaCl declined growth parameters, chlorophyll, and the photosynthetic gas exchange parameters however, Tre supplementation alleviated the decline in the attributes considerably at all levels and evident effects were observed at 10 mM Tre. Treatment of Tre resulted in reduced hydrogen peroxide, electrolyte leakage, and lipid peroxidation in both controls (non-stressed) and NaCl-treated plants. The accumulation of glycine betaine, sugars, proline, and trehalose increased by the supplementation of Tre at all levels and caused further increase when added to NaCl-stressed plants. The decline in relative water content was alleviated by the Tre supplementation. In addition, the application of Tre imparted an apparent augmentation of antioxidant activities. Furthermore, the concentrations of ascorbic acid and reduced glutathione increased by the Tre application under normal and NaCl stressed plants enabling the antioxidant system to eliminate the free radicals more effectively. Salinity increased the total phenols and flavonoids, and Tre application imparted further enhancement in their accumulation. The accumulation of sodium in Tre-supplied plants was reduced compared to NaCl-stressed plants. The effects of Tre were concentration dependent and the most beneficial was 10 mM.
Keywords: *antioxidants, secondary metabolites, osmolytes, photosynthesis, Trigonella foenum-graecum, trehalose, salinity*

Introduction

Salt stress restricts the growth of crop plants and affects their productivity significantly. Worldwide the saline polluted soils are increasing, thus raising concerns over global sustainable food production. Salt stress occurs due to surplus increase of toxic ions like sodium etc. in the soil which results in both ionic and osmotic stress to growing plants (Xiao and Zhou, 2023). Salinity stress exerts a wide range of negative effects on plant development from germination to yield production. Salinity reduces germination, shoot and root growth, mineral uptake, enzyme functioning, photosynthesis etc. therefore influencing the overall metabolic functioning and yield of plants (Ahanger et al., 2019; Barhoumi et al., 2022). It is established now that the basic reasons for growth and yield retardation under salt stress is the oxidative and ionic stress which severely influence the metabolism (Shu et al., 2012; Ahanger and Agarwal, 2017; Arif et al., 2020). The acclimation of plants to salinity results from the better functioning of tolerance pathways which includes the antioxidant mechanism, osmolyte buildup, neutralisation of toxic metabolic products like free radicals, the compartmentation of toxic salt ions like Na into the vacuole and their less absorption from the soil (Choudhary et al., 2023; Chakraborty and Kumari, 2024). The antioxidants can be enzymatic or non-enzymatic which work in

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_37213740

coordination to eliminate the toxic radicals and bring relief to plants by preventing damage to major processes (Gharsallah et al., 2016; Kapoor et al., 2019; Qin et al., 2024). The accumulation of osmolytes is another strategy that helps plants to endure the salt stress and show alleviation of the stress (Kumar et al., 2024). Research evidences are enough that the innate ability of plants to activate the antioxidant functioning and the osmolyte accumulation is associated with their potential to tolerate salt stress (Ahanger and Agarwal, 2017; Parwez et al., 2021; Kosar et al., 2021). Greater functioning of the antioxidant system and the osmolyte synthesis exclusively contribute to redox homeostasis, radical neutralisation, and protection of major cellular strictures under stress (Xiao and Zhou, 2023; Azeem et al., 2023; Kumar et al., 2024). More importantly, osmolyte accumulation maintains the ionic balance by restricting the entry of toxic salts and also contributes to tissue water status (Xiao and Zhou, 2023; Waheed et al., 2024).

Trehalose (Tre) is a non-reducing sugar found in a diverse range of organisms and serves many functions like osmoprotectant, regulation of carbohydrate metabolism, stress mitigation, energy storage/source, etc. (Kosar et al., 2019; Raza et al., 2024). Sugars have a myriad of important roles in several metabolic processes from germination to the reproductive stage throughout the plant life and also share interplay with the phytohormones during signalling (Kosar et al., 2019; Hassan et al., 2023). Trehalose is a compatible solute which gets accumulated in plants under various stresses like salinity, drought, etc. (Chang et al., 2014; Mostofa et al., 2015; Mohanan et al., 2023). Accumulation of Tre in plants provides stability to major metabolic pathways and cellular structures (Kosar et al. 2019; Mohanan et al. 2023). It also imparts stress tolerance by improving the antioxidant and glyoxalase activity, accumulation of other osmolytes, and also reduces the accumulation of toxic ions (Mostofa et al., 2015; Li et al., 2023; Zafar et al., 2024). Trehalose alleviated the reduction in growth, photosynthesis, and gas exchange parameters by maintaining ROS-antioxidant balance in drought stressed rice cultivars (Mohanan et al., 2023). Supplementing Tre to cucumber prevented the drought mediated inhibition of adventitious root formation through maintenance of ROS and phytohormone homeostasis (Li et al., 2014). Therefore, it's evident that Tre has important role in stress tolerance of plants.

Fenugreek (*Trigonella foenum-graecum*) is important legume crop belonging to Fabaceae family which is widely used for culinary and clinical purposes throughout the world. It has medical importance as antidiabetic, anticancerous, expectorant, laxative and also strengthens immunity and mental health (Singh et al., 2022). The nutritionally and pharmaceutically essential bioactive components of fenugreek include trigonelline and diosgenin. Fenugreek is a stress sensitive crop and faces several challenges in natural habitat which make it tough and challenging to get optimal productivity (Parwez et al., 2021). Increased salinity in agricultural soil can be one of the factors that can decline the productivity of fenugreek to appreciable levels. Applying the beneficial metabolites during seedling growth can improve the management practices and also prevent damages done by stresses. Therefore, the efficiency of Tre was investigated in reducing the salt stress damage.

Material and methods

Fenugreek (*Trigonella foenum-graecum*) seeds were sterilized by keeping them in 5% sodium hypochlorite for five minutes. These sterilized seeds were then washed with distilled water and dried using sterile tissue paper. These seeds were then sown at uniform

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depth (3 cm) in pots filled with soil, sand, and vermicompost (4:2:1). All pots were fully saturated by applying full strength nutrient solution. The composition of nutrient solution used was 3 mM KNO_3, 2 mM $Ca(NO_3)_2$, 2 mM $MgSO_4$, 1 mM $NH_4H_3PO_4$, 50 μM KCl, 25 μM H_3BO_4, 2 μM $MnCl_2$, 20 μM $ZnSO_4$, 0.5 μM $CuSO_4$, 0.5 μM $(NH_4)_6Mo_7O_{24}$, and 20 μM Na_2Fe-EDTA (Ahanger et al., 2017). After germinations, the healthy growing fifteen seedlings per pot were maintained and irrigated regularly with nutrient solution for two weeks. Thereafter, half of the pots were irrigated with normal nutrient solution while as other half received Haogland nutrient solution containing NaCl (100 mM) to initiate salt stress. At the same time within both groups, trehalose (Tre) treatments in different concentrations i.e., 0, 1, 5, 10, and 20 mM was also started. Plants were grown for three weeks and monitored regularly. Trehalose was applied once a week. There were three pots (one pot is one replicate) for every treatment and all pots were kept in net house under natural climate conditions. Three weeks (i.e., five weeks old) after the salinity and Tre treatments were started, plants were used for analysing the different physiological and biochemical parameters using standard protocols.

Morphological and growth characteristics

The measurement of plant height was done using a scale while as the fresh weight was noted immediately after harvesting the plants. The plant tissue was dried in oven which was set at 60 ^oC for 48 hours and the dry weight was taken.

Determination of chlorophylls, carotenoids, and photosynthetic parameters

The chlorophyll pigments were extracted by macerating the fresh leaf in acetone by using a pestle and mortar. Homogenate was centrifuged for 20 min at 3000g and the supernatant was read at 480, 645, and 663 nm against blank acetone using spectrophotometer (Beckman 640 D, USA) (Arnon, 1949). The portable photosynthetic machineLi-6400 (LI-COR Inc., USA) was used to measure the photosynthetic parameters like net photosynthesis, transpiration, intercellular CO_2 concentration, and stomatal conductance.

Measurement of the activities of antioxidants

Fresh 2.0 g leaves from each treatment were macerated in ice cold 100 mM extraction buffer (pH 7.8) which contained 1% polyvinyl pyrolidine, 1 mM of each PMSF and EDTA. After centrifuging the extract at 15,000 g for 10minutes at 4^oC, the supernatant was used for measuring the activity of antioxidant enzymes. The content of protein in supernatant was estimated according to Lowry et al. (1951).

The activity of guaiacol peroxidase (POD) was measured by the method of Zhou and Leul (1998) with slight modification. The reaction mixture contained potassium phosphate buffer, 50 μl of the enzyme extract, 1% H_2O_2, and 1% guaiacol. Optical density of the mixture was recorded at 470 nm for 2 minutes.

Method of van Rossum et al. (1997) was used to assay the activity of superoxide dismutase. The reaction mixture components were phosphate buffer, methionine, riboflavin, nitroblue tetrazolium, sodium carbonate, EDTA, and enzyme. The samples were incubated under light for 15 minutes and the absorbance was taken at 560 nm.

The activity of ascorbate peroxidase was measured using the method of Nakano and Asada (1981) and the change in optical density was measured at 290 nm for 2 minutes.

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Method of Carlberg and Mannervik (1975) was adopted to measure the activity of Glutathione reductase and the reaction mixture contained phosphate buffer, oxidised glutathione and NADPH. Change in the optical density was measured at 340 nm for 2 min.

