Bangladesh Agron. J. 2023, 26(1): 75-83

ENHANCING SALT STRESS TOLERANCE AND YIELD PARAMETERS OF PROSO MILLET THROUGH EXOGENOUS PROLINE AND GLYCINE BETAINE SUPPLEMENTATION

M.M. Alam and M. Hasanuzzaman*

Department of Agronomy, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh.
*Corresponding author, Email: mhzsauag@yahoo.com

(**Received:** 13 July 2023, **Accepted:** 01 September 2023)

Keywords: Proso millet, abiotic stresses, salinity, relative water content, ionic toxicity

Abstract

Proline (Pro) and glycine betaine (GB) act as significant osmoprotectants, potentially mitigating the detrimental effects of various abiotic stresses in plants. Given the growth-enhancing capabilities and other regulatory roles of Pro and GB, the current study was conducted to assess their function in imparting salt stress resilience in proso millet (*Panicum miliaceum* L). Proso millet plants were subjected to two levels of salt stress (S_1 = 150 mM; S_2 = 300 mM). Foliar applications of Pro (0.5 mM) and GB (0.5 mM) were provided under control and salt-stressed conditions at 10-day intervals twice. Compared to control conditions, plant growth, fresh and dry weight, leaf relative water content (RWC), SPAD value, and yield-contributing attributes noticeably decreased under salt stress. In contrast, plants supplemented with Pro and GB exhibited enhanced characteristics. Moreover, growth and yield parameters improved in salt-treated proso millet plants when supplemented with Pro and GB. These results suggest that the foliar application of Pro and GB can alleviate salt-induced oxidative stress in proso millet plants by modulating the antioxidative defense.

Introduction

Agricultural productivity across the globe is critically endangered by an array of abiotic stresses, such as drought, salinity, flooding, and extreme temperatures. Salinity, prevalent in over 100 countries, is particularly concerning as it degrades agricultural quality and yield, posing a significant environmental challenge (Dey *et al.*, 2021; Sabagh *et al.*, 2021). Studies reveal that nearly 20% of the Earth's arable land grapples with the harmful impacts of salt stress (Hasanuzzaman and Fujita, 2022). This stress leads to excessive absorption of sodium (Na^+) and chloride (Cl^-) ions, triggering ionic stress and cytotoxicity that severely hamper plant growth and development (Shah *et al.*, 2021).

Among the affected crops, proso millet (*Panicum miliaceum* L.), a drought-resistant variety, is significantly vulnerable to salinity stress. Despite its drought resistance, it demonstrates limited tolerance to elevated salt levels (Zou *et al.*, 2019). High soil salinity upsets the plant's water balance, creating a water deficit and inhibiting nutrient uptake. The resulting osmotic stress from salt accumulation hinders growth, diminishes photosynthetic activity, and disrupts reproductive development (Akhter *et al.*, 2021; Shah *et al.*, 2021; Mushtaq *et al.*, 2023). The escalation of oxidative damage due to reactive oxygen species (ROS) accumulation also results in cell membrane and cellular dysfunction (Hasanuzzaman *et al.*, 2020; Saleem *et al.*, 2021; Hasanuzzaman and Fujita, 2022).

Managing salinity stress requires an integrated approach, incorporating agronomic practices, breeding initiatives, and innovative biotechnological tools. The development of salt-resistant varieties, refining irrigation techniques, and deploying effective soil management strategies are all steps towards mitigating the adverse effects of salinity stress. Additionally, researchers were exploring various biochemical strategies to amplify the salinity tolerance of proso millet, including the external application of compounds such as proline (Pro) and glycine betaine (GB).

Pro, an osmolyte integral to osmotic adjustment and stress resistance in plants, and GB, a crucial chemical osmoregulator, have both shown potential in alleviating salinity stress. They contribute to maintaining cellular osmotic balance, protect cells from oxidative damage, regulate stress response enzymes, and enhance the overall salt tolerance of proso millet (Shafi *et al.*, 2019; Hosseinifard *et al.*, 2022; Zhu *et al.*, 2022; Bai *et al.*, 2022).

However, the efficiency of Pro and GB is contingent on a myriad of factors such as concentration, method of application, and specific proso millet genotypes. With this in mind, this study was undertaken to evaluate the effects of applying Pro and GB under varying salinity stress levels in proso millet cultivation.

