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ENHANCING MAIZE GROWTH AND MITIGATING SALINITY STRESS THROUGH FOLIAR APPLICATION OF PROLINE AND GLYCINE BETAINE

INAM IRSHAD[1], MUHAMMAD ANWAR-UL-HAQ[1]*, JAVAID AKHTAR[1] AND MUHAMMAD MAQSOOD[2]

[1]*Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad, Pakistan*
[2]*Department of Agronomy, University of Agriculture, Faisalabad, Pakistan*
[*]*Corresponding author's email: Haqgondal@gmail.com*

Abstract

Salinity stress is a major problem for crop productivity worldwide. It reduces crop growth and yield by disrupting the plant's physiological and metabolic processes. However, osmolytes usage as foliar is suggested to overcome this issue to some extent in plants. These osmolytes have the potential to decrease the uptake of toxicity-generating ions, thus playing a vital role in the regulation of plant growth. However, regarding use of their best application rate still needs scientific attention. That's why the current study was conducted with the objective of determining the effectiveness of osmolytes, i.e., proline (Pro) and glycine betaine (GB) usage, on maize under salinity stress. For that hybrid maize variety (Monsanto DK-6789) was sown on soils having EC2 (control), 5, and 10 dSm^{-1}. Osmolytes proline and glycine betaine were applied as foliar at concentrations of 20mM, 40mM, and 60mM. The results showed that osmolytes foliar application, i.e., proline 60mM and glycine betaine 60mM performed significantly best for improving maize growth, chlorophyll contents, and potassium uptake. At the highest salinity level of 10 dS/m, the Pro20 treatment showed a 6.0% reduction in electrolyte leakage compared to the control group, while the Pro40 and Pro60 treatments demonstrated significant reductions of 24.1% and 30.0%, respectively. Likewise, the GB20, GB40, and GB60 treatments exhibited reductions of 17.4%, 32.1%, and 40.2%, respectively, compared to the control group. In conclusion, the foliar proline 60mM and glycine betaine 60mM application is an effective strategy for mitigating the negative impact of salinity stress on maize growth and productivity.

Key words: Osmolytes; Growth attributes; Chlorophyll contents; Potassium; Sodium; Electrolyte leakage.

Introduction

Salinity is a common issue in arid and semi-arid climates because of the limited water availability and high evaporation rates, which can lead to the accumulation of salts in soil and water (Pitman & Läuchli, 2002, Zhao *et al*., 2021). In arid and semi-arid regions, water is scarce and often comes from underground sources, such as wells or aquifers (Asgari *et al*., 2012, Ahmed *et al*., 2020). These sources are typically rich in dissolved salts, which can accumulate in the soil as the water evaporates (Zafar-ul-Hye *et al*., 2019, Zhao *et al*., 2021). The salts can also be brought to the surface through capillary action, where they can accumulate and create salt pans or salt flats (Ahmadvand *et al*., 2019). High salinity levels in the soil can be detrimental to plant growth and can limit agricultural productivity (Zhao *et al*., 2021). Salt can damage plant roots, hinder water uptake, and cause osmotic stress, which can ultimately lead to reduced crop yields (Adnan *et al*., 2020). In addition, high levels of salt in the soil can also impact soil structure and reduce soil fertility (Majeed & Muhammad, 2019). To address the issue of salinity in arid and semi-arid regions, various strategies can be employed. The use of osmolytes is one such technology (Nxele *et al*., 2017).

Osmolytes, also known as compatible solutes, are small organic molecules (Sharma *et al*., 2019) that accumulate in cells in response to stress to maintain cellular function and protect against damage caused by salt (Sharma *et al*., 2019). Proline and glycine betaine are two common osmolytes that are known to play an important role in alleviating salinity stress in plants (Khalid *et al*., 2022). Proline is a well-known osmolyte that is synthesized in response to salinity stress. It accumulates in plants in high concentrations and helps to maintain cellular water balance, stabilize proteins, scavenge free radicals, and regulate gene expression. Proline also acts as a signaling molecule that regulates plant growth and development under stress conditions (Hosseinifard *et al*., 2022). Glycine betaine is another osmolyte that is synthesized in response to salinity stress. It accumulates in plants and acts as a compatible solute to maintain cellular water balance and protect against salt-induced damage. Glycine betaine also acts as an osmoprotectant by stabilizing membrane structure and protecting against oxidative stress. In addition to proline and glycine betaine, other osmolytes such as betaine, trehalose, and sugars have also been shown to play a role in salinity stress alleviation. The accumulation of osmolytes is regulated by several factors, including salt concentration, water availability, and plant species (Desoky *et al*., 2019). Maize is one of such important cereal crops in the world, providing food and feed for humans and livestock (Rohman *et al*., 2019). It is a staple food crop in many countries, particularly in sub-Saharan Africa, where it is an important source of carbohydrates and protein (Grote *et al*., 2021). However, salinity stress is a significant threat to maize production, particularly in arid and semi-arid regions where water is scarce and the soil salinity levels are high (Zafar-ul-Hye *et al*., 2014). Salinity stress affects maize growth and development by altering water uptake, nutrient availability, and physiological processes such as photosynthesis, respiration, and transpiration (Khan, 2001, Rohman *et al*., 2019). So far, maize plants have developed various mechanisms to cope with salinity stress, i.e., changes in root morphology, osmotic adjustment, and ion homeostasis; the current study was planned to explore the effectiveness of proline and glycine betaine to mitigate salinity stress in maize. This study covers the knowledge gap regarding the use of proline and glycine betaine best application rate for improvement in maize growth under variable levels of salinity. It is hypothesized that proline and glycine betaine might have the potential to alleviate moderate and high levels of salinity stress in maize when applied in low concentrations.