Non-enzymatic antioxidants

The content of ascorbic acid was estimated by the method of Mukherjee and Choudhuri (1983). Plant tissue was extracted in 6% trichloroacetic avid and the extract was centrifuged for 10 minutes. The supernatant was boiled in water bath for 15 minutes after adding thiourea and dinitrophenylhydrazine. After cooling the sample, sulphuric acid (80%) was added and the optical density was taken at 530 nm. Calculation was carried from the standard curve of ascorbic acid. Method described by Ellman (1959) was used for estimation of reduced glutathione. The plant tissue was extracted in phosphate buffer and centrifuged at 10000g for 10 minutes. The supernatant was mixed with 5, 5-dithiobis-2-nitrobenzoic acid and after 10 minutes the optical density of the samples was taken at 412 nm. Standard curve of reduced glutathione was for calculation.

Measurement of lipid peroxidation

Fresh plant tissue was homogenised in 1% trichloroacetic acid and extract was centrifuged for 10 minutes at 10000 g. One mL supernatant was mixed with 4 mL thiobarbituric acid and incubated at 95^{o}C. Samples were cooled and centrifuged again at 5000 g for 5 minutes. The absorbance of the supernatant was taken at 532 and 600 nm according to Heath and Packer (1968).

Estimation of hydrogen peroxide

Method of Velikova et al. (2000) was used for estimation of hydrogen peroxide. Fresh 100 mg leaf was extracted in 0.1% trichloro acetic acid and the homogenate was centrifuged at 10,000 gfor 10 minutes. Thereafter, potassium phosphate buffer (pH 7.0) and the potassium iodide were added to supernatant and the absorbance of mixture was taken at 390 nm.

Measurement of electrolyte leakage

Method described by Dionisio-Sese and Tobita (1998) was used to measure the electrolyte leakage in all treatments. The percent EL of the leaves was calculated using following formulae I:

$$\text{EL}\ (\%) = \frac{\text{EC}_\text{b} - \text{EC}_\text{a}}{\text{EC}_\text{c} - \text{EC}_\text{a}}\ \text{X}\ 100 \qquad \text{(Eq.1)}$$

where;
EC_a = electrical conductivity at room temperature,
EC_b = electrical conductivity at 50 ^{o}C,
EC_c = electrical conductivity at 100 ^{o}C.

Measurement of relative water content and osmolytes

The relative water content (RWC) of leaves was measured according to Smart and Bingham (1974). Among the osmolytes, proline, trehalose, glycine betaine and sugars

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were estimated. For the extraction of soluble sugar content tissue was extracted in ethanol and the homogenate was centrifuged at 5000g for 20 minutes. Then the sugars were determined using anthrone reagent and glucose was used as standard (Shields and Burnet, 1960). Proline in plant tissue was extracted by homogenising the tissue in 3% sulphosalicylic acid and the extract was centrifuged at 3000g. Supernatant, glacial acetic acid and nihydrin reagent were incubated in boiling water bath for one hour and samples were cooled on ice. Proline was separated from cooled samples using toluene. Optical density was taken at 520 nm (Bates et al., 1973).

Content of trehalose was estimated by the method described by Li et al. (2014). The plant tissue was extracted in ethanol and the homogenate was centrifuged at 11500 g for 20 minutes. The collected supernatant was dried and re-suspended in water. Then 200 µL of this solution was mixed with 300 µL of 0.2 N H_2SO_4 and boiled for ten minutes followed by cooling on ice. Thereafter, 200 µL of sodium hydroxide was added and again incubated in boiling water bath. Samples were cooled and anthrone reagent (2 mL) was added and samples were again kept incubated in boiling water bath for ten minutes. Thereafter, samples cooled on ice and the absorbance was recorded at 630 nm. Calculation was carried using standard curve of trehalose.

The content of glycine betaine was estimated using method of Grieve and Grattan (1983). The plant material was extracted in distilled water for 24 hours and 2 N $H_2SO_4$was added to extract. To 0.5 mL aliquot was added cold potassium iodide-iodine reagent and were gently stirred. Samples were kept at 4 ^oC for sixteen hours followed by centrifugation at 10000 g for 15 minutes. Thereafter, deionized water and 1, 2-di-chloroethane were added and the optical density of the lower layer was taken at 365 nm.

Estimation of flavonoids

Method described by Zhishen et al. (1999) was followed for the estimation of flavonoids. Dried 100 mg of the plant sample was homogenised in 3 mL methanol and the homogenate was subjected to centrifugation for ten minutes at 10000 g. Supernatant was collected and 1 mL supernatant was taken from each tube was made to 4 mL using distilled water. Thereafter, 5% $NaNO_2$ and 10% $AlCl_3$were added and allowed to stand for 5 minutes followed by addition of NaOH. Optical density was taken at 510 nm and quercetin was used as standard.

Total phenols

The extraction of phenols was done by macerating dry powdered 100 mg plant tissue in ethanol for three hours at room temperature with constant shaking. Extract was centrifuged at 10000g for 10 minutes and the supernatant was reacted with 1 mL Folin–Ciocalteu reagent. After incubation of three minutes at room temperature, 1 mL of Na_2CO_3 was added to all samples and subsequently incubated at room temperature for 1 h in dark. After that, the optical density was taken at 765 nm and the content of phenol was expressed as gallic acid equivalent (Singleton and Rossi, 1965).

Estimation of sodium

Plant tissue was digested in acid (sulphuric acid, nitric acid and perchloric acid) and digested samples were diluted using distilled water. The content of sodium was estimated using flame photometer.

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Statistical analysis

For performing the statistical analysis of the data one-way analysis of variance (ANOVA) was used followed by Duncan's Multiple Range Test (DMRT). The values are mean (±SE) standard error of three replicates in each group. Different letters on bars signify the significant difference at $P \leq 0.05$.

Results

The salinity stress resulted in decline of 35.76%, 54.00% and 55.09% in shoot length, fresh weight and dry weight. Treatment of Tre caused increase in these attributes with increasing concentration and the highest increase in shoot length, fresh and dry plant weight was reported in 10 mM Tre treatment. When Tre was applied to NaCl stressed plants the decline was mitigated. Compared to NaCl treated plants all concentrations of Tre resulted in mitigation of the decline. Contrary to the NaCl treated plants, highest increase in shoot length, fresh weight and dry weight was 32.80%, 56.74% and 45.52% in plants treated with NaCl + 10 mM Tre. Other concentrations of Tre also mitigated the decline (*Figure 1A-C*).

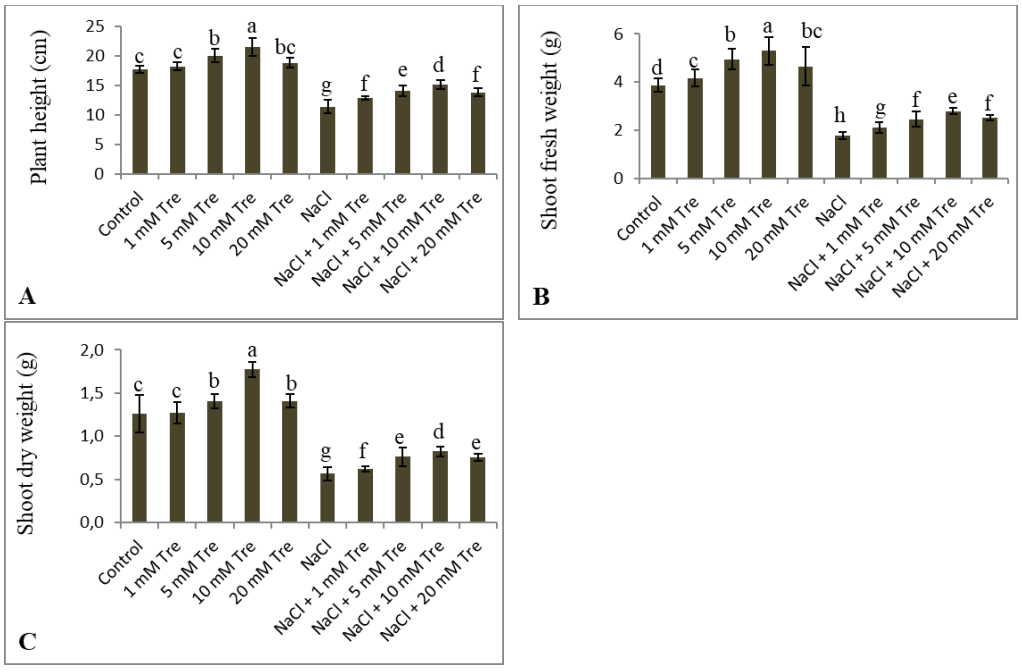

Figure 1. *Effect of different concentrations of trehalose (0, 1, 5, 10 and 20 mM Tre) on (A) shoot length, (B) shoot fresh weight and (C) shoot dry weight of Trigonella foenum-graecum with and without salt stress (100 mM NaCl). Data is mean (±SE) of three replicates and the different letters on bars depict the significant difference at P<0.05*

Sodium (Na) increased by 112.23% in NaCl treated plants however, Tre treatment resulted in reduced accumulation of Na. Contrary to NaCl treated counterparts, the Na declined by 6.92% in NaCl + 1 mM Tre, by 17.24% in NaCl + 5 mM Tre, by 25.29% in NaCl + 10 mM Tre and by 16.33% in NaCl + 20 mM Tre (*Figure 2*).