Materials and Methods

Plant materials and experimental design

Healthy proso millet (*Panicum miliaceum* L.var. BARI Cheena-1) seeds were carefully chosen and consistently sown in 16 L plastic containers. Baseline applications of organic manure and chemical fertilizers, including urea, triple superphosphate, and muriate of potash, were used. Twenty days after sowing (DAS), along with the control group (0 mM NaCl), we introduced mild (150 mM NaCl) and severe salinity (300 mM NaCl) to the plants. Supplements of proline (Pro) (50 μM) and glycine betaine (GB) (50 mM) were given at 10 and 20 DAS under both normal and saline conditions. The study employed a completely randomized design (CRD) with three repetitions.

Evaluation of plant height

Proso millet plant heights were measured at 20, 30 DAS, and upon harvesting. A standard measuring scale was used to gauge the distance from the base level to the uppermost leaf tip of each plant. The average measurements were taken from five arbitrarily chosen plants from each pot.

To calculate the Fresh Weight (FW) per plant, randomly uprooted three sample plants from each repetition and used a balance for weighing. After determining FW, the samples were dried in an oven at 80°C for 48 h. Following this, they were weighed again to measure the Dry Weight (DW), with the average dry weight recorded as DW per plant.

Assessment of SPAD values

The SPAD meter (Minolta Camera Co., Osaka, Japan) was utilized to evaluate the top, middle, and base sections of five randomly selected leaves from each pot. Measurements from each section were then averaged (Yuan et al., 2016).

Estimation of yield contributing parameters

Standard procedures were employed to measure parameters contributing to yield, including the number of tillers per hill, panicle length, filled and unfilled grains per panicle, 1000-seed weight, and grain yield.

Measurement of relative water content

Five leaves were randomly chosen from each pot, cleaned, and weighed to determine the fresh weight (FW). The samples were then soaked in distilled water for 24 h, after which excess surface water was removed to measure the turgid weight (TW). The samples were then oven-dried for 72 h to obtain the dry weight (DW). The relative water content (RWC) was calculated using the formula: RWC (%) = (FW–DW)/(TW–DW) × 100 (Barrs and Weatherley, 1962).

Statistical analysis

The collected data from the three repetitions were statistically evaluated using Staistix 10 software. A one-way analysis of variance (ANOVA) was performed. Fisher's least significant difference (LSD) test was applied at the 5% significance level to compare the mean differences.

Results and Discussion

When subjected to S_1 and S_2, a reduction of 5% and 18% in plant height was observed at 30 DAS, respectively. This reduction progressively intensified over time, with the most significant decrease recorded during harvest—13% and 27% for S_1 and S_2, respectively. Nonetheless, the application of Pro and GB counteracted the adverse effects of salt stress on plant height. While the impact wasn't considerable at 30 DAS under both S_1 and S_2, these compounds proved more effective at 50 DAS. Pro, in particular, was highly successful in counterbalancing the detrimental effects of both levels of salt stress on proso millet.

Table 1. Plant height at 30 DAS, 50 DAS, and at harvest of proso millet under salt stress supplemented by Pro and GB

Treatment	Plant height (cm)		
	30 DAS	50 DAS	at harvest
C	18.77 0.62 ab	50.72 0.97 a	74.60 1.39 a
Pro	19.26 0.58 a	50.86 2.39 a	74.48 0.69 a
GB	18.46 1.05 ab	51.36 0.99 a	75.22 0.72 a
S_1	14.71 0.52 ab	44.71 0.05 d	65.12 3.87 de
S_1+Pro	16.33 0.32 bc	49.22 2.34 b	71.02 1.52 bc
S_1+GB	15.63 0.86 bc	47.56 1.58 c	68.38 3.01 cd
S_2	11.82 0.24 e	39.08 0.58 e	54.55 4.91 f
S_2+Pro	14.25 0.51 cd	46.44 0.61 c	66.21 1.00 de
S_2+GB	13.26 0.74 d	44.93 2.19 d	63.28 4.59 e

Plant morphological attributes, such as plant height, were significantly affected by salt stress. This is due to a decrease in cell growth, chlorophyll content, nutrient absorption, relative water content, transpiration rate, and stomatal conductance (Khalid *et al.*, 2022; Rasool *et al.*, 2023). However, the accumulation of Pro and GB can play a pivotal role in maintaining cellular osmotic balance, scavenging reactive oxygen species, and stabilizing proteins and membranes. Consequently, these osmo-protectants effectively counteract the adverse effects of salinity stress on plant growth, allowing plants to flourish even under demanding conditions (Desok *et al.*, 2019; Dikilitas *et al.*, 2020; Khalid *et al.*, 2022).

