Pak. J. Bot., 55(2): DOI: http://dx.doi.org/10.30848/PJB2023-2(37)

FOLIAR APPLICATION OF MANNITOL AS AN EFFECTIVE APPROACH TO AMELIORATE THE SALT STRESS-INDUCED TOXICITY IN TWO VARIETIES OF MAIZE (*ZEA MAYS* L.)

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Abstract

The present research work was done to assess the role of mannitol in the amelioration of adverse effects of NaCl stress on two maize varieties i.e. Salaar (V1) and LS-787(V2). For this purpose, a pot experiment with Completely Randomized Design (CRD) was conducted at Botanical Garden of University of Gujrat, Gujrat, Pakistan. For this study, 64 pots, half (32) for V1 and half (32) for V2 were filled with river sand. There were four replicates for each treatment. NaCl stress (100mM) was applied at the time of sowing. Full strength Hoagland's nutrient solution was given once in a week. After two weeks of germination, foliar application of mannitol was applied in three levels (25mM, 50mM. 75mM). Data was collected for biochemical, physiological and yield parameters after one month of foliar application of mannitol. Data was analyzed using COSTAT software for Analysis of Variance (ANOVA) and mean values were compared with DMRT. Results indicated a considerable decrease in chlorophyll pigments, antioxidant activities (SOD, POD and CAT), protein and carbohydrate accumulation, anthocyanin contents and yield of both varieties which were not treated with mannitol spray under induced salt stress (100mM NaCl) as compared to the plants that were treated with foliar spray of mannitol under salt stress. There was a rise in the electrolyte leakage in the presence of induced salt stress. Physiological activities of maize were also reduced by the salt stress. This indicated a positive role of mannitol in the amelioration of harmful effects of salt stress in maize.

Key words: Maize, Foliar, Mannitol, NaCl, Growth, Yield, Salt stress.

Introduction

Maize (*Zea mays* L.) belongs to the family Poaceae and it fits in to the Tribe Maydae. It has Mexican and Central American origin. It has above 32000 genes, number of somatic chromosomes is 20 and the size of genome is 2.3 gigabase (Schnable *et al*., 2009). Maize in the form of sweet maize and popcorn is one of the routinely used food crops by humans and also used in renewable energy sector as raw material (Gyori, 2016). Moreover, for human body, maize is a good source of essential minerals and vitamins. To above 4.5 billion people in almost 94 emerging countries, about 30% of the food calories are provided by maize (Shiferaw *et al*., 2011). Because of the quality of nutrients present in maize grains, it fulfills the demands of diet for human, but in the case of production, unfavorable abiotic conditions such as salinity, cold temperature and water stress are main limiting factors in its performance (Fita *et al*., 2015).

Salt stress is among the major abiotic stress that are adversely affecting the growth of crops, especially in arid and semi-arid lands of the globe (Shahzad *et al*., 2019). There is a Worldwide estimate that upto 20 % of the irrigated land of the world is salt – affected (Munns & Gilliham, 2015). Salinization of soil is among the major environmental hazards that reduce the growth and development of plant, leading to excessive loss of yield, especially in arid and semi-arid soils (Porcel *et al*., 2012). In arid areas, soil salinity as a key agricultural problem affects the majority of land capable of being ploughed and used to grow crops. Remarkably influencing on growth and yield of agricultural crops, soil salinization is affecting above 800 million hectares of the total land surface (Shelden *et al*., 2016).

In higher plants, osmolytes and solutes that are responsible for resistance against a wide range of abiotic stresses include alditols and mannitol (Hema *et al*., 2014). Mannitol is a six carbon liquid sugar that is abundantly found in plants and fungi. Mannitol is present widely in plant species in every way and found in more than 70 families (Ruijter *et al*., 2003). Major functions in physiology of plants that include storage of carbon and the defense mechanism against environmental stress are carried out by Mannitol (Iwamoto & Shiraiwa, 2005; Patel & Williamson, 2016). Mannitol certainly has the ability to perform its role as compound which is compatible with cellular mechanism, act as osmoprotectant, protect from heat and avoid oxidation. It is also stated that Mannitol has central importance in decreasing the osmotic stress and the stresses caused by the salts in majority of plant species (Bhauso *et al*., 2014). In the symplast of expanding tissues, there is 64% decline in potassium amount during salinity stress (Shahzad *et al*., 2012). According to an observation, in sandy soils, there is a decrease in height of plant, elongation of root, biomass of shoot and root, harvest index, area of leaf, mitotis, photosynthesis and the yield of straw and grains of maize plants per pot affected by salinity stress (El Sayed, 2011). It has been observed that in maize plants that are under salt stress, photosynthetic pigments and biomass level increases by the foliar application of Mannitol (Kaya *et al*., 2013). So this study was undertaken to find efficacy of mannitol to reduce the effect of salt stress in maize.