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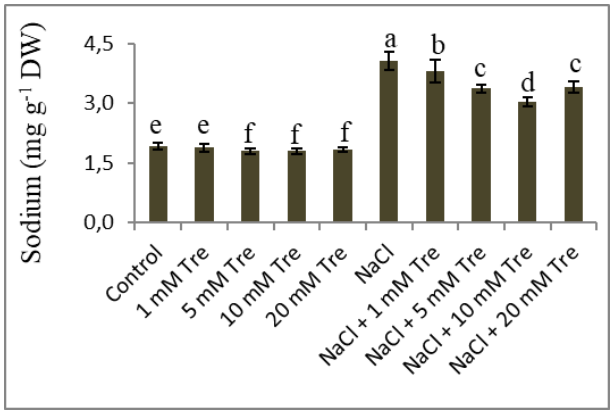

Figure 2. *Effect of different concentrations of trehalose (0, 1, 5, 10 and 20 mM Tre) on the sodium content of Trigonella foenum-graecum with and without salt stress (100 mM NaCl). Data is mean (±SE) of three replicates and the different letters on bars depict the significant difference at P<0.05*

Supplementing the Tre to normal and salinity stressed plants resulted in enhanced accumulation of the osmolytes like glycine betaine, sugars and proline as well as the Tre levels. Compared to control, NaCl stress caused an increase of 95.04%, 127.22%, 132.30% and 48.76% in glycine betaine, sugars, proline and trehalose content. In unstressed plants, glycine betaine and sugars showed highest increase of 71.07% and 48.59% respectively in 10 mM Tre treatment while as proline and trehalose showed highest increase of 69.89% and 155.37% due to 20 mM Tre when compared to control. When the Tre was applied to NaCl stressed plants it also resulted in increased accumulation of osmolytes. The maximum increase in glycine betaine, sugars, proline and trehalose content was 216.52%, 208.52%, 263.50% and 107.43% respectively in NaCl + 10 mM Tre treatment over the control. Increase in their accumulation was concentration dependent and after 10 mM a decline was observed due to 20 mM Tre (*Figure 3A-D*). The RWC reduced by 35.74% due to NaCl while as increased by 3.30%, 4.57%, 5.01% and 0.059% due to 1, 5, 10 and 20 mM Tre. Decline in RWC was mitigated by Tre treatment and compared to NaCl treatment the increase of 8.91% in NaCl + 1 mM Tre, 15.37% in NaCl + 5 mM Tre, 24.78% in NaCl + 10 mM Tre and 9.66% in NaCl + 20 mM Tre was observed in RWC (*Figure 3E*).

Salt stress resulted in increased hydrogen peroxide, electrolyte leakage and lipid peroxidation which were reduced by the treatment of Tre. Compared to control, NaCl increased hydrogen peroxide, electrolyte leakage and lipid peroxidation by 151.72%, 124.23% and 75.38% respectively. When Tre was given to NaCl stressed plants, these parameters reduced and the highest decline of 30.95%, 31.48% and 29.97% in hydrogen peroxide, electrolyte leakage and lipid peroxidation was in NaCl + 10 mM Tre treatment compared to NaCl treatment. In control plants, the treatment of Tre resulted in declined hydrogen peroxide, electrolyte leakage and lipid peroxidation in a concentration dependent manner up to 10 mM, and at 20 mM an increase was observed. The decline in hydrogen peroxide, electrolyte leakage and lipid peroxidation was 29.82%, 29.34% and 35.58% respectively due to 10 mM Tre as compared to the control plants (*Figure 4A-C*).

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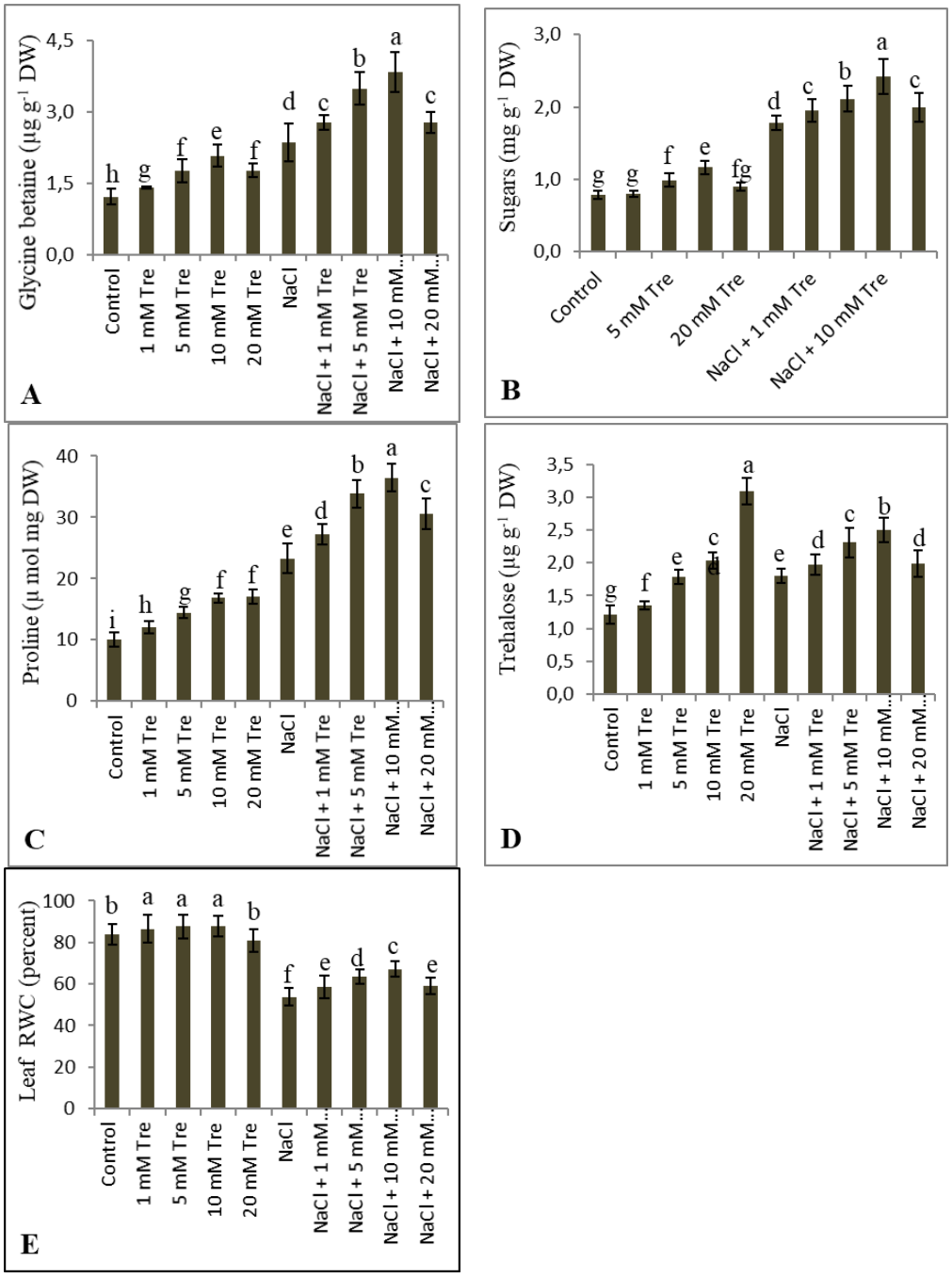

Figure 3. *Effect of different concentrations of trehalose (0, 1, 5, 10 and 20 mM Tre) on (A) glycine betaine, (B) sugars, (C) proline, (D) trehalose and (E) relative water content of Trigonella foenum-graecum with and without salt stress (100 mM NaCl). Data is mean (±SE) of three replicates and the different letters on bars depict the significant difference at P<0.05*

The content of chlorophylls and carotenoid pigments decreased by 55.55% and 33.14% in NaCl treated plants. In normal plants the treatment of Tre caused increase in pigments. As compared to control, chlorophylls and carotenoids increased by 5.55% and 10.37% in 1 mM Tre, by 19.84% and 24.85% in 5 mM Tre, by 32.53% and 36.65% in 10 mM Tre and by 11.90% and 21.97% in 20 mM Tre treated plants. When Tre was supplied to NaCl treated plants it resulted in mitigation of the decline at all concentrations.

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Contrary to NaCl treated plants, highest increase of 53.57% in chlorophylls and 35.26% in carotenoids was observed in plants treated with NaCl + 10 mM Tre (*Figure 5A and B*).