For instance, de Freitas *et al.* (2019) reported increased plant growth, gas exchange, and relative water content when *Sorghum bicolor* plants were sprayed with 30 mM of Pro under 75 mM of NaCl stress. Similarly, enhanced plant height and chlorophyll content were observed in *Oryza sativa* when treated with GB spray under salt stress (Annunziata *et al.*, 2019). Moreover, Abbas *et al.* (2012) found that salt-stressed *Citrus sinensis* plants, sprayed with Pro (25 mg L^{-1}), showed an increased production rate of salt-responsive proteins and a decreased salt impact on plant height and leaf numbers, aligning with our current findings.

Under salt stress conditions, both fresh weight (FW) and dry weight (DW) decrease in a dose-dependent manner. When exposed to S1 and S2, FW reduced by 18% and 27%, respectively. However, the application of SA proved to be more effective under both S1 and S2, enhancing FW by 13% and 12%, respectively. A similar trend was observed for DW. The most significant decrease (34%) in DW occurred under S2, but SA and Pro were able to enhance it by 12% and 17%, respectively, under S1. Meanwhile, under S2, these agents could only increase DW by 17% and 12%, respectively.

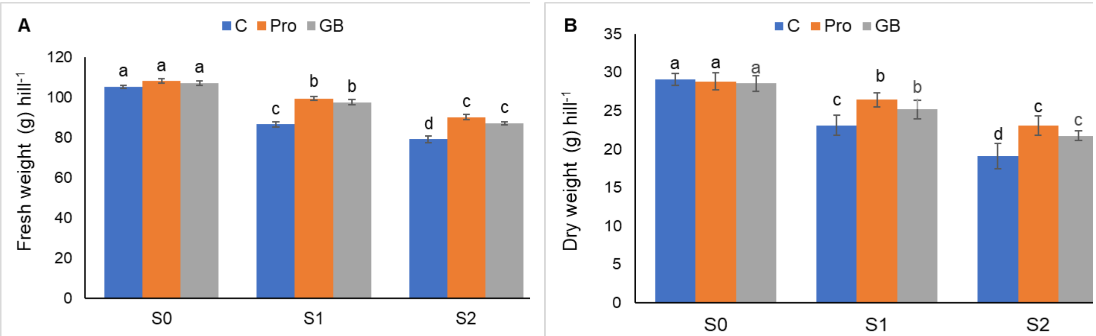

Fig. 1. Fresh weight (A) and dry weight (B) of proso millet under salt stress supplemented by salicylic acid and Pro.Here, Pro and GB represent Pro and GB, respectively.

Elevated salinity levels can significantly negatively impact plants, leading to noticeable effects such as plant mortality and reduced productivity (Hannachi *et al.*, 2022). Increased soil salinity can trigger osmotic stress, resulting in a prolonged water deficit within plant cells. At the same time, it can induce severe ion toxicity and oxidative damage, collectively inflicting detrimental effects on plant growth, which consequently reduces both the fresh weight (FW) and dry weight (DW) of the plants (Desok *et al.*, 2019; Qaoud *et al.*, 2023).

As illustrated in (Figure 1), NaCl stress has noticeably reduced the assessed parameters in proso millet. This reduction could likely be due to two reasons. First, the presence of salt in the soil solution hinders water uptake from the plant root zone to the cell, leading to a decreased growth rate. Second, excessive salt infiltration through the transpiration streams can cause cellular injuries to the transpiring leaves. These injuries could potentially lead to further damage, resulting in diminished photosynthetic and growth capacities (Elhindi *et al.*, 2017; Shehata and Nosir, 2019).

However, Pro plays a pivotal role in maintaining ion homeostasis within plants. It helps mitigate the uptake of toxic ions such as sodium and facilitate the uptake of essential nutrients like potassium. This ion balance supports critical metabolic processes, bolsters energy production, and aids nutrient transport. As a result, it fosters increased biomass accumulation, leading to a higher FW and DW in plants (Ibrahim *et al.*, 2019).

Conversely, GB, a naturally occurring compound, has been found to significantly enhance plant growth and promote dry weight accumulation under salt stress conditions (Joushan *et al.*, 2020).