Materials and Methods

A pot experiment was performed to study the role of foliar application of mannitol in the amelioration of induced salinity stress on two varieties of maize at the botanical garden of University of Gujrat. Two maize varieties (Salaar and LS-787) were used in this experiment. Salt stress comprising of 100mM NaCl in the solution form was given at the time of sowing. River sand was used to fill the pots. After one week of sowing, germination was started. Thinning in the pots was done after 10 days of germination of plants. For proper irrigation of plants Hoagland's nutrient solution was used one time per week. After two weeks of the germination of maize plants, mannitol was applied in the form of foliar spray in three levels. The application of mannitol was done early in the morning by using manual spray in controlled conditions. 0.1% of Tween-20 solution was also mixed with the mannitol solution for the better absorption of mannitol in the leaves of maize plants.

Following levels of salt stress and mannitol spray were applied to the maize varieties.

T0: Control
T1: 100mM (NaCl)
T2: 25mM (Mannitol)
T3: 50mM (Mannitol)
T4: 75mM (Mannitol)
T5: 25mM (Mannitol) + 100 mM (NaCl)
T6: 75mM (Mannitol) + 100 mM (NaCl)
T7: 50mM (Mannitol) + 100 mM (NaCl)

Data was collected from the current experiment after one month of foliar application of mannitol and given below parameters were noted.

Biochemical parameters were also studied by using the standard protocols. Chlorophyll contents (chl a, b and carotenoids) were measured by using the method given by Arnon (1949). Glycine betaine content was measured by using the method given by Grieve & Grattan, (1983). Antioxidant enzymes activity was also measured by different method, the Superoxide dismutase content was measured by using method described by Giannopolitis and Ries, (1977). Method given by Chance & Maehly, (1955) was used to measure the Catalase activity. In measuring Peroxidase content, each enzyme activity of extract was noted by using the method stated by Bradford, (1976).Malondialdehyde content was measured by the method given by Carmak & Horst (1991). Total carbohydrate content was measured by using the method given by Anthron and total soluble proteins were measured by the using the standard conditions explained by Bradford (1976). The electrical conductivity of plants was measured by using the electrical conductivity (EC) meter. Procedure given by Krizek *et al*., (1993) was used to measure the Anthocyanin content of plants.

The Physiological parameters including photosynthetic rate A (μmol CO_2 m-2s-1), transpiration rate E (mmol H_2Om-2s-1), stomatal conductance gs (mol H_2O m-2s-1) and intercellular CO_2 concentration Ci (μmol CO_2 mol-1) of the maize plants were measured by using IRGA (infrared gas analyzer) apparatus having model LCi Console (ADC) Bioscientific Ltd, serial number 32641. Yield parameters including the number of cobs per plant, number of seeds per cob and 100 grain weight (g) were measured.

Statistical Analysis

Analysis of variance was computed by using the COSTAT computer software. Duncan's New Multiple Range test (DMRT) was used for the comparison of mean values at 5% of probability (Steel & Torrie, 1980).

Results

Results were obtained for the above mentioned biochemical, physiological and yield parameters.

Biochemical parameters: Salt stress significantly decreased the amounts of various biochemical parameters studied in this experiment. Glycine betaine contents significantly increased with the salt stress that is because of the capability of plants to cope with stress conditions (Table 1). The increase in the glycine betaine content showed the activation of mechanism of stress tolerance in these plants. The maximum value of chlorophyll 'a' and carotenoid content was obtained by the application of 50mM and 75mM Mannitol during salt stress In case of chlorophyll 'b' the highest value during salt stress was obtained by application of 50mM mannitol in V1 and 75mM mannitol in V2 (Fig. 1A-D). The highest value for protein content of both varieties under salt stress was obtained by application of 75mM mannitol. For carbohydrate contents, 50 and 75mM was effective in both varieties for the amelioration of the adverse effect of salt stress (Table 2). The Malondialdehyde content was also increased by the spray of mannitol. This showed the improved tolerance of maize plants against salt stress (Fig. 1E). For the reduction of electrolyte leakage during salt stress, the most effect treatment was 75mM mannitol spray. During salt stress, the anthocyanin contents were also improved by the foliar spray of mannitol especially 25mM and 75mM mannitol. In terms of antioxidant enzymes, 50 and 75 mM manitol spray was effective for increasing the amounts of catalase and peroxidase. All mannitol treatment (25mM, 50mM and 75mM) were effective equally in increasing the amount of Superoxide dismutase (Fig. 1F-K).