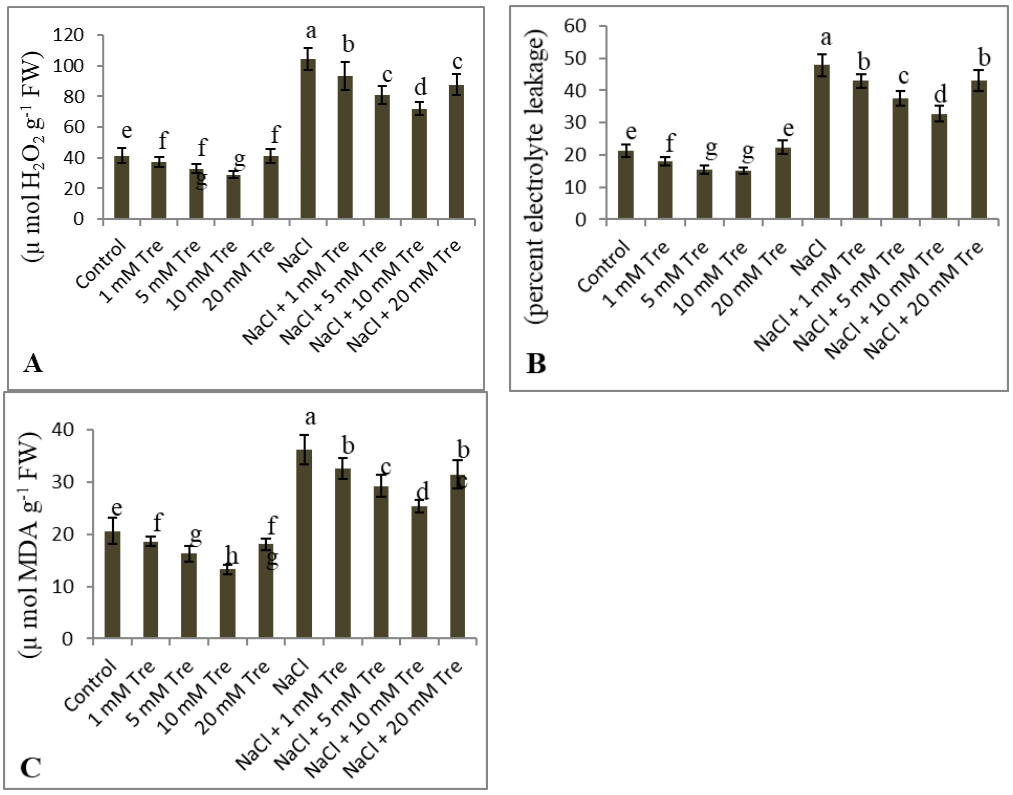

Figure 4. *Effect of different concentrations of trehalose (0, 1, 5, 10 and 20 mM Tre) on (A) hydrogen peroxide, (B) electrolyte leakage and (C) lipid peroxidation of Trigonella foenum-graecum with and without salt stress (100 mM NaCl). Data is mean (±SE) of three replicates and the different letters on bars depict the significant difference at P<0.05*

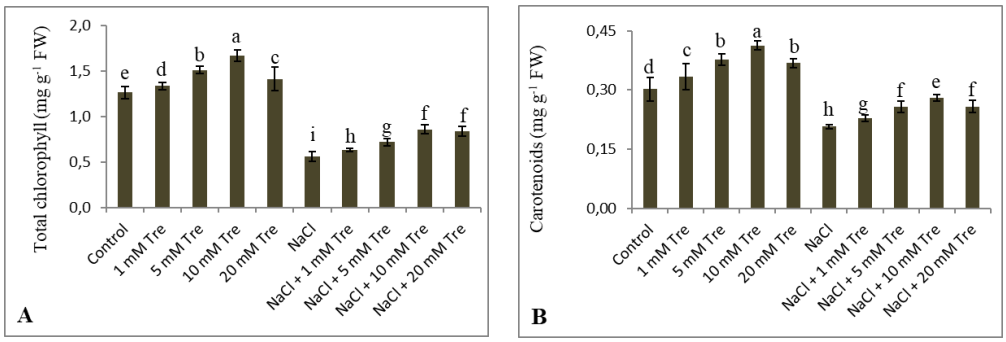

Figure 5. *Effect of different concentrations of trehalose (0, 1, 5, 10 and 20 mM Tre) on (A) total chlorophyll and (B) carotenoids of Trigonella foenum-graecum with and without salt stress (100 mM NaCl). Data is mean (±SE) of three replicates and the different letters on bars depict the significant difference at P<0.05*

Salt stress reduced photosynthesis, transpiration, stomatal conductance and intercellular CO_2 concentration however, Tre treatment resulted in increase in these attributes. The decrease caused by NaCl stress in photosynthesis was 39.33%,

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transpiration was 48.36%, stomatal conductance was 43.66% and intercellular CO_2 was 31.49% as compared to control. Treatment of Tre to NaCl stressed plants imparted mitigation of this decline. Contrary to NaCl stressed counterparts, greater increase in photosynthesis, transpiration, stomatal conductance and intercellular CO_2 concentration was 51.52%, 52.83%, 54.16% and 36.03% respectively observed in plants grown with NaCl + 10 mM Tre. Though the effect was concentration dependent, 10 mM Tre increased the parameters most evidently. In normal plants, the highest increase in photosynthesis, transpiration, stomatal conductance and intercellular CO_2 concentration was 52.58%, 40.55%, 52.62% and 36.63% respectively by the treatment of 10 mM Tre, nevertheless, other concentrations also imparted increase in these parameters (*Figure 6A-D*).

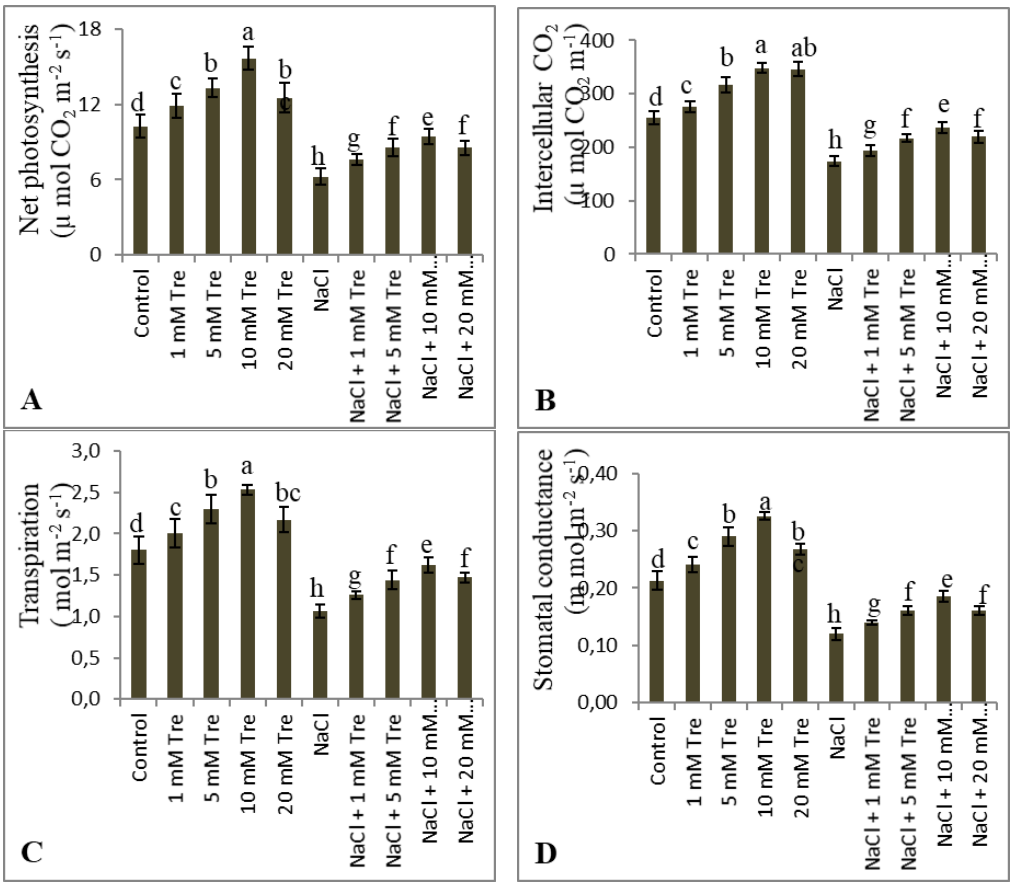

Figure 6. *Effect of different concentrations of trehalose (0, 1, 5, 10 and 20 mM Tre) on (A) net photosynthesis, (B) intercellular CO_2 concentrations, (C) transpiration rate and (D) carotenoids of Trigonella foenum-graecum with and without salt stress (100 mM NaCl). Data is mean (±SE) of three replicates and the different letters on bars depict the significant difference at $P<0.05$*

Highest increment of the activities of enzymes of antioxidant system was exhibited by plants raised with Tre in normal and NaCl stress conditions. NaCl treatment resulted in increase of 110.76% in SOD, 115.46% in POD, 69.49% in APX and 74.86% in GR contrary to the control. Trehalose treatment to NaCl stressed plants resulted in further increase of the activities and reaching the highest increase of 181.53%, 200.21%, 113.06% and 118.26% for SOD, POD, APX and GR in NaCl + 10 mM treated plants as compared to control. In unstressed plants, Tre supplementation increased the activities of

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these enzymes in concentration dependent manner upto 10 mM and at 20 mM decline in their activities started.The highest increase of 38.46%, 58.01%, 34.92% and 30.47% was observed in SOD, POD, APX and GR respectively in 10 mM Tre treated plants as compared to control (*Figure 7A-D*).