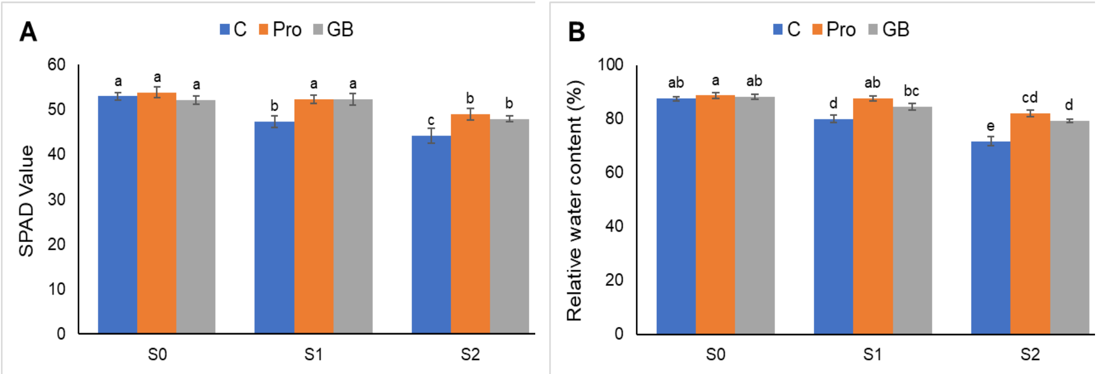

Fig. 2. SPAD value (A) and RWC (B) of proso millet under salt stress supplemented by salicylic acid and Pro. Here, Pro and GB represent Pro and GB, respectively.

At 30 DAS, the SPAD value decreased by 11% and 17% under S_1 and S_2 conditions, respectively, compared to the control plants. However, the application of Pro and GB to stressed plants demonstrated a similar trend in mitigating salt stress-induced damage in proso millet. Proso millet was affected more severely by a higher salt stress dose, S_2 (18%), than the lower dose, S_1 (9%), in terms of RWC. However, the application of Pro and GB helped alleviate these negative effects. Pro was found to be more effective under lower levels of salt stress compared to the higher one.

Under stress conditions, plants adopt an essential survival strategy by adjusting their photosynthetic capacity. This involves altering various photosynthetic attributes, including the SPAD value, to ensure their survival and optimal functioning despite adverse conditions. Our findings conclusively demonstrate that applying exogenous Pro and GB to leaves effectively counteracts the salt-induced decrease in SPAD value. This outcome is due to the osmoprotectant properties of Pro and GB, which act as antitranspirants, allowing the plant to access more water over a prolonged period. Consequently, photosynthesis is facilitated, contributing to the observed reduction in stress-related effects on the SPAD value (Dawood *et al.,* 2021).

Relative water content (RWC) serves as a reliable indicator of plant stress tolerance, offering valuable insights into their resilience and adaptability to challenging conditions. Salinity stress induces osmotic stress, which has been shown to impact plants' RWC (Mushtaq *et al.*, 2023). Accumulation of phytotoxic ions such as Na^+ and Cl^- in leaves disrupts nutrient balance, significantly reducing the concentrations of essential elements like K^+ and Ca^{2+}. This disruption in ion equilibrium negatively affects plant water relations, leading to decreased RWC and leaf water potential (Acosta-Motos *et al.*, 2017). In this study observed that increased salt concentrations resulted in a decrease in RWC. However, foliar application of Pro and GB reinstated RWC, corroborating earlier findings by Tang *et al.* (2015).

Salt stress significantly affected the yield-contributing characteristics (e.g., tiller number hill^{-1}) of proso millet. The most significant reduction (41%) occurred under S_2 compared to S_1, measured at 30, 50 DAS, and during harvest. However, Pro proved more effective than GB in counteracting the negative effects of salt stress on tiller number hill^{-1} under both salt stress doses from 30 DAS to harvest. Salinity severely affects plants by impairing their reproductive capacity through hindrances in photosynthesis and assimilate production, leading to diminished yield and overall productivity (Arif *et al.*, 2020; Shah *et al.*, 2021). In reaction to increased soil salinity, plants initiate expedited water loss from their cells. For proso millet, water is critical for flowering and panicle formation, especially during the reproductive stage. Therefore, water scarcity significantly impacts the plant's reproductive processes (Yuan *et al.*, 2022).

The current study's results demonstrated that salt stress decreased panicle length, filled grain numbers, and 1000-grain weight, correlating with the reduction of proso millet's grain yield. Under salt stress conditions, Pro proved more effective than GB in mitigating negative effects on yield-contributing parameters like tiller number hill^{-1}.

Exposure to S1 and S2 resulted in a decrease in panicle length by 22% and 47%, respectively, compared to the control. However, both Pro and GB performed better under S2 than S1, increasing the panicle length by 17 and 11%, respectively (Table 2).