Material and Methods

Maize seeds and pot preparation: In the experiment, the hybrid maize variety Monsanto DK-6789, sourced from Monsanto private limited, was utilized. The soil for the pot experiment was obtained from a university field and was sieved prior to use in the containers. Subsequently, 12 kg pots were filled with soil in which salinity was developed as per the treatment plan.

Seeds sowing and thinning: Six seeds of the hybrid maize variety were sown in each pot. Following one week of seedling germination, hoeing was carried out in the soil, and the seeds were sown, and subsequently, three healthy and uniformly sized seedlings were retained in each pot by thinning.

Treatment plan: In this study, the potential of two osmolytes, proline, and glycine betaine, to alleviate the toxic effects of salt stress on maize plants was investigated. Foliar spray was developed for the osmolytes at different concentrations, namely 20 mM, 40 mM, and 60 mM. To impose salinity stress, NaCl salt was used in the pots, and 21 treatments were divided into seven treatment groups. Six of these groups were treated with different osmolytes, resulting in a total of 27 treatments. In the first treatment group, maize was grown without any organic osmolyte under different electrical conductivity (EC) levels of 2 dS m^{-1}, 5 dS m^{-1}, and 10 dS m^{-1}. In the second treatment group, 20 mM of proline was foliar applied under the same EC levels. In the third treatment group, proline was applied at a concentration of 40 mM, and in the fourth treatment group, it was applied at a concentration of 60 mM, both under the same EC levels. In the fifth, sixth, and seventh treatment groups, glycine betaine was applied foliar at concentrations of 20 mM, 40 mM, and 60 mM, respectively, to evaluate its effects on alleviating the harmful impact of salinity on maize. The treatments were applied to the pots, which were arranged in a completely randomized design (CRD) with 3 replicates.

Fertilizer and irrigation: The application of the treatments began one week after the germination of seedlings, and the recommended amounts of nitrogen, phosphorus, and potassium fertilizers were also applied, with a recommended ratio of 92-58-35 kg/acre, respectively. The moisture in the pots was maintained at 65% field capacity throughout the experiment by using tap water as a source of irrigation (Danish *et al*., 2020).

Harvesting and data collection: Plants were harvested 60 days after sowing, and various growth attributes were measured, including shoot fresh weight (g), shoot dry weight (g), root fresh weight (g), root dry weight (g), root length (cm), and plant height (cm). The length of the plant was measured using a meter rod after harvesting. Digital weighing balance (SF-400, China origin) was used to measure fresh and dry weights. To determine dry weights, plant samples were kept in an oven at 65 to 67°C for 48 hours.

Chlorophyll contents: The relative chlorophyll content was measured using a SPAD-502 meter (Neufeld *et al*., 2006) of Japanese origin.

Nutrients concentration in plants: The wet digestion method was used for Na^+, K^+, and Ca^{+2}. To do this, dried plant samples were ground into a fine powder, digested in an acid mixture ratio of 2:1 (HNO_3:HCl) (Miller, 1998), and then filtered with Whatman filter paper No. 40. Na^+ and K^+ were measured using Jenway PFP-7 flame photometer (Donald & Hanson, 1998), while Ca^{+2} was measured using atomic adsorption spectrophotometer from digested plant samples (Hanlon, 1998).

Proline contents: The method used to analyze the proline content of plant samples was based on the procedure described by Bates *et al*., (1973). A 0.5 g fresh plant sample was ground with HCl using a mortar and pestle and then filtered through Whatman No. 1 filter paper. The filtrate was made up to 20 mL by adding sulfosalicylic acid. Next, 2 mL of the resulting material was transferred into a falcon tube kept in an ice bath. To this, 2 mL of ninhydrin, 2 mL of glacial acetic acid, and 4 mL of toluene were added and carefully mixed for 15-20 seconds. The toluene layer containing the chromophore was separated using a separatory funnel, and the absorbance was measured at 520 nm with a blank using a spectrophotometer. The proline content of plant samples was calculated using a standard curve, and the results were expressed in mg g^{-1} DW.

Glycine-betaine contents: To determine the glycine betaine (GB) content of the plant sample, 500 mg of dried and powdered material was mixed with 20 mL of distilled water and shaken on a mechanical shaker at room temperature for 24 hours. The resulting mixture was filtered through Whatman filter paper no. 1, and the extracted material was diluted with 2 N sulfuric acid (H_2SO_4) and cooled in an ice bath for 1 hour. Then, 0.2 mL of potassium triiodide solution was added, and the samples were stored at 4°C and centrifuged at 10,000 rpm for 15 minutes. The supernatant was collected and stored separately in a glass tube. The iodide crystals were dissolved in 9 mL of 1,2-dichloroethane with vigorous stirring, and after 3 hours, the absorbance of the sample at 365 nm was measured using a spectrophotometer with a blank. The GB content of the plant sample was calculated using a standard curve, and the results were expressed in µg g^{-1} DW units (Grattan & Grieve, 1993).

Statistical analysis

Standard statistical analysis was done for analysis of data (Steel *et al*., 1997). Two factorial ANOVA was applied along with pair comparison for the differentiation of treatments. Principal component analysis was done using OriginPro 2021 (Origin Lab Corporation, 2021).