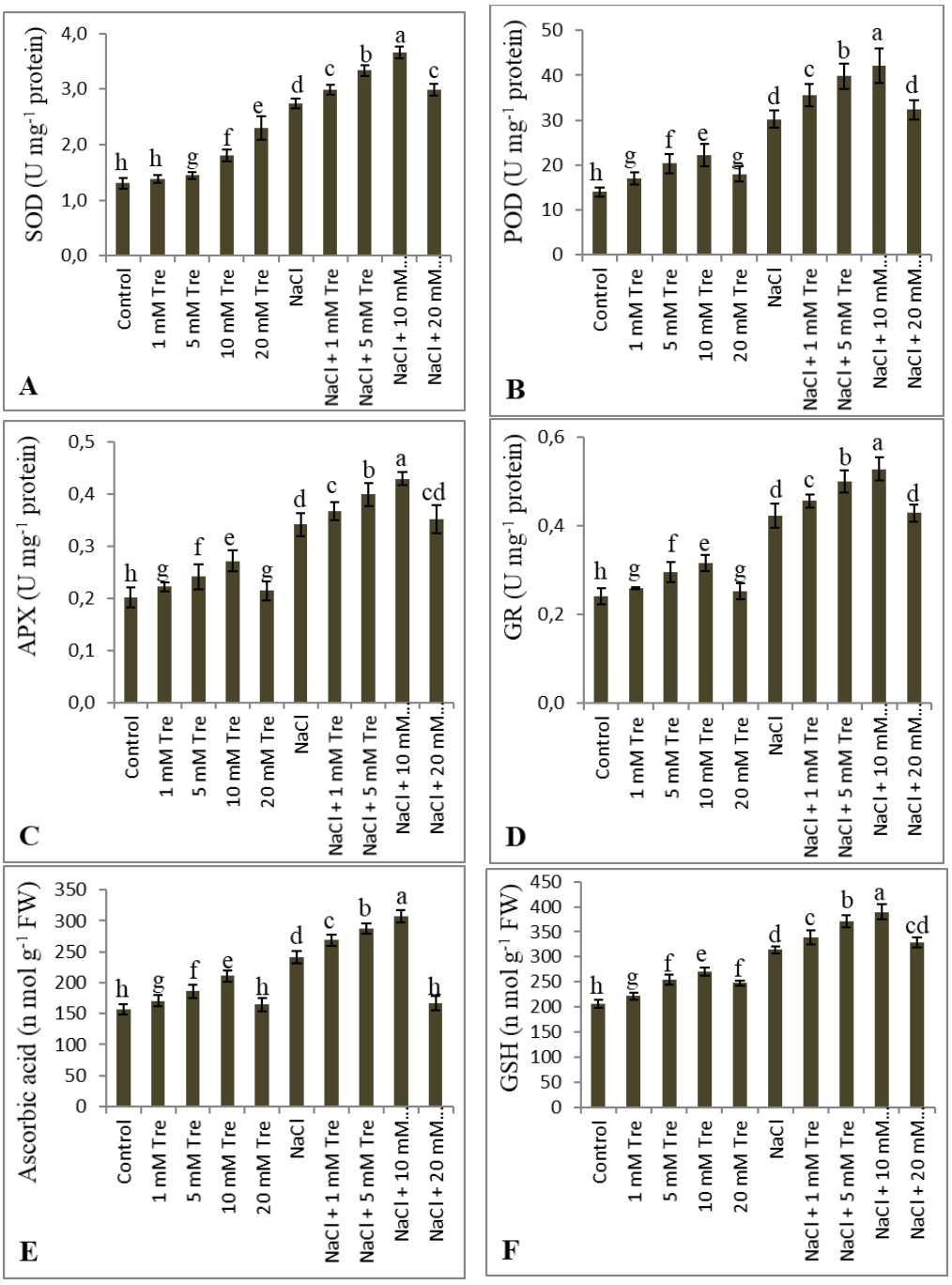

Figure 7. *Effect of different concentrations of trehalose (0, 1, 5, 10 and 20 mM Tre) on the activity of (A) superoxide dismutase (B) guaiacol peroxidase, (C) ascorbate peroxidase, (D) glutathione reductase, (E) ascorbic acid and (F) reduced glutathione of Trigonella foenum-graecum with and without salt stress (100 mM NaCl). Data is mean (±SE) of three replicates and the different letters on bars depict the significant difference at P<0.05*

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Trehalose treatment increased ascorbic acid and reduced glutathione in both growth conditions. Contrary to control, an enhancement of 53.39% and 52.20% was recorded in ascorbic acid and reduced glutathione due to NaCl. Highest increase in ascorbic acid was 94.92% and reduced glutathione was 88.89% in plants grown with NaCl + 10 mM Tre over the control. In normal plants, all concentrations of Tre applied caused increase in ascorbic acid and reduced glutathione although highest values were recorded in 10 mM Tre (*Figure 7E and F*).

Trehalose application resulted in increase of the flavonoids and phenols accumulation. Contrary to control, total flavonoids and total phenols increase by 7.54% and 5.12% in 1 mM Tre, by 17.92% and 8.87% in 5 mM Tre, by 27.35% and 16.05% in 10 mM Tre and by 7.54% and 2.72% in 20 mM Tre applications. Salt stress caused an increase of 49.05% in flavonoids and 67.27% in phenols over the control plants. Trehalose treatment imparted further increase in NaCl stressed plants exhibiting the highest increase of 86.79% and 121.60% in NaCl + 10 mM Tre treatment contrary to control (*Figure 8A and B*).

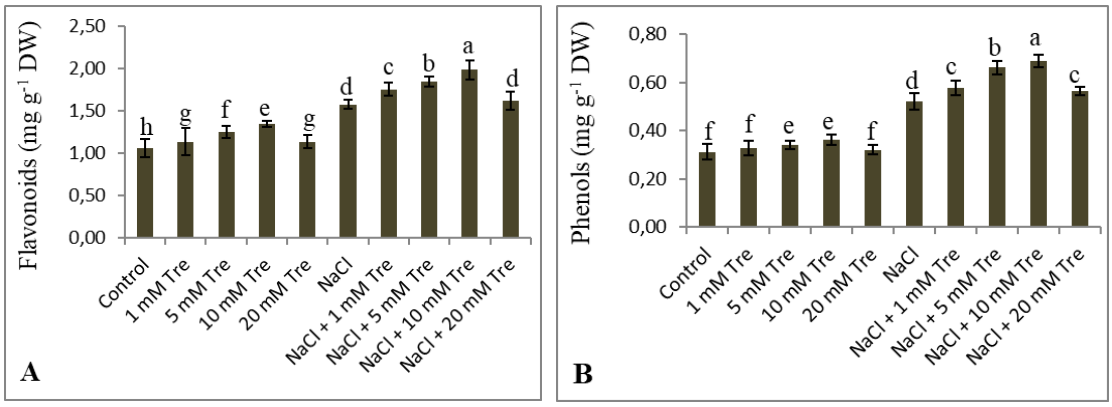

Figure 8. *Effect of different concentrations of trehalose (0, 1, 5, 10 and 20 mM Tre) on the activity of (A) flavonoids and (B) phenols of Trigonella foenum-graecum with and without salt stress (100 mM NaCl). Data is mean (±SE) of three replicates and the different letters on bars depict the significant difference at P<0.05*

Discussion

The salt concentrations are increasing in soil and this result in conversion of agricultural land into unproductive land. This increases the pressure on global food security therefore to adapt new management techniques which can help plants to counter the undesirable effects of excess salts. Accretion of osmolytes is a natural potential of plants to counter the stresses therefore in this study the effect of applied Tre was investigated on some physiological and biochemical parameters. Different concentrations of Tre applied resulted in increased growth of the *Trigonella foenum-graecum*. Salt stress has been reported to reduce the growth of plants by impeding the root growth, water uptake, nutrient assimilation, phytohormone metabolism and cell division and proliferation (Arif et al., 2020). Similar to these results, the decline in growth characteristics by NaCl has been observed by others (Gharsallah et al., 2016; Taibi et al., 2016; Soliman et al., 2020). Increased accumulation of sodium, reduced water content and restricted photosynthesis can be suggested as possible reasons for the growth reductions in salt stressed fenugreek. The exogenous supplementation of Tre was

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affective in increasing the growth and also in mitigating the salinity damage. At 10 mM, Tre has the maximal impact and increased growth and dry weight relatively better than other concentrations. Earlier, Sadak et al. (2019) has also observed mitigation of growth parameters in *Triticum aestivum* by Tre treatment. In rice, treatment of Tre alleviated the decline in shoot length and shoot fresh and dry weight (Abdallah et al., 2016). This improvement in growth parameters by the Tre treatment can be due to increased water content and reduced sodium accumulation. In present study the effect of 10 mM Tre was more apparent as compared to other concentrations. Trehalose application reduced the accumulation of sodium which contributed to lessen the negative effects of salinity. The exclusion and selective uptake of toxic ions like sodium is regulated by the proficient activity of transport proteins and channels which mediate selective uptake of ions (Mansour et al., 2003; Saddhe et al., 2021). External supply of Tre reportedly alleviated the damaging impact of salinity in *Catharanthus roseus* by declining the uptake and build up of Na while as increasing potassium causing significant decline in Na/K ratio. All the concentrations of trehalose used reduced the Na ion accumulation which can also contribute to growth improvement by cutting down the ill effects of excess Na accumulation. In *Citrullus lanatus*, exogenous Tre application has been observed improve K/Na ratio by lessening the Na ion accumulation within tissues (Yuan et al., 2022).

Trehalose itself is an important osmolyte that accumulates in cells so as to prevent the negative effects of adverse growth conditions including stresses. It was observed that exogenous Tre caused substantial increment in the accumulation of tissue compatible metabolites like glycine betaine, sugars and proline. These compatible osmolytes are having very beneficial function in defending cells from the damaging impact of stresses like excess salinity (Kumar et al., 2024; Chakraborty and Kumari, 2024). Osmolytes bring respite to plants from stresses by maintaining cellular turgor, redox homeostasis, eliminating excess accumulated radicals, protecting enzyme functioning, fine tuning physiological mechanisms and crosstalk with phytohormones (Choudhary et al., 2023; Kumar et al., 2024; Chakraborty and Kumari, 2024).The accretion of glycine betaine, proline, sugars and trehalose has been reported to increase in plants grown on excess NaCl (Chang et al., 2014; Mostofa et al., 2015; Ahanger et al., 2019; Soliman et al., 2020; Ferdosi et al., 2022). Similar to the results of this study, trehalose treatment to salt stressed rice increased proline and also the endogenous trehalose accumulation (Mostofa et al., 2015). In *Cucumis sativus*, it has been reported that exogenous Tre triggered the accumulation of other sugars and trehalose resulting in improved radical scavenging and growth under drought stress (Li et al., 2023). Similarly, Razzaq et al. (2024) have also observed increased glycine betaine and proline in *Zea mays* grown on chromium. Mohanan et al. (2023) have also reported increased trehalose accumulation in drought stressed rice after exogenous Tre supplementation reflected in improved chlorophyll and photosynthesis. Increase in the levels of sugars and free amino acids following external Tre supplementation in *Catharanthus roseus* was observed to be associated with improved growth, photosynthetic gas exchange parameters and salt stress mitigation (Chang et al., 2014). The positive influence of this increased osmolyte accumulation due to applied Tre on the growth parameters, chlorophyll synthesis, photosynthesis, and enzyme activity was also evident in this study. Further studies can be very helpful in understanding the role of exogenous Tre in mitigating salt stress in *Trigonella foenum-graecum*. Increased accumulation of the osmolyte compounds in Tre treatments also improved the RWC thereby reflecting in maintenance of cellular water concentrations

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DOI: http://dx.doi.org/10.15666/aeer/2302_37213740

and also protecting the major cellular processes from salinity mediated negative influence (Chang et al., 2014; Sadak, 2019; Samadi et al., 2019; Mohanan et al., 2023).