Table 2. The number of tillers hill^{-1} at 30 DAS, 50 DAS, harvest and panicle length of proso millet under salt stress supplemented by Pro and GB.

Treatment	Number of tiller hill^{-1}			Panicle length (cm)
	30 DAS	50 DAS	harvest	
C	1.444 0.040 a	3.989 0.016 a	3.6696 0.1372 a	18.8 0.62 ab
Pro	1.455 0.093 a	3.874 0.147 ab	3.6482 0.1401 a	19.3 0.58 a
GB	1.479 0.041 a	3.737 0.074 b	3.6397 0.0452 a	18.5 1.05 b
S1	1.094 0.030 c	2.964 0.045 e	2.9582 0.0453 c	14.7 0.52 d
S1 + Pro	1.234 0.068 b	3.517 0.130 c	3.3957 0.0596 b	16.3 0.32 c
S1 + GB	1.220 0.033 b	3.345 0.126 cd	3.0477 0.1162 c	15.6 0.86 c
S2	0.851 0.047 e	2.519 0.064 f	2.1923 0.1233 e	11.8 0.24 f
S2 + Pro	0.971 0.039 d	3.183 0.130 d	2.6402 0.0991 d	14.2 0.51 d
S2 + GB	0.943 0.047 d	2.821 0.109 e	2.6010 0.0950 d	13.3 0.74 e

The application of various salt levels had a negative impact on other yield-contributing parameters, such as filled grain panicle^{-1}, unfilled grain panicle^{-1}, 1000-grain weight, and the total yield of proso millet. Exposure to S2 led to a 40% decrease in filled grain panicle^{-1} compared to the control. Nevertheless, both Pro and GB were more effective under S1 than S2, increasing the number of filled grain panicle^{-1}by 15- and 12%, respectively (Figure 3A).

Similarly, there was a significant increase in unfilled grain panicle^{-1} by 28% under S2 compared to the control. However, the application of supplemental SA and Pro increased the number of unfilled grain panicle^{-1} under both S1 and S2, with Pro proving more effective than GB (Figure 3B).

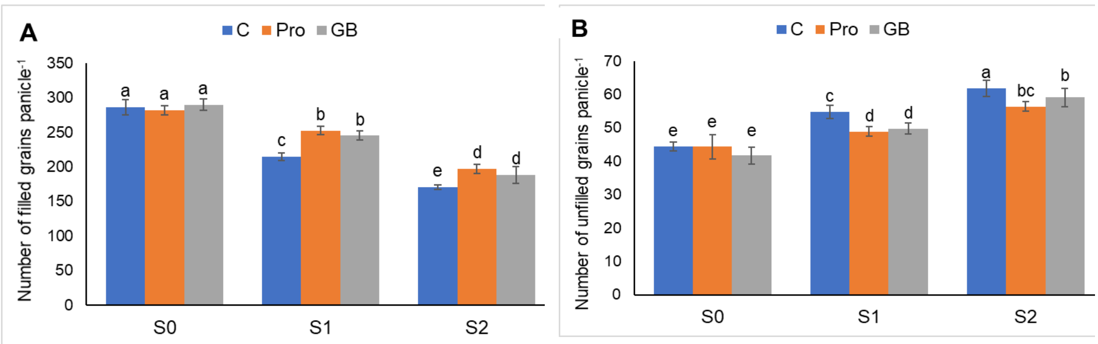

Fig. 3. The number of filled (A) and unfilled (B) grains panicle^{-1} of proso millet under salt stress supplemented by salicylic acid and Pro. Here, Pro and GB represent Pro and GB, respectively

A significant decrease in 1000-grain weight was observed under S2, with a reduction of 39%, while it was only 23% under S1 compared to the control. Pro demonstrated better efficacy than GB, resulting in a 21% increase in 1000-grain weight under S2 (Figure 4A). Under salt stress conditions, grain yield hill^{-1} was diminished by 19% and 41% under S1 and S2, respectively, compared to the control. However, the supplementation of Pro enhanced the grain yield hill^{-1} by 19% at S2 compared to S1 (Figure 4B).