Results

Shoot and root length: The shoot length results indicate that the different treatments had a significant impact on the growth of the plant shoots. Under 2 dS/m, the Pro20 treatment had a 20.2% increase in shot length compared to the control. The 40mM proline (Pro40) treatment had the most significant effect, with a 56.3% increase in shoot length compared to the control. The Pro60 and 60mM

glycine betaine (GB60) treatments also had a substantial increase in shoot length, with 114.2% and 79.8% increase, respectively, compared to the control. At 5 dS/m, the Pro40 treatment had the most significant effect on shoot length, with a 69.3% increase compared to the control. The Pro60 and GB60 treatments also had a substantial increase in shoot length, with 120.2% and 85.9% increase, respectively, compared to the control. Under 10 dS/m, the Pro40 treatment had the most significant effect on shoot length, with a 367.9% increase compared to the control. The Pro60 and GB60 treatments also had a substantial increase in shoot length, with 479.2% and 436.4% increase, respectively, compared to the control (Fig. 1A).

For root length under electrical conductivity (EC) of 2 dS/m, the Pro20 treatment showed a 17% increase in comparison to the control, while the GB20 treatment had no significant difference. The Pro40 treatment had a 24% increase, and the Pro60 treatment had the highest percentage increase of 29% in root length, being significantly higher than all other treatments at this EC level. At the same EC level, the GB40 treatment had a 22% increase, and the GB60 treatment showed a 30% increase, which was the highest percentage increase among all treatments. In the case of 5 dS/m EC, the Pro20 treatment showed a 15% increase compared to the control, while the GB20 treatment had no significant difference. The Pro40 treatment had a 23% increase, and the Pro60 treatment had the highest percentage increase of 27%. At the same EC level, the GB40 treatment showed a 21% increase, and the GB60 treatment had a 26% increase, which was the second highest among all treatments. At an EC of 10 dS/m, the Pro20 treatment showed a 7% increase compared to the control, while the GB20 treatment had a 13% increase. The Pro40 treatment had a 20% increase, and the Pro60 treatment had the highest percentage increase of 26%. At the same EC level, the GB40 treatment had a 19% increase, and the GB60 treatment had a 23% increase, which was the second highest among all treatments (Fig. 1B).

Root and shoot fresh and dry weight: The results for root fresh weight show a clear trend of increased growth with increasing salinity levels, although the degree of growth varies depending on the treatment. For the 2 dS/m treatment, the Pro60 treatment had the greatest increase in root fresh weight, showing a 342% increase over the control treatment, followed by the GB60 treatment which showed a 278% increase over the control. The Pro40 treatment also showed a significant increase, with a 50% increase over the control. For the 5 dS/m treatment, the Pro60 treatment again had the greatest increase in root fresh weight, showing a 213% increase over the control treatment, followed by the GB60 treatment, which showed a 294% increase over the control. The Pro40 treatment also showed a significant increase, with a 57% increase over the control. For the 10 dS/m treatment, the Pro60 treatment had the greatest increase in root fresh weight, showing a 388% increase over the control treatment, followed by the GB60 treatment, which showed a 611% increase over the control. The Pro40 treatment also showed a significant increase, with a 187% increase over the control (Fig. 2A).

Compared to the control group, the results of the root dry weight data showed various degrees of percentage increase or decrease in the different treatments. In the 2 dS/m treatment, the Pro20 treatment had a 14% increase in root dry weight compared to the control, while the Pro40 treatment had a 28% increase, and the Pro60 treatment had a significant 103% increase. The GB20 treatment had a 16% increase, GB40 had a 36% increase, and GB60 had an impressive 86% increase in root dry weight. In the 5 dS/m treatment, Pro20 had a 20% increase, Pro40 had a 32% increase, and Pro60 had a 73% increase compared to the control group. The GB20 treatment had an 8% increase, GB40 had a 46% increase, and GB60 had a 84% increase. In the 10 dS/m treatment, the Pro20 treatment had a 14% increase compared to the control group, while the Pro40 treatment had a 43% increase, and the Pro60 treatment had a 73% increase. The GB20 treatment had a 23% increase, GB40 had a 56% increase, and GB60 had a 89% increase in root dry weight (Fig. 2B).

For shoot fresh weight, there was a significant increase in growth with increasing salinity levels. At 2 (dS/m), Pro60 had the highest shot fresh weight with 119.67%, followed by GB60 with 86.33%. Pro40 also showed a significant increase in shoot fresh weight by 70% compared to the control, which had a shoot fresh weight of 35.67%. At 5 (dS/m), Pro60 had the highest shot fresh weight with 101.67%, followed by GB60 with 79.67%. Pro40 also showed a significant increase in shot fresh weight by 61% compared to the control, which had a shot fresh weight of 29.67%. At 10 (dS/m), Pro60 had the highest shot fresh weight with 87.67%, followed by GB60 with 72%. Pro40 also showed a significant increase in shot fresh weight with 52.33% compared to the control, which had a shot fresh weight of 16.33%. GB20, Pro20, and GB40 also showed increases in shoot fresh weight at varying salinity levels (Fig. 2C).

The shoot dry weight results show varying percentage increases or decreases compared to the control group at different salinity levels and with different treatments. At 2 dS/m salinity level, Pro20 treatment showed a 17.5% increase in shoot dry weight compared to the control group, while Pro40 treatment exhibited a 55% increase, and Pro60 treatment demonstrated a significant 168% increase. GB20 treatment also showed a 43% increase compared to the control group, while GB40 treatment exhibited a 50% increase, and GB60 treatment demonstrated a 100% increase in shoot dry weight. At 5 dS/m salinity level, Pro20 treatment showed a 12.5% increase in shot dry weight compared to the control group, while Pro40 treatment exhibited a 65.5% increase and Pro60 treatment demonstrated a significant 158% increase. GB20 treatment also showed a 43% increase compared to the control group, while GB40 treatment exhibited a 77.5% increase and GB60 treatment demonstrated a 120% increase in shoot dry weight. At the highest salinity level of 10 dS/m, Pro20 treatment showed a 19% increase in shoot dry weight compared to the control group, while Pro40 treatment exhibited a 104% increase, and Pro60 treatment demonstrated a significant 180% increase. GB20 treatment also showed a 46% increase compared to the control group, while GB40 treatment exhibited a 150% increase and GB60 treatment demonstrated a 170% increase in shoot dry weight (Fig. 2D).