The external supplementation of Tre accrued the total phenols and the flavonoids in both normal and NaCl treated plants. The plant metabolites regulate several developmental and defence mechanisms including the tolerance to stresses (Kumar et al., 2023). These metabolites act as antioxidants, osmolytes and also contribute to regulation of stress signalling (Kumar et al., 2023; Saini et al., 2024). Salt stress caused increase in phenols and flavonoids. Improved accumulation of phenols and flavonoids by the salinity stress has been reported in several plant species by others as well (Ahanger et al., 2019; Benjamin et al., 2019; Soliman et al., 2020). Increased synthesis of secondary compounds may protect plant cells from the ionic and oxidative stresses through their ability to bind toxic ions causing reduction in their impact on the structure and function of cytoplasmic components (Sytar et al., 2018). Trehalose resulted in improved build up of metabolites and which may help in protecting the seedlings from the salt stress by helping in radical scavenging and improving the functioning other related mechanisms. Trehalose treatments increased phenols in *Raphanus sativa* and sunflower under drought (Shafiq et al., 2015), *Zea mays* under chromium (Razzaq et al., 2024), and quinoa (Sadak et al., 2019) and wheat (Sadak, 2019) under salt stress. Similarly, Samadi et al. (2019) has observed increased flavonoids in Tre treated strawberry plants reflecting in improved growth. Greater phenol and flavonoid accumulation due to external Tre supplementation results in greater radical scavenging efficiency in stressed *Zea mays* (Ali et al., 2012).

Salt stress caused significant decline in the chlorophyll pigment synthesis and the gas exchange characteristics of fenugreek however, applied Tre proved beneficial by improving the parameters most consciously at 10 mM concentrations. In corroboration to these results, salt stress reduced the chlorophyll pigments, photosynthesis and other stomatal parameters of photosynthesis in other crop plants like *Dianthus caryophyllus* (Kwon et al., 2019), wheat (Ahanger et al., 2019), soybean (Soliman et al., 2020), *Oenanthe javanica* (Kumar et al., 2021) and *Avicennia marina* (Barhoumi et al., 2022). The chloroplast development and chloroplast ultrastructure are negatively influenced by salt stress (Lu et al., 2023) which can directly influence the photosynthetic parameters and the chlorophyll synthesis. In stress plants the chlorophyll synthesis goes down due to increased activity of degradation enzymes and considerable decline in the synthesising ones (Turan and Tripathy, 2015). Treatment of Tre proved beneficial in increasing the chlorophyll pigments and the photosynthetic gas exchange which can help in growth enhancement through higher carbon metabolism. Earlier, in drought stressed rice (Mohanan et al., 2023) and chromium stressed *Zea mays* (Razzaq et al., 2024) has also observed the mitigation of the reduction in chlorophyll and the photosynthetic gas exchange by Tre application. Similarly, exogenous Tre application to *Catharanthus roseus* resulted in mitigation of decline in photosynthesis and gas exchange parameters (Chang et al., 2014). Trehalose reduced the toxic radical buildup which lead to improved chlorophyll synthesis and the photosynthetic performance of *Trigonella foenum-graecum*.

The beneficial effects of added Tre were marked as significant decline in the lipid peroxidation, hydrogen peroxide and electrolyte leakage. Salt stress increases the accumulation of toxic radicals and causes the membrane damage which mediates electrolyte leakage and similar reports in other crop plants are available (Taibi et al., 2016; Soliman et al., 2020; Kumar et al., 2021; Lu et al., 2023). Excess accumulated radicals prove dangerous for the metabolism and cellular structures. Salinity stress increased the

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DOI: http://dx.doi.org/10.15666/aeer/2302_37213740

hydrogen peroxide and peroxidation, and the damaging effects were apparent as damage to chloroplast structure (Shu et al., 2012; Lu et al., 2023). Trehalose imparted decline in radical accumulation and also protected membrane damage which was reflected as reduced lipid peroxidation and electrolyte leakage. Mostofa et al. (2015) and Samadi et al. (2019) also observed reduced radicals in Tre treated rice and strawberry respectively under salt stress. Reduced reactive species accumulation and lipid peroxidation after the application of Tre has been also reported in *Raphanus sativus* (Shafiq et al., 2015) and *Zea mays* (Razzaq et al., 2024) grown in water deficit and chromium stress. Decline in the electrolyte leakage by Tre application has been observed by Mohanan et al. (2023) in drought stressed rice. This declined radical accumulation and the oxidative damage in Tre supplied seedlings can be ascribed to greater antioxidant system functioning which was clearly evident. Though all levels of Tre caused boosted the antioxidant enzymes, however, 10 mM Tre proved much impactful. To lessen the harm caused by salinity plants increase the activities of antioxidant enzymes and comparable observations have been reported earlier in several crop species (Taibi et al., 2016; Ahanger et al., 2019; Soliman et al., 2020; Kumar et al., 2021; Lu et al., 2023). Greater functioning of antioxidant system is correlated with the better stress tolerance and improved protection to metabolism. The activities of antioxidant enzymes including superoxide dismutase, guaiacol peroxidase, ascorbate peroxidase and glutathione reductase showed increase owning to the treatment of Tre. Similar to these results, Mostofa et al. (2015) and Samadi et al. (2019) have also reported increase in the antioxidant enzyme activities by the application of Tre in rice and strawberry lessening the oxidative damage of salt stress. Li et al. (2023) has observed significant augmentation of the activities and the transcript levels of antioxidant enzymes easing the effects of drought in *Cucumis sativus*. Greater antioxidant functioning confers the lesser accumulation of toxic radicals and defending the chief pathways of metabolism like photosynthesis, electron transport chain etc. (Kapoor et al., 2019). Greater antioxidant functioning in Tre supplied seedlings reduces the oxidative effects on growth and photosynthesis (Zafar et al., 2024; Elkelish et al., 2024). In addition, the accumulation of ascorbic acid and glutathione was increased in Tre treated plants which further strengthens the radical scavenging system so that tolerance against salinity is increased. Both ascorbic acid and glutathione are the key components of antioxidant system, redox buffer and also neutralise the radicals individually (Hasanuzzaman et al., 2020). Increased ascorbic acid and glutathione in Tre supplemented seedlings can improve the functioning of pathways like ascorbate-glutathione cycle which is aimed to neutralise hydrogen peroxide. Earlier, increase in ascorbic acid and glutathione by the application of Tre has been reported in rice (Mostofa et al., 2015), *Raphanus sativa* (Shafiq et al., 2015) and *Zea mays* (Razzaq et al., 2024) grown under salt, drought and chromium stress. Further improvement in the functioning of antioxidant enzymes and the accumulation of non-enzymatic antioxidant in Tre supplied seedlings confirms its valuable role in mitigating the adverse influence of salinity on fenugreek growth.

Conclusion

The growth restriction and decline in photosynthesis triggered by salinity was alleviated by the Tre supplementation at all levels, more conspicuously at 10 mM. Exogenously supplied Tre regulated the osmolyte and the secondary metabolite accumulation contributing to alleviation of undesirable effects of salt stress on water relations, enzyme functioning and photosynthesis. Moreover, the reduced accumulation

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of Na ions and the augmented functioning antioxidant system confers the alleviation of oxidative effects of salinity by Tre in *Trigonella foenum-graecum*. Further research can be fruitful to know the exact mechanisms using omic approaches.

Acknowledgments. The author extends their appreciation to the Deanship of Research and Graduate Studies at King Khalid University for funding this work through Large Research Project under grant number RGP2/323/45.

Conflicts of interest. The author declares no competing financial interests.

REFERENCES

[1] Abdallah, M. M. S., Abdelgawad, Z. A., El-Bassiouny, H. M. S. (2016): Alleviation of the adverse effects of salinity stress using trehalose in two rice varieties. – South African Journal of Botany 103: 275-282. https://doi.org/10.1016/j.sajb.2015.09.019.

[2] Ahanger, M. A., Agarwal, R. M. (2017): Salinity stress induced alterations in antioxidant metabolism and nitrogen assimilation in wheat (*Triticum aestivum* L) as influenced by potassium supplementation. – Plant Physiology and Biochemistry 115: 449-460.

[3] Ahanger, M. A., Tittal, M., Mir, R. A., Agarwal, R. M. (2017): Alleviation of water and osmotic stress-induced changes in nitrogen metabolizing enzymes in *Triticum aestivum* L. cultivars by potassium. – Protoplasma 254(5): 1953-1963.