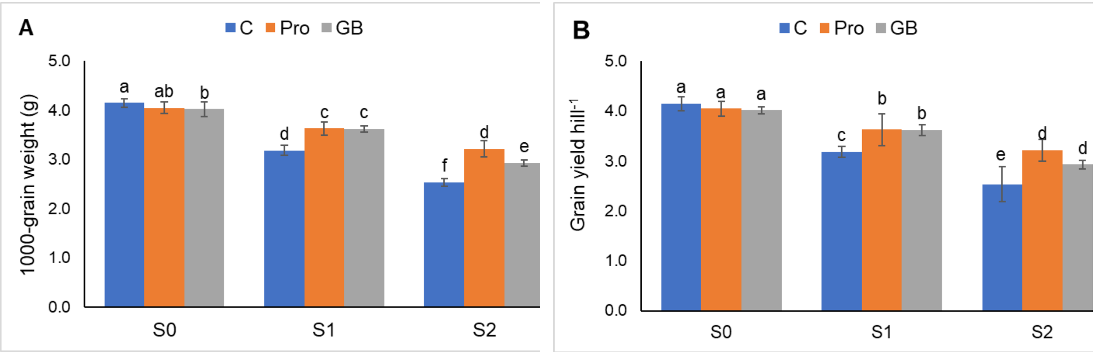

Fig. 4. The 1000-grain weight (A) and grain yield (B) of proso millet under salt stress supplemented by salicylic acid and Pro. Here, Pro and GB represent Pro and GB, respectively.

The results of this experiment underscored the vital role of externally applied Pro and GB in maintaining optimal physiological processes under salt stress. As osmo-protectants, Pro and GB have the potential to reduce excessive water loss from cells, enabling plants to cope with water stress during periods of salt-induced osmotic imbalance. By promoting photosynthesis, they contribute to increased carbohydrate production, which is crucial for improving yield-contributing characteristics such as biomass accumulation and grain filling.

Moreover, by mitigating the adverse effects of salinity, these compounds enhance yield-contributing parameters, including biomass production, grain filling, and overall crop productivity. Thus, they play indispensable roles in improving crop performance in saline environments (Tabssum *et al.*, 2018; Shemi *et al.*, 2021). These findings underscore the valuable benefits of Pro and GB in supporting plant growth and productivity in challenging environments.

Conclusion

The results of this study provide compelling evidence that the individual and combined supplementation of Pro and GB successfully mitigated the adverse effects of salt stress on proso millet. These treatments demonstrated a significant enhancement in the salt tolerance of proso millet. Under salt stress conditions, the exogenous application of Pro and GB resulted in notable improvements in both morphological and yield parameters of proso millet. Moreover, the application of Pro exhibited superior efficacy compared to GB in conferring resistance to different levels of salt stress. These findings highlight the potential of Pro and GB as valuable tools for enhancing salt stress tolerance in proso millet and potentially other crops. Further research and field trials will be required to explore their application in agricultural practices and their long-term effects on crop productivity.

Acknowledgements

We would like to express our appreciation to the Ministry of Science and Technology, Government of the People's Republic of Bangladesh, for their valuable financial support in enabling the execution of this research work. The authors extend their sincere gratitude and appreciation to Abdul Awal Chowdhury Masud, Assistant Professor in the Department of Agronomy at SAU, for his assistance in updating the manuscript.

References

Abbas, M.F., A.M. Jasim and H.A. Al-Taha. 2012. Effect of exogenous proline on protein pattern changes in *Citrus sinensis* (L.) Osbeck under *in vitro* salt stress. Adv. Agric. Bot. 4: 36-41.

Acosta-Motos, J.R., M.F. Ortuño, A. Bernal-Vicente, P. Diaz-Vivancos, M.J. Sanchez-Blanco and J.A. Hernandez. 2017. Plant responses to salt stress: adaptive mechanisms. Agronomy 7: 18.

Akhter, M.S., S. Noreen, S. Mahmood, M. Ashraf, A.A. Alsahli and P. Ahmad. 2021. Influence of salinity stress on PSII in barley (*Hordeum vulgare* L.) genotypes, probed by chlorophyll-a fluorescence. J. King Saud Univ. Sci. 33: 101239.

Annunziata, M.G., L.F. Ciarmiello, P. Woodrow, E. Dell'Aversana and P. Carillo. 2019. Spatial and temporal profile of glycine betaine accumulation in plants under abiotic stresses. Front. Plant Sci. 10: 230.

Arif, Y., P. Singh, H. Siddiqui, A. Bajguz and S. Hayat. 2020. Salinity induced physiological and biochemical changes in plants: An omic approach towards salt stress tolerance. Plant Physiol. Biochem. 156, 64–77.

Bai, M., W. Zeng, F. Chen, X. Ji, Z. Zhuang and Jin, B. 2022. Transcriptome expression profiles reveal response mechanisms to drought and drought-stress mitigation mechanisms by exogenous glycine betaine in maize. Biotechnol. Lett. 44: 367–386.