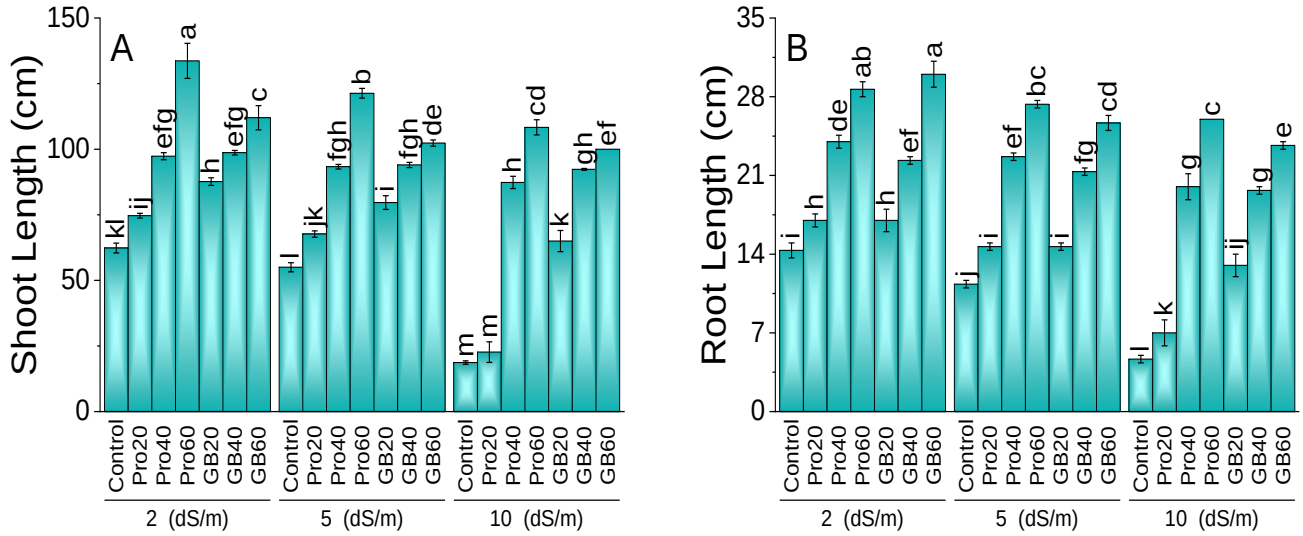

Fig. 1. Effect or different concentrations of proline and glycine betaine on shoot length (A) and root length (B) of maize. Bars are means of three replicates ± SE compared with Fisher LSD; $p \leq 0.05$. Proline (Pro); Glycine betaine (GB).