[4] Ahanger, M. A., Qin, C., Begum, N., Maodong, Q., Dong, X. X., El-Esawi, M., El-Sheikh, M. A., Alatar, A. A., Zhang, L. (2019): Nitrogen availability prevents oxidative effects of salinity on wheat growth and photosynthesis by up-regulating the antioxidants and osmolytes metabolism, and secondary metabolite accumulation. – BMC Plant Biology 19: 479. https://doi.org/10.1186/s12870-019-2085-3.

[5] Ali, Q., Ashraf, M., Anwar, F., Al-Qurainy, F. (2012): Trehalose-induced changes in seed oil composition and antioxidant potential of maize grown under drought stress. – Journal of the American Oil Chemists' Society 89(8): 1485-1493. DOI 10.1007/s11746-012-2032-z.

[6] Arif, Y., Singh, P., Siddiqui, H., Bajguz, A., Hayat, S. (2020): Salinity induced physiological and biochemical changes in plants: An omic approach towards salt stress tolerance. – Plant Physiology and Biochemistry 156: 64-77. https://doi.org/10.1016/j.plaphy.2020.08.042.

[7] Arnon, D. I. (1949): Copper enzymes in isolated chloroplast polyphenol oxidase in Beta vulgaris. – Plant Physiol 24: 1-15.

[8] Azeem, M., Pirjan, K., Qasim, M., Mahmood, A., Javed, T., Muhammad, H., Yang, S., Dong, R., Ali, B., Rahimi, M. (2023): Salinity stress improves antioxidant potential by modulating physio-biochemical responses in *Moringa oleifera* Lam. – Scientific Reports 13: 2895. https://doi.org/10.1038/s41598-023-29954-6.

[9] Barhoumi, Z., Atia, A., Hussain, A. A., Albinhassan, T. H., Saleh, K. A. (2022): Effects of high salinity on photosynthesis characteristics, leaf histological components and chloroplasts ultrastructure of *Avicennia marina* seedlings. – Acta Physiologiae Plantarum 44: 85. https://doi.org/10.1007/s11738-022-03418-2.

[10] Bates, L. S., Waldren, R. P., Teare, I. D. (1973): Rapid determination of free proline for water-stress studies. – Plant Soil 39: 205-207.

[11] Benjamin, J. J., Lucini, L., Jothiramshekar, S., Parida, A. (2019): Metabolomic insights into the mechanisms underlying tolerance to salinity in different halophytes. – Plant Physiology and Biochemistry 135: 528-545. DOI:10.1016/j.plaphy.2018.11.006.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_37213740

[12] Carlberg, I. N., Mannervik, B. E. (1975): Purification and characterization of the flavoenzyme glutathione reductase from rat liver. – Journal of Biological Chemistry 250: 5475-5480.
[13] Chakraborty, P., Kumari, A. (2024): Role of compatible osmolytes in plant stress tolerance under the influence of phytohormones and mineral elements. – In: Improving Stress resilience in plants: Physiological and Biochemical Basis and Utilization in Breeding. Chapter 9, pp. 165-201. https://doi.org/10.1016/B978-0-443-18927-2.00011-X.
[14] Chang, B., Yang, L., Cong, W., Zu, Y., Tang, Z. (2014): The improved resistance to high salinity induced by trehalose is associated with ionic regulation and osmotic adjustment in *Catharanthus roseus*. – Plant Physiology Biochemistry 77: 140-148. http://dx.doi.org/10.1016/j.plaphy.2014.02.001.
[15] Choudhary, S., Wani, K. I., Naeem, M., Khan, M. M. A., Aftab, T. (2023): Cellular responses, osmotic adjustments, and role of osmolytes in providing salt stress resilience in higher plants: polyamines and nitric oxide crosstalk. – Journal of Plant Growth Regulation 42: 539-553. https://doi.org/10.1007/s00344-022-10584-7.
[16] Dionisio-Sese, M., Tobita, S. (1998): Antioxidant responses of rice seedlings to salinity stress. – Plant Science 135(1): 1-9. https://doi.org/10.1016/s0168-9452(98)00025-9.
[17] Elkelish, A., Alhudhaibi, A. M., Hossain, A. S., Haouala, F., Alharbi, B. M., El-Banna, M. F., Rizk, A., Badji, A., Aljwaizea, N. I., Sayed, A. A. S. (2024): Alleviating chromium-induced oxidative stress in *Vigna radiate* through exogenous trehalose application: insights into growth, photosynthetic efficiency, mineral nutrient uptake, and reactive oxygen species scavenging enzyme activity enhancement. – BMC Plant Biology 24: 460. https://doi.org/10.1186/s12870-024-05152-y.
[18] Ellman, G. L. (1959): Tissue sulphydryl groups. – Archives of Biochemistry and Biophysics 82: 70-77.
[19] Ferdosi, M. F. H., Shoaib, A., Habib, S., Khan, K. A. (2021): Modulation of salt-induced stress impact in *Gladiolus grandifloras* L. by exogenous application of salicylic. – Scientific Reports 11: 15597. https://doi.org/10.1038/s41598-021-95243-9.
[20] Gharsallah, C., Fakhfakh, H., Grubb, D., Gorsane, F. (2016): Effect of salt stress on ion concentration, proline content, antioxidant enzyme activities and gene expression in tomato cultivars. – AoB Plants 8: plw055. doi: 10.1093/aobpla/plw055.
[21] Grieve, C. M., Grattan, S. R. (1983): Rapid assay for determination of water soluble quaternary ammonium compounds. – Plant Soil 70: 303-307.
[22] Hasanuzzaman, M., Bhuyan, M. H. M. B., Zulfiqar, F., Raza, A., Mohsin, S. M., Al Mahmud, J., Fujita, M., Fotopoulos, V. (2020): Reactive oxygen species and antioxidant defense in plants under abiotic stress: revisiting the crucial role of a universal defense regulator. – Antioxidants (Basel) 9(8): 681. doi:10.3390/antiox9080681.
[23] Hassan, M. U., Nawaz, M., Shah, A. N., Raza, A., Barbanti, L., Hashem, M., Brestic, M., Pandey, S., Alamri, S., Mostafa, Y. S., Sabagh, A. E. L., Qari, S. H. (2023): Trehalose: A Key Player in Plant Growth Regulation and Tolerance to Abiotic Stresses. – Journal of Plant Growth Regulation 42: 4935-4957. https://doi.org/10.1007/s00344-022-10851-7.
[24] Heath, R. L., Packer, L. (1968): Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. – Archives of Biochemistry and Biophysics 125: 189-198.
[25] Kapoor, D., Singh, S., Kumar, V., Romero, R., Prasad, R., Singh, J. (2019): Antioxidant enzymes regulation in plants in reference to reactive oxygen species (ROS) and reactive nitrogen species (RNS). – Plant Gene 19: 100182. https://doi.org/10.1016/j.plgene.2019.100182.
[26] Kosar, F., Akram, N. A., Sadiq, M., Al-Qurainy, F., Ashraf, M. (2019): Trehalose: a key organic osmolyte effectively involved in plant abiotic stress tolerance. – Journal of Plant Growth Regulation 38: 606-618. https://doi.org/10.1007/s00344-018-9876-x.
[27] Kosar, F., Akram, N. A., Ashraf, M., Ahmad, A., Alyemeni, M. N., Ahmad, P. (2021): Impact of exogenously applied trehalose on leaf biochemistry, achene yield and oil

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_37213740

composition of sunflower under drought stress. – Physiologia Plantarum 172(2): 317-333. https://doi.org/10.1111/ppl.13155.

[28] Kumar, S., Li, G., Yang, J., Huang, X., Ji, Q., Liu, Z., Ke, W., Hou, H. (2021): Effect of salt stress on growth, physiological parameters, and ionic concentration of water dropwort (*Oenanthe javanica*) cultivars. – Front Plant Science 12. https://doi.org/10.3389/fpls.2021.660409.

[29] Kumar, K., Debnath, P., Singh, S., Kumar, N. (2023): An overview of plant phenolics and their involvement in abiotic stress tolerance. – Stresses 3(3): 570-585. https://doi.org/10.3390/stresses3030040.

[30] Kumar, P., Kumar, A., Agrawal, A., Bhusan, R., Kumar, S., Rehman, S., Kumar, R. (2024): Osmolytes as stress sensors in plants: acclimatizing plants under stress conditions. – In: Singh, L. R., Dar, T. A., Kumari, K. (eds.) Cellular Osmolytes. Springer, Singapore. https://doi.org/10.1007/978-981-97-6001-5_11.

[31] Kwon, O. K., Mekapogu, M., Kim, K. S. (2019): Effect of salinity stress on photosynthesis and related physiological responses in carnation (*Dianthus caryophyllus*). – Horticulture, Environment, and Biotechnology 60: 831-839. https://doi.org/10.1007/s13580-019-00189-7.

[32] Li, Z. G., Luo, L. J., Zhu, L. P. (2014): Involvement of trehalose in hydrogen sulfide donor sodium hydrosulfide-induced the acquisition of heat tolerance in maize (*Zea mays* L.) seedlings. – Botany Study 55: 20.

[33] Li, C., Lu, X., Liu, Y., Xu, J., Yu, W. (2023): Trehalose alleviates the inhibition of adventitious root formation caused by drought stress in cucumber through regulating ROS metabolism and activating trehalose and plant hormone biosynthesis. – Plant Physiology and Biochemistry 205: 108159. https://doi.org/10.1016/j.plaphy.2023.108159.