Barrs, H.D. and P.E. Weatherley. 1962. A re-examination of the relative turgidity technique for estimating water deficits in leaves. Aust. J. Biol. Sci. 15: 413–428.

Dawood, M.G., M.A. Khater and M.E. El-Awadi. 2021. Physiological role of osmoregulators proline and glycinebetaine in increasing salinity tolerance of Chickpea. Egyptian J. Chem. 64: 7637-48.

de Freitas, P.A., H.H. de Carvalho, J.H. Costa, R.D. Miranda, K.D. Saraiva, F.D. de Oliveira, D.G. Coelho, J.T. Prisco, and E. Gomes-Filho. 2019. Salt acclimation in sorghum plants by exogenous proline: physiological and biochemical changes and regulation of proline metabolism. Plant Cell Rep. 8: 403-16.

Desok, E.M., S.A. Ibrahim and A.M. Merwad. 2019. Mitigation of salinity stress effects on growth, physio-chemical parameters and yield of snapbean (*Phaseolus vulgaris* L.) by exogenous application of glycine betaine. Int. Lett. Nat. Sci. 76: 60-71.

Dey, G., P. Banerjee, P.K. Sharma, J.P. Maity, H. Etesami and A.K. Shaw. 2021. Management of phosphorus in salinity-stressed agriculture for sustainable crop production by salt-tolerant phosphate solubilizing bacteria—a review. Agronomy 11: 1552.

Dikilitas, M., E. Simsek. and A. Roychoudhury. 2020. Role of Proline and Glycine Betaine in Overcoming Abiotic Stresses. In: Amelioration of Plant Abiotic Stress: Biochemical and Molecular Perspectives, A. Roychoudhury and D.K. Tripathi (Eds.). Protective Chemical Agents in the. John Wiley & Sons Ltd., New York. Pp:1-23.

Elhindi, K.M., A.S. El-Din and A.M. Elgorban. 2017. The impact of arbuscular mycorrhizal fungi in mitigating salt-induced adverse effects in sweet basil (*Ocimum basilicum* L.). Saudi J. Biol. Sci. 24: 170-9.

Hannachi, S., K. Steppe, M. Eloudi, L. Mechi, I. Bahrini and M.C. Van Labeke. 2022. Salt stress induced changes in photosynthesis and metabolic profiles of one tolerant ('Bonica') and one sensitive ('Black beauty') eggplant cultivars (*Solanum melongena* L.). Plants 11: 590.

Hasanuzzaman, M. and M. Fujita. 2022. Plant Responses and Tolerance to Salt Stress: Physiological and Molecular Interventions. Int. J. Mol. Sci. 9: 4810.

Hasanuzzaman, M., M.H.MB. Bhuyan, F. Zulfiqar, A. Raza, S.M. Mohsin, J.A. Mahmud, M. Fujita and V. Fotopoulos. 2020. Reactive Oxygen Species and Antioxidant Defense in Plants under Abiotic Stress: Revisiting the Crucial Role of a Universal Defense Regulator. Antioxidants 9: 681.

Hosseinifard, M., S. Stefaniak., J.M. Ghorban, E. Soltani, L. Wojtyla and M. Garnczarska. 2022. Contribution of Exogenous Proline to Abiotic Stresses Tolerance in Plants: A Review. Int. J. Mol. Sci. 23: 5186.

Ibrahim, A., A. Awad, A. Gendy and M. Abdelkader. 2019. Effect of proline foliar spray on growth and productivity of sweet basil (*Ocimum basilicum* L.) plant under salinity stress conditions. Zagazig J. Agric. Res. 46: 1877-1889.

Joushan, Z., H. Sodeizadeh, M.A.H. Ardakani, R. Yazdani Biouki and S. Kajahhosseini. 2020. Investigating the effect of foliar application of glycine betaine on some quantitative and qualitative characteristics of mint (*Mentha spicata* L). under salinity stress. Plant Prod. 43: 267-280.

Khalid, M., H.M. Rehman, N. Ahmed, S. Nawaz, F. Saleem, S. Ahmad, M. Uzair, I.A. Rana, R.M. Atif, Q.U. Zaman and H.M. Lam. 2022. Using exogenous melatonin, glutathione, proline, and glycine betaine treatments to combat abiotic stresses in crops. Int. J. Mol. Sci. 23: 12913.