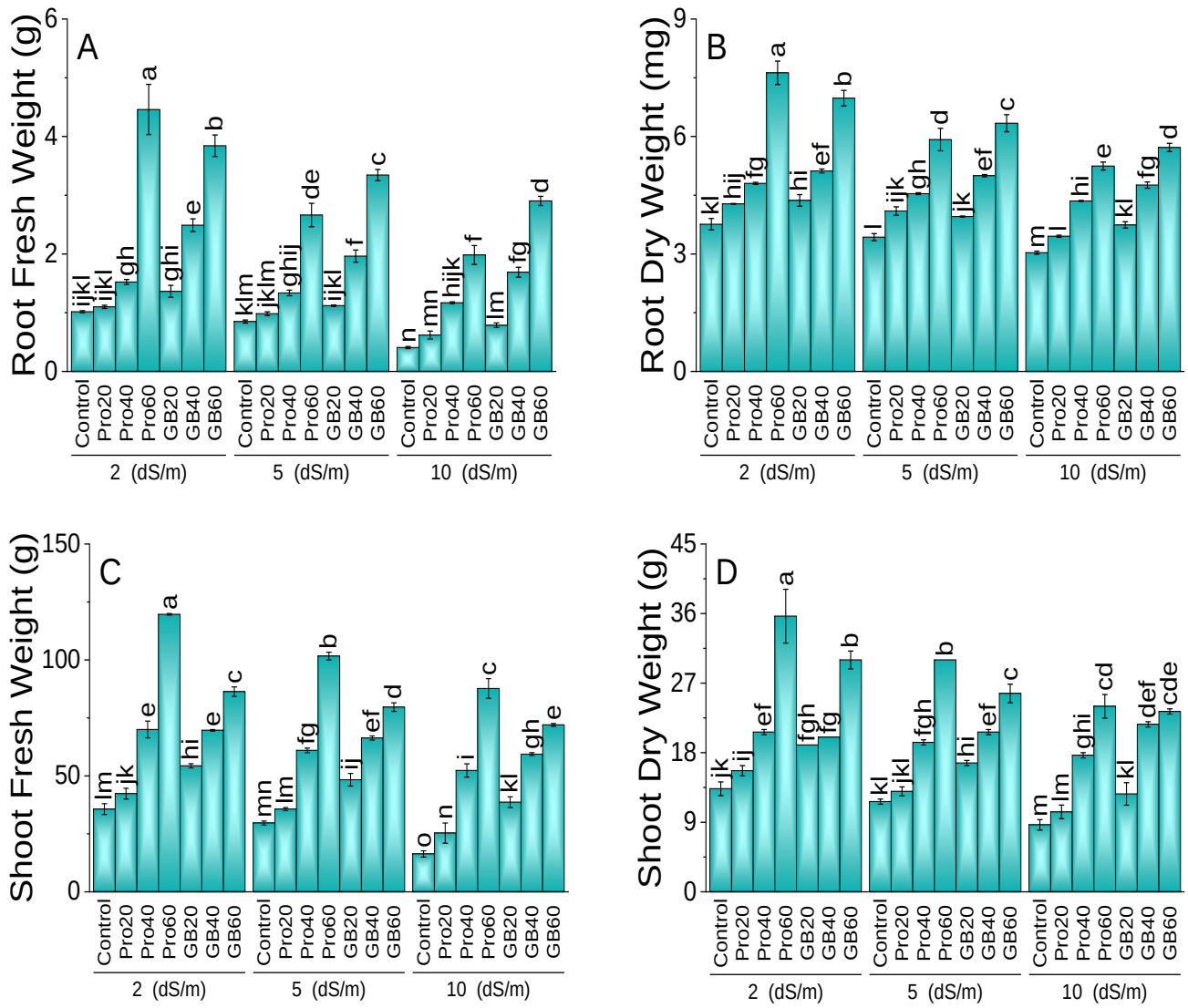

Fig. 2. Effect or different concentrations of proline and glycine betaine on root fresh weight (A), root dry weight (B), shoot fresh weight (C) and shoot dry weight (D) of maize. Bars are means of three replicates ± SE compared with Fisher LSD; $p \leq 0.05$. Proline (Pro); Glycine betaine (GB).

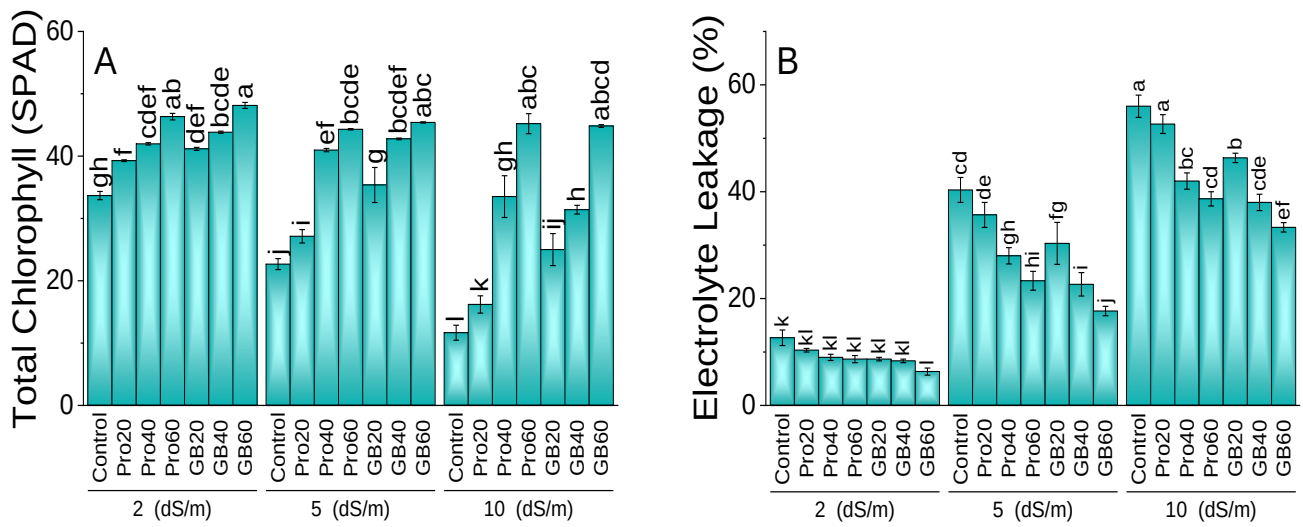

Fig. 3. Effect or different concentrations of proline and glycine betaine on total chlorophyll (A) and electrolyte leakage (B) of maize. Bars are means of three replicates ± SE compared with Fisher LSD; $p \leq 0.05$. Proline (Pro); Glycine betaine (GB).

Chlorophyll contents and electrolyte leakage: The results for the total chlorophyll content also indicate varying percentage increases or decreases compared to the control group at different salinity levels and with different treatments. For instance, at the 2 dS/m salinity level, Pro20 treatment showed a 16.5% increase in total chlorophyll content compared to the control group, while Pro40 treatment exhibited a 24.9% increase, and Pro60 treatment demonstrated a significant 37.4% increase. Similarly, GB20 treatment also showed a 22.1% increase compared to the control group, while GB40 treatment exhibited a 29.8% increase, and GB60 treatment demonstrated a significant 42.9% increase in total chlorophyll content. Moving on to the 5 dS/m salinity level, Pro20 treatment showed a 19.5% increase in total chlorophyll content compared to the control group, while Pro40 treatment exhibited a substantial 80.9% increase, and Pro60 treatment demonstrated a significant 95.7% increase. Similarly, GB20 treatment also showed a significant 56.2% increase compared to the control group, while GB40 treatment exhibited a considerable 88.8% increase, and GB60 treatment demonstrated a significant 100.9% increase in total chlorophyll content. Finally, at the highest salinity level of 10 dS/m, Pro20 treatment showed a 38.3% increase in total chlorophyll content compared to the control group, while Pro40 treatment exhibited a remarkable 185.4% increase, and Pro60 treatment demonstrated a significant 287.4% increase. Similarly, GB20 treatment also showed a substantial 115.2% increase compared to the control group, while GB40 treatment exhibited a significant 169.4% increase, and GB60 treatment demonstrated a significant 284.3% increase in total chlorophyll content (Fig. 3A).