[34] Lowry, O., Rosebrough, A., Far, A., Randall, R. (1951): Protein measurement with folin phenol reagent. – J. Biol. Chem. 193: 680-685.

[35] Lu, C., Li, L., Liu, X., Chen, M., Wan, S., Li, G. (2023): Salt stress inhibits photosynthesis and destroys chloroplast structure by down regulating chloroplast development–related genes in *Robinia pseudoacacia* Seedlings. – Plants 12(6): 283. https://doi.org/10.3390/plants12061283.

[36] Mansour, M. M. F., Salama, K. H. A., Al-Mutawa, M. M. (2003): Transport proteins and salt tolerance in plants. – Plant Science 164(6): 891-900. https://doi.org/10.1016/S0168-9452(03)00109-2.

[37] Mohanan, A., Kodigudla, A., Raman, D. R., Bakka, K., Challabathula, D. (2023): Trehalose accumulation enhances drought tolerance by modulating photosynthesis and ROS-antioxidant balance in drought sensitive and tolerant rice cultivars. – Physiology and Molecular Biology of Plants 29: 2035-2049. https://doi.org/10.1007/s12298-023-01404-7.

[38] Mostofa, M. G., Hossain, M. A., Fujita, M. (2015): Trehalose pretreatment induces salt tolerance in rice (*Oryza sativa* L.) seedlings: oxidative damage and co-induction of antioxidant defense and glyoxalase systems. – Protoplasma 252(2): 461-475. doi:10.1007/s00709-014-0691-3.

[39] Mukherjee, S. P., Choudhuri, M. A. (1983): Implications of water stress-induced changes in the levels of endogenous ascorbic acid and hydrogen peroxide in *Vigna* seedlings. – Physiologia Plantarum 58: 166-170.

[40] Nakano, Y., Asada, K. (1981): Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. – Plant Cell Physiology 22: 867-880.

[41] Parwez, R., Nabi, A., Mukarram, M., Aftab, T., Khan, M. M. A., Naeem, M. (2021): Various Mitigation Approaches Applied to Confer Abiotic Stress Tolerance in Fenugreek (*Trigonella foenum-graecum* L.): A Review. – In: Naeem, M., Aftab, T., Khan, M. M. A. (eds.) Fenugreek. Springer, Singapore. https://doi.org/10.1007/978-981-16-1197-1_8.

[42] Qin, C., Lian, H., Zhang, B., He, Z., Alsahli, A. A., Ahanger, M. A. (2024): Synergistic influence of selenium and silicon supplementation prevents the oxidative effects of arsenic stress in wheat. – Journal of Hazardous Materials 465: 133304.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_37213740

https://doi.org/10.1016/j.jhazmat.2023.133304.
[43] Raza, A., Bhardwaj, S., Rahman, M. A., Garcia-Caparros, P., Habib, M., Saeed, F., Charagh, S., Foyer, C. H., Siddique, K. H. M., Varshney, R. K. (2024): Trehalose: A sugar molecule involved in temperature stress management in plants. – The Crop Journal 12(1): 1-16. https://doi.org/10.1016/j.cj.2023.09.010.
[44] Razzaq, M., Akram, N. A., Chen, Y., Samdani, M. S., Ahmad, P. (2024): Alleviation of chromium toxicity by trehalose supplementation in *Zea mays* through regulating plant biochemistry and metal uptake. – Arabian Journal of Chemistry 17(2): 105505. https://doi.org/10.1016/j.arabjc.2023.105505.
[45] Sadak, M. S. (2019): Physiological role of trehalose on enhancing salinity tolerance of wheat plant. – Bulletin of the National Research Centre 43: 53. https://doi.org/10.1186/s42269-019-0098-6.
[46] Sadak, M. S., El-Bassiouny, H. M. S., Dawood, M. G. (2019): Role of trehalose on antioxidant defense system and some osmolytes of quinoa plants under water deficit. – Bulletin of the National Research 43: 5. https://doi.org/10.1186/s42269-018-0039-9.
[47] Saddhe, A. A., Mishra, A. K., Kumar, K. (2021): Molecular insights into the role of plant transporters in salt stress response. – Physiologia Plantarum 173(4): 1481-1494. doi: 10.1111/ppl.13453.
[48] Saini, N., Anmol, A., Kumar, S., Wani, A. W., Bakshi, M., Dhiman, Z. (2024): Exploring phenolic compounds as natural stress alleviators in plants- a comprehensive review. – Physiological and Molecular Plant Pathology 133: 102383. https://doi.org/10.1016/j.pmpp.2024.102383.
[49] Samadi, S., Habibi, G., Vaziri, A. (2019): Exogenous trehalose alleviates the inhibitory effects of salt stress in strawberry plants. – Acta Physiologiae Plantarum 41: 112. https://doi.org/10.1007/s11738-019-2905-y.
[50] Schields, R., Burnett, W. (1960): Determination of protein-bound carbohydrate in serum by a modified anthrone method. – Analytical Chemistry 32: 885-886. DOI: 10.1021/ac60163a053.
[51] Shafiq, S., Akram, N. A., 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 185: 68-75. https://doi.org/10.1016/j.scienta.2015.01.010.
[52] Shu, S., Guo, S. R., Sun, J., Yuan, L. Y. (2012): Effects of salt stress on the structure and function of the photosynthetic apparatus in *Cucumis sativus* and its protection by exogenous putrescine. – Physiologiae Plantarum 146(3): 285-296. doi: 10.1111/j.1399-3054.2012.01623.x.
[53] Singh, N., Yadav, S. S., Kumar, S., Narashiman, B. (2022): Ethnopharmacological, phytochemical and clinical studies on Fenugreek (*Trigonella foenum-graecum* L.). – Food Science 46: 101546. https://doi.org/10.1016/j.fbio.2022.101546.
[54] Singleton, V. L., Rossi, I. A. (1965): Colorimetry of total phenolics with phospho-molybdic-phosphotungstic acid reagents. – American Journal of Enology Viticulture 16: 144-158. DOI:10.5344/ajev.1965.16.3.144.
[55] Smart, R. E., Bihgham, G. E. (1974): Rapid estimates of relative water content. – Physiologiae Plantarum 53: 258-260.
[56] Soliman, M., Elkelish, A., Souad, T., Alhaithloul, H., Farooq, M. (2020): Brassinosteroid seed priming with nitrogen supplementation improves salt tolerance in soybean. – Physiology and Molecular Biology of Plants 26(3): 501-511. https://doi.org/10.1007/s12298-020-00765-7.
[57] Sytar, O., Mbarki, S., Zivcak, M., Brestic, M. (2018): The involvement of different secondary metabolites in salinity tolerance of crops. – In: Kumar, V., Wani, S., Suprasanna, P., Tran, L. S. (eds.) Salinity responses and tolerance in plants. Volume 2. Springer, Cham. https://doi.org/10.1007/978-3-319-90318-7_2.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_37213740

[58] Taibi, K., Taibi, F., Abderrahim, L. A., Ennajah, A., Belkhodja, M., Mulet, J. M. (2016): Effect of salt stress on growth, chlorophyll content, lipid peroxidation and antioxidant defence systems in *Phaseolus vulgaris* L. – South African Journal of Botany 105: 306-312. https://doi.org/10.1016/j.sajb.2016.03.011.
[59] Turan, S., Tripathy, B. C. (2015): Salt-stress induced modulation of chlorophyll biosynthesis during de-etiolation of rice seedlings. – Physiologiae Plantarum 153(3): 477-91. doi:10.1111/ppl.12250.
[60] van Rossum, M. W. P. C., Alberda, M., van der Plas, L. H. W. (1997): Role of oxidative damage in tulip bulb scale micropropagation. – Plant Science 130: 207-216.
[61] Velikova, V., Yordanov, I., Edreva, A. (2000): Oxidative stress and some antioxidant systems in acid rain-treated bean plants. – Plant Sci 151: 59-66.
[62] Waheed, A., Zhuo, L., Wang, M., Hailiang, X., Tong, Z., Wang, C., Aili, A. (2024): Integrative mechanisms of plant salt tolerance: Biological pathways, phytohormonal regulation, and technological innovations. – Plant Stress 14: 100652. https://doi.org/10.1016/j.stress.2024.100652.
[63] Xiao, F., Zhou, H. (2023): Plant salt response: Perception, signaling, and tolerance. – Front. Plant Sci. 13. https://doi.org/10.3389/fpls.2022.1053699.
[64] Yuan, G., Sun, D., An, G., Li, W., Si, W, Liu, J., Zhu, Y. (2022): Transcriptomic and metabolomic analysis of the effects of exogenous trehalose on salt tolerance in watermelon (*Citrullus lanatus*). – Cells 11(15): 2338. doi: 10.3390/cells11152338.
[65] Zafar, S., Khan, I. M., Ashraf, M. A., Zafar, M., Ahmad, M., Rasheed, R., Mehmood, A., Ahmad, K. S. (2024): Insights into trehalose mediated physiological and biochemical mechanisms in *Zea mays* L. under chromium stress. – BMC Plant Biology 24: 783. https://doi.org/10.1186/s12870-024-05514-6.
[66] Zhishen, J., Mengcheng, T., Jianming, W. (1999): The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. – Food Chemistry 64: 555-559.
[67] Zhou, W., Leul, M. (1998): Uniconazole-induced alleviation of freezing injury in relation to changes in hormonal balance, enzyme activities and lipid peroxidation in winter rape. – Plant Growth Regulation 26(1): 41-47. https://doi.org/10.1023/A:1006004921265.

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DOI: http://dx.doi.org/10.15666/aeer/2302_37213740