Physiological parameters: Induced salt stress of 100mM NaCl significantly reduced the physiological parameters of both varieties of maize. The photosynthetic rate, transpiration rate and stomatal conductance was significantly increased by the application of 75mM mannitol under salt stress conditions (Table 3). Intercellular CO2 Concentration was also improved by the foliar application of 50 and 75mM mannitol under saline conditions (Fig. 2M-Q). This showed the improved tolerance of maize varieties against the salt stress. The application of mannitol turned out to be very effective to improve the physiological parameters of plants in stress conditions and allow them to better survive.

Table 1. Mean squares of ANOVA for various biochemical parameters of Maize (*Zea mays* L.) under NaCl and mannitol treatment.

Sources	df	MS of glycine betaine content	MS of chlorophyll 'a'	MS of chlorophyll 'b'	MS of carotenoid content	MS of protein content	MS of carbohydrate content	MS of MDA content
Salt	1	0.0190***	6.6424***	6.6865*	3.4222ns	0.0265**	0.0029***	62.679***
Mani	3	0.0173***	1.5401***	2.0089***	0.0280***	0.1611***	0.0094***	29.038***
Var	1	3.4689***	1.4792ns	0.0016***	0.0017*	0.0048ns	3.9062ns	55.943***
Salt x Mani	3	0.0176***	3.0195***	0.0011***	9.9948*	0.1185***	0.0013***	70.662***
Salt x Var	1	3.3764***	8.0918ns	2.2017***	9.6874ns	0.0025ns	7.6562ns	0.1076ns
Mani x Var	3	6.7643***	8.6339ns	6.6962ns	1.6650ns	0.0052ns	4.4768***	5.6818*
Salt x Mani x Var	3	4.3293***	3.9136ns	1.8459***	7.1757*	0.0109*	0.0010***	19.586***
Error	48	1.5911	6.0738	1.1651	2.5082	0.0030	6.0291	1.4167
Total	**63**							

Table 2. Mean squares of ANOVA for various biochemical parameters of Maize (*Zea mays* L.) Under NaCl and mannitol treatment.

Sources	Df	MS of electrolyte leakage	MS of anthocyanin	MS of catalase	MS of peroxidase	MS of superoxide dismutase
Salt	1	8.0827*	3.1220ns	0.0084**	0.0105***	0.0059***
Mani	3	55.733***	2.8517*	0.0057***	0.0075***	0.0039***
Var	1	93.887***	8.649**	0.0089**	0.0102***	6.0025*
Salt x Mani	3	38.177***	2.6711*	0.0153***	0.0044***	0.0064***
Salt x Var	1	1.3040ns	8.3265ns	8.2656ns	2.9326ns	0.0013***
Mani x Var	3	38.627***	2.4426ns	9.1858ns	0.0030**	0.0041***
Salt x Mani x Var	3	1.6155ns	2.1404ns	0.0014ns	0.0010ns	0.0022***
Error	48	1.5851	9.3113	8.6475	6.3754	9.8802
Total	**63**					

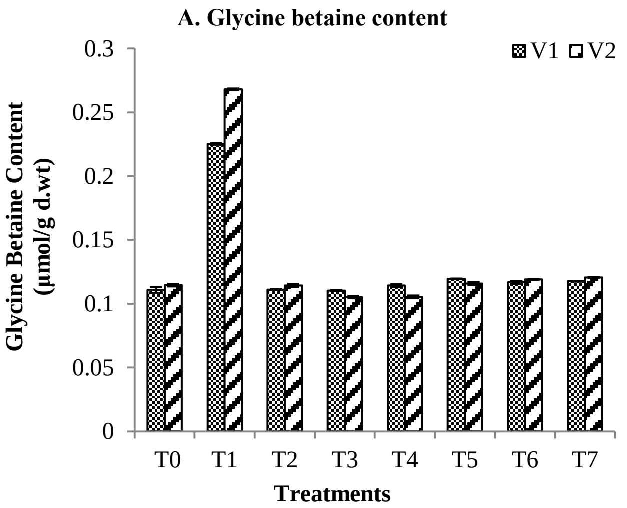
Table 3. Mean squares of ANOVA for physiological parameters of Maize (*Zea mays* L.) under NaCl and mannitol treatment.

Sources	df	MS of photosynthetic rate	MS of transpiration rate	MS of stomatal conductance	MS of intercellular CO_2 concentration	MS of water use efficiency
Salt	1	35.0908*	0.1216***	3.0625ns	35.4024**	62.2078*
Mani	3	35.1776**	0.3806***	8.2083***	11.0068ns	176.174***
Var	1	48.9825*	16.090***	0.0115***	73.96***	17649.4***
Salt x Mani	3	31.0122**	0.1692***	6.1875***	34.3462***	75.9637**
Salt x Var	1	14.8321ns	0.0129ns	2.25ns	0.03062ns	52.7252ns
Mani x Var	3	3.73850ns	0.0473**	1.9375ns	0.95791ns	54.7622*
Salt x Mani x Var	3	4.01612ns	0.1111***	2.625*	7.78270ns	106.053***
Error	48	7.25262	0.0084	8.3333	3.95927	15.0536
Total	**63**					

Table 4. Mean squares of ANOVA for yield parameters of Maize (*Zea mays* L.) under NaCl and mannitol treatment.