The electrolyte leakage results demonstrate varying percentage changes relative to the control group for different salinity levels and treatments. At a salinity level of 2 dS/m, the Pro20 treatment displayed an 18.3% reduction in electrolyte leakage compared to the control group, while the Pro40 and Pro60 treatments showed even greater reductions of 29.1% and 31.7%, respectively. Similarly, the GB20, GB40, and GB60 treatments demonstrated significant reductions of 31.7%, 34.3%, and 50.0%, respectively. Moving to a higher salinity level of 5 dS/m, the Pro20 treatment exhibited an 11.2% reduction in electrolyte leakage compared to the control group, while the Pro40 and Pro60 treatments showed significant reductions of 30.6% and 42.3%, respectively. In comparison, the GB20, GB40, and GB60 treatments showed reductions of 24.8%, 43.9%, and 56.3%, respectively. At the highest salinity level of 10 dS/m, the Pro20 treatment showed a 6.0% reduction in electrolyte leakage compared to the control group, while the Pro40 and Pro60 treatments demonstrated significant reductions of 24.1% and 30.0%, respectively. Likewise, the GB20, GB40, and GB60 treatments exhibited reductions of 17.4%, 32.1%, and 40.2%, respectively, compared to the control group.

Ions and osmolytes: The results showed that at an EC of 2 dS/m, the Pro20 treatment caused a 38.8% decrease in Na uptake compared to the control, while the GB20 treatment caused a 45.6% decrease. In contrast, the Pro40 treatment showed a 17.2% increase in Na uptake, whereas the GB60 treatment showed a 3.2% increase. The Pro60 and GB40 treatments resulted in a slight increase in Na uptake compared to the control. At an EC of 5 dS/m, the Pro20 treatment caused a 42.9% decrease in Na uptake, while the GB20 treatment caused a 31.9% decrease compared to the control. However, the Pro40 and Pro60 treatments showed a 6.3% and 4.9% increase in Na uptake, respectively, while the GB60 treatment showed a 20.6% increase. The GB40 treatment also showed a slight increase in Na uptake compared to the control. Finally, at an EC of 10 dS/m, the Pro20 treatment caused a 30.6% decrease in Na uptake, while the GB20 treatment caused a 37.1% decrease compared to the control. On the other hand, the Pro40 treatment showed a 15.4% increase in Na uptake, whereas the GB40 treatment showed a 25.8% increase.

The K concentration in plants was evaluated at three different EC levels (2, 5, and 10 dS/m) in response to different treatments. The results showed that the K concentration varied among treatments and EC levels. The GB20 treatment consistently showed the highest K concentration across all EC levels, while the Pro60 treatment had the lowest K concentration at all EC levels. At an EC of 2 dS/m, the GB20 treatment had the highest K concentration (15.59 mg/g DW), while the Pro60 treatment had the lowest (8.31 mg/g DW). At an EC of 5 dS/m, the GB20 treatment again had the highest K concentration (14.18 mg/g DW), while the Pro60 treatment had the lowest concentration (9.17 mg/g DW). Similarly, at an EC of 10 dS/m, the GB20 treatment had the highest K concentration (14.11 mg/g DW), while the Pro60 treatment had the lowest concentration (7.86 mg/g DW). In contrast, the K concentration in the control group remained relatively stable across all EC levels, ranging from 10.00 to 13.00 mg/g DW. Overall, the results suggest that the different treatments and EC levels have variable effects on K uptake in plants, and the GB20 treatment may be a more effective method to increase K concentration in plants.

At an EC of 2 dS/m, the Pro20 treatment had a 15.6% decrease in Pro compared to the control, while the GB20 treatment had a 19.0% increase. The Pro40 treatment showed a 28.1% increase in Pro, while the GB60 treatment showed a 15.9% increase. The Pro60 treatment had the largest decrease in Pro at 32.1%, compared to the control. In the case of EC 5 dS/m, the Pro20 treatment showed a 21.3% decrease in Pro, while the GB20 treatment had a 4.3% increase compared to the control. The Pro40 treatment showed a 32.5% increase in Pro, while the GB60 treatment showed a 24.9% increase. The Pro60 treatment had the largest decrease in Pro at 46.2%, compared to the control. However, at EC 10 dS/m, the Pro20 treatment showed a 38.5% decrease in Pro, while the GB20 treatment had a 22.3% increase compared to the control. The Pro40 treatment showed a 27.2% increase in Pro, while the GB40 treatment had the largest increase in Pro at 45.9%. The Pro60 and GB60 treatments both had decreases in Pro compared to the control, at 23.0% and 22.6%, respectively.

Based on the results, at an EC of 2 dS/m, the GB60 treatment had the lowest GB concentration (9.33 µmol g^{-1}), while the GB40 treatment had the highest GB concentration (27.67 µmol g^{-1}), representing a 196% increase compared to the control. At an EC of 5 dS/m, the GB60 treatment still had the lowest GB concentration (11.67 µmol g^{-1}), while the GB40 treatment had the highest GB concentration (33.33 µmol g^{-1}), representing a 236% increase compared to the control. Similarly, at an EC of 10 dS/m, the GB60 treatment had the lowest GB concentration (18.67 µmol g^{-1}), while the GB40 treatment had the highest GB concentration (38.33 µmol g^{-1}), representing a 264% increase compared to the control. Overall, the results indicate that GB concentration in the plants is influenced by different treatments and EC levels, with the GB40 treatment showing consistently higher GB concentrations across all EC levels (Table 1).