Mushtaq, N. U., K. M. Alghamdi, S. Saleem, I. Tahir, A. Bahieldin, B. Henrissat, M.K. Alghamdi, R.U. Rehman, and K. R. Hakeem. 2023. Exogenous zinc mitigates salinity stress by stimulating proline metabolism in proso millet (*Panicum miliaceum* L.). Front. Plant Sci. 14: 1053869.

Qaoud, H.A., H.A. Ali, H. Al-Fares, T. Qubbaj and M.J.Y. Shtaya. 2023. Effect of salinity on the growth and some morphological traits of pearl millet. Pak. J. Bot. 55: 807-811.

Rasool, A., W. Hafiz Shah, S.A. Padder, I. Tahir, H.F. Alharby, K.R. Hakeem and R. Rehman. 2023. Exogenous selenium treatment alleviates salinity stress in Proso Millet (*Panicum miliaceum* L.) by enhancing the antioxidant defence system and regulation of ionic channels. Plant Growth Regul. 100: 479–494.

Sabagh, E.L., M.S. Islam, M. Skalicky, M.A. Raza, K. Singh and M.A. Hossain, M., et al. 2021. Salinity stress in wheat (*Triticum aestivum* L.) in the changing climate: Adaptation and management strategies. Front. Agron. 3: 661932.

Saleem, S., N.U. Mushtaq, W.H. Shah, A. Rasool, K.R. Hakeem and R.U. Rehman. 2021. Morpho-physiological, biochemical and molecular adaptation of millets to abiotic stresses: A review. Phyton. 90: 1363-1385.

Shafi, A., I. Zahoor and U. Mushtaq. 2019. Proline Accumulation and Oxidative Stress: Diverse Roles and Mechanism of Tolerance and Adaptation Under Salinity Stress. In: Salt Stress, Microbes, and Plant Interactions: Mechanisms and Molecular Approaches, M. Akhtar (Eds.). Springer, Singapore. Pp. 269-300.

Shah, W.H., A. Rasool, S. Saleem, N.U. Mushtaq, I. Tahir and K.R. Hakeem, et al. 2021. Understanding the Integrated Pathways and mechanisms of transporters, protein kinases, and transcription factors in plants under salt stress. Int. J. Genom. 2021: 5578727.

Shehata, A.M and W.S. Nosir. 2019. Response of sweet basil plants (*Ocimum basilicum*, L.) grown under salinity stress to spraying seaweed extract. Future J. Biol. 2: 16-28.

Shemi, R., R. Wang, E.S. Gheith, H.A. Hussain, S. Hussain, M. Irfan, L. Cholidah, K. Zhang, S. Zhang and L. Wang. 2021. Effects of salicylic acid, zinc and glycine betaine on morpho-physiological growth and yield of maize under drought stress. Sci. rep. 11: 3195.

Tabssum, F., Q. Zaman, Y. Chen, U. Riaz, W. Ashraf, A. Aslam, N. Ehsan, R. Nawaz, H. Aziz and S.S. Shah. 2018. Exogenous application of proline improved salt tolerance in rice through modulation of antioxidant activities. Pak. J. Agric. Res. 32: 140-151.

Tang, X., X. Mu, H. Shao, H. Wang and M. Brestic. 2015. Global plant-responding mechanisms to salt stress: physiological and molecular levels and implications in biotechnology. Crit. Rev. Biotechnol. 35: 425-37.

Yuan, Y., J. Liu, Q. Ma, Y. Gao, Q. Yang, X. Gao and B. Feng. 2022. Cleaner production of proso millet (*Panicum miliaceum* L.) in salt-stressed environment using re-watering: From leaf structural alleviations to multi-omics responses. J. Clean. Prod. 334: 130205.

Yuan, Z., Q. Cao, K. Zhang, S.T. Ata-Ul-Karim, Y. Tian, Y. Zhu, W. Cao and X. Liu. 2016. Optimal leaf positions for SPAD meter measurement in rice. Front. Plant Sci. 7: 719.

Zhu, M., Q. Li, Y. Zhang, M. Zhangand and Z. Li. 2022. Glycine betaine increases salt tolerance in maize (*Zea mays* L.) by regulating Na^+ homeostasis. Front. Plant Sci. 13: 978304.

Zou, C.S., L.T. Li, D. Miki, D.L. Li, Q.M. Tang, L.H. Xiao, S. Rajput, P. Deng, L. Peng, W. Jia, R. Huang, M.L. Zhang, Y.D. Sun, J.M. Hu, X. Fu, P.S. Schnable, YX. Chang, F. Li, H. Zhang and H. Zhang. 2019. The genome of broomcorn millet. Nat. Commun. 10: 436.