Sources	Df	MS of No. of cobs/Plant	MS of No. of grains/cob	MS of 100 grains weight (g)
Salt	1	0.25ns	467.64***	1.2825
Mani	3	0.9375**	1887.4***	4.8389
Var	1	0ns	159.39*	2.2575
Salt x Mani	3	0.5416ns	2034.7***	2.8018
Salt x Var	1	0.0625ns	0.0156ns	0.1463
Mani x Var	3	0.0416ns	35.807ns	0.8741
Salt x Mani x Var	3	0.0208ns	122.09*	0.2165
Error	48	0.1979	37.671	0.3887
Total	**63**			

Yield parameters: The effect of mannitol spray on maize varieties was very effective in the amelioration of adverse effects of salt stress. The yield of maize plants was also highly improved by the foliar application of mannitol despite of the induced salt stress (Table 4). The number of cobs per plant, grains per cob and 100 grains weight was also significantly reduced by the 100mM NaCl (Fig. 3R-T). However, the application of 50mM mannitol spray was effective for improving yield of maize. But the most effective treatment was 75mM Mannitol for the improvement of these yield parameters.



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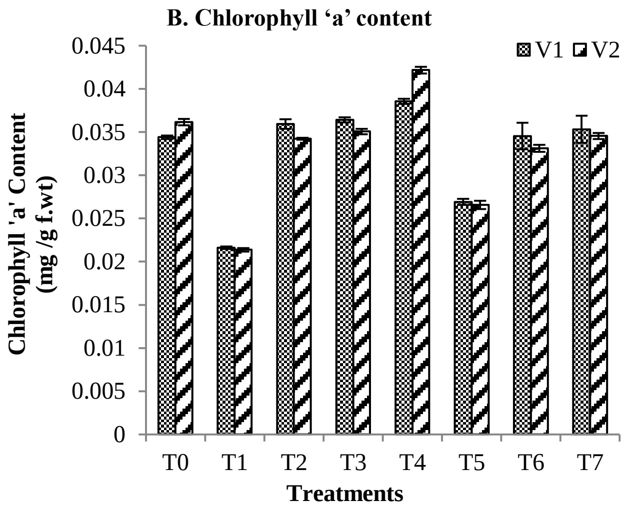

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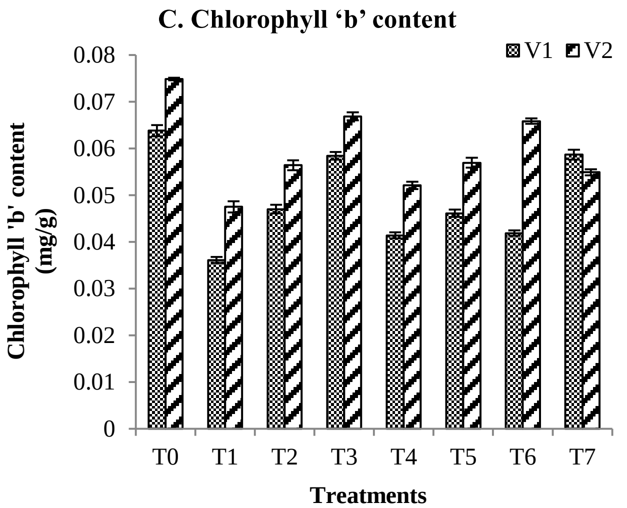

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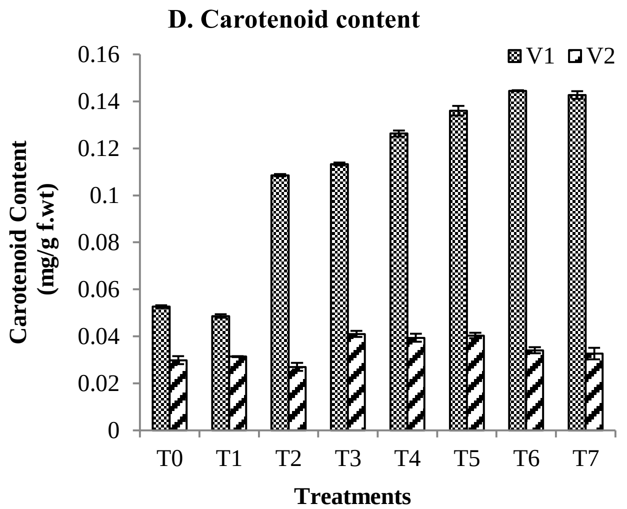

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