Table 1. Effect or different concentrations of proline and glycine betaine on Na, K, proline and glycine betaine contents of maize.

EC (dS/m)	Treatment	Na (mg/g DW)	K (mg/g DW)	Pro (µmol g^{-1})	GB (µmol g^{-1})
2 (dS/m)	Control	2.50 h-j	13.00 cd	22.33 j	9.33 m
	Pro20	1.53 j	14.18 b	26.33 hi	11.33 lm
	Pro40	2.07 j	11.74 ef	28.67 h	12.00 l
	Pro60	1.83 j	10.10 hi	34.00 g	12.67 kl
	GB20	1.36 j	15.59 a	25.33 i	19.00 g
	GB40	4.32 g-i	12.60 de	24.67 ij	22.67 f
	GB60	2.40 ij	10.51 gh	28.67 h	27.67 d
5 (dS/m)	Control	7.83 c-e	11.49 fg	31.67 g	11.67 l
	Pro20	4.47 gh	13.60 b-d	38.33 f	14.33 jk
	Pro40	7.33 c-e	10.80 f-h	44.67 c	15.33 ij
	Pro60	7.45 c-e	8.31 jk	50.33 b	16.33 ij
	GB20	5.33 fg	14.11 b	31.67 g	25.33 e
	GB40	4.57 g	11.37 fg	40.67 ef	33.33 c
	GB60	6.23 e-g	10.00 hi	44.00 cd	38.00 b
10 (dS/m)	Control	12.13 a	9.17 ij	42.00 de	15.33 ij
	Pro20	8.43 b-d	11.74 ef	44.67 c	16.67 hi
	Pro40	10.27 ab	9.97 hi	49.33 b	19.00 g
	Pro60	7.64 c-e	7.33 k	53.00 a	18.67 gh
	GB20	7.63 c-e	13.74 bc	41.00 e	29.00 d
	GB40	9.01 bc	10.67 f-h	44.33 cd	38.33 b
	GB60	6.70 d-f	7.86 k	51.67 ab	43.00 a

Values are means (n=3) ± SE. Different letters are showing significant changes at $p \leq 0.05$

Table 2. Eigenvalues obtained after principal component analysis for studied attributes.

Principal component number	Eigenvalue	Percentage of variance (%)	Cumulative (%)	PC1 (59.7%)	PC2 (24.3%)
Root length (cm)	7.16724	59.72702	59.72702	0.36042	0.04773
Shoot length (cm)	2.91628	24.3023	84.02932	0.35667	0.01215
Root fresh weight (g)	0.84062	7.00521	91.03452	0.34625	0.05711
Root dry weight (mg)	0.39888	3.32397	94.35849	0.35774	0.0601
Shoot fresh weight (g)	0.26248	2.18733	96.54582	0.35768	0.08354
Shoot dry weight (g)	0.1862	1.55166	98.09749	0.35903	0.07347
Total chlorophyll (SPAD)	0.08502	0.70849	98.80598	0.34003	-0.08516
Electrolyte leakage (%)	0.05086	0.42383	99.22981	-0.25653	0.39637
Na (mg/g DW)	0.04242	0.35352	99.58333	-0.17087	0.47397
K (mg/g DW)	0.02461	0.20509	99.78842	-0.1281	-0.46522
Proline (µmol g^{-1})	0.01777	0.14811	99.93653	0.00869	0.54866
Glycine betaine (µmol g^{-1})	0.00762	0.06347	100	0.10518	0.26808

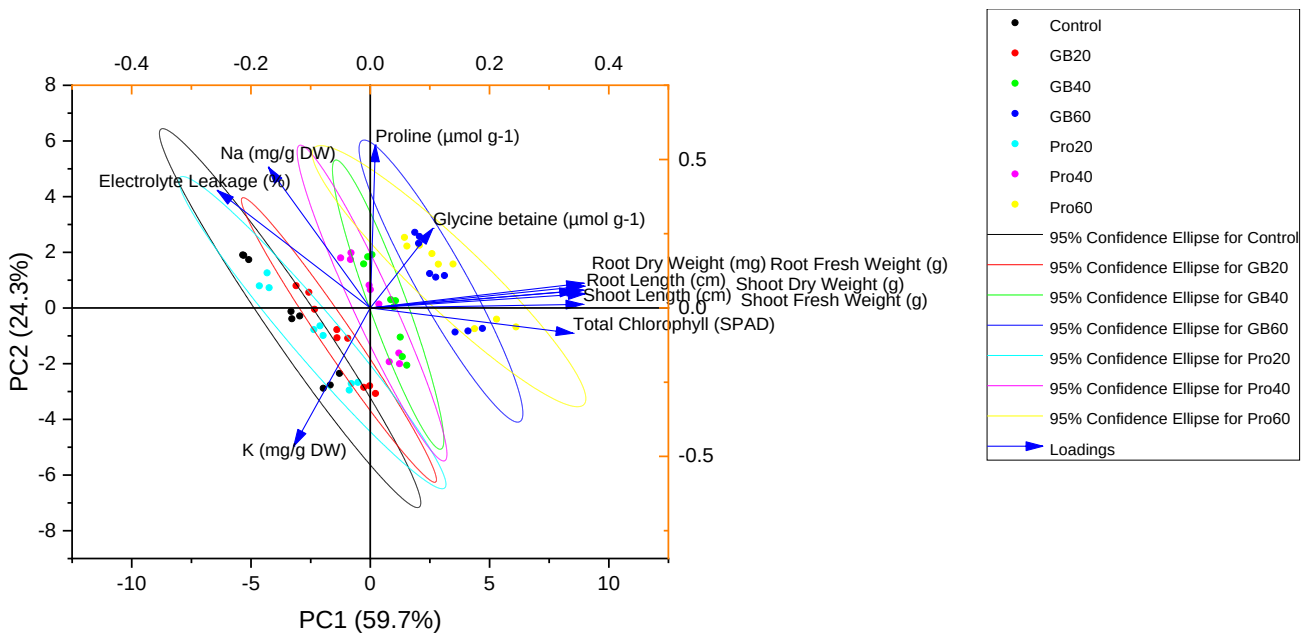

Fig. 4. Principal component analysis for studied attributes.

Principal components analysis: Results showed that there are 12 variables included in the analysis, and the first two principal components account for 84.0% of the total variance in the data. The first principal component (PC1) has an eigenvalue of 7.16724, which explains 59.7% of the total variance in the data. This component is strongly positively associated with all the variables, with the highest loadings on root length (0.36042) and shoot length (0.35667). The second principal component (PC2) has an eigenvalue of 2.91628, which explains 24.3% of the total variance in the data. This component is strongly positively associated with root fresh weight (0.34625) and root dry weight (0.35774), but negatively associated with electrolyte leakage (-0.25653) and Na content (-0.17087). The cumulative percentage of variance explained by the first two components is 84.0%. The other variables have very small eigenvalues and loadings on the first two components, indicating that they do not contribute much to the variance in the data and are not strongly related to the main patterns identified by the analysis. The variables with the highest loadings on PC1 (root length and shoot length) are likely the most important factors contributing to the variability in the data, while those with high loadings on PC2 (root fresh weight and root dry weight) are more strongly associated with a second pattern of variability (Table 2; Fig. 4).

Discussion

Results of current study showed that application of proline and glycine betaine (GB) played a vital role in improvement of growth attributes in maize. This improvement in growth was due to less uptake of Na and Better uptake of K in the maize plants. Proline is an amino acid that plays an important role in plant responses to salinity stress. It has been found to improve shoot length, root length, and fresh and dry weight of shoot and root in maize under salinity stress. One of the ways proline helps in improving these parameters is by acting as an osmoprotectant. Salinity stress causes a water deficit in plants, which leads to the

accumulation of salts in the plant tissues (El Moukhtari *et al*., 2020). Proline helps maintain cellular water balance by accumulating in the cytoplasm and acting as an osmoprotectant, thereby reducing salt stress (Wu *et al*., 2017). Proline also plays a role in stabilizing the structure and function of proteins and membranes in plant cells (Ben Rejeb *et al*., 2014). Salinity stress can cause damage to proteins and membranes, leading to reduced growth and productivity (Jamil *et al*., 2007). Proline helps stabilize these structures and protect them from damage, thereby improving plant growth and productivity. In addition, proline can also regulate the expression of stress-responsive genes, i.e., DREB (dehydration-responsive element binding) genes (Gupta *et al*., 2014), LEA (late embryogenesis abundant) genes, and MAPK (mitogen-activated protein kinase) genes which are involved in various physiological processes related to plant growth and stress tolerance (Sun *et al*., 2021). This can lead to improved root and shoot growth, as well as increased fresh and dry weight of shoot and root. Similar kinds of findings were also noted in the current study where proline was applied as treatment. Glycine betaine (GB) can enhance nutrient uptake by improving root morphology and physiology (Tuteja, 2007), thereby increasing shoot length, root length, and fresh and dry weight of shoot and root (Dawood, 2016). Salinity stress causes damage to the photosynthetic machinery, leading to reduced chlorophyll content and photosynthetic activity. Addition of GB can help protect the photosynthetic machinery from damage by stabilizing the membrane structure and enhancing the activity of antioxidant enzymes, such as superoxide dismutase (SOD) and catalase (CAT) (Haider *et al*., 2021). It also enhances the synthesis of chlorophyll by regulating the expression of genes involved in chlorophyll biosynthesis, such as Mg-chelatase and protochlorophyllide oxidoreductase. In addition to the above, proline regulates the expression of ion transporters involved in Na^+ uptake in plants, i.e., GmHKT1 and GmSOS1 (Bilal *et al*., 2023). By regulating the expression of these transporters, proline can reduce the uptake of Na^+ into plant tissues. It also regulates the regulate hormone signaling in plants, including the signaling pathways of abscisic acid (ABA) and jasmonic acid (JA) (Sofy *et al*., 2020). These hormones play a role in regulating Na^+ uptake in plants, and proline can help regulate their signaling pathways to reduce Na^+ uptake.

Conclusion

Based on data, it appears that increasing the salinity level had a negative effect on the plant growth and biochemical parameters of the tested plants. Foliar applications of proline and glycine betaine have been found to be effective in reducing the adverse effects of salts on maize crops. These applications have been shown to improve the internal nutrient concentration and chlorophyll contents of maize plants, thus enhancing their growth and development. Among the osmolytes tested, 60mM proline and glycine betaine have shown the best results in minimizing the negative effects induced by salts on maize. More research is recommended to declare 60mM proline and glycine betaine as the most effective treatments for maize cultivation in salinity stress under variable agroclimatic conditions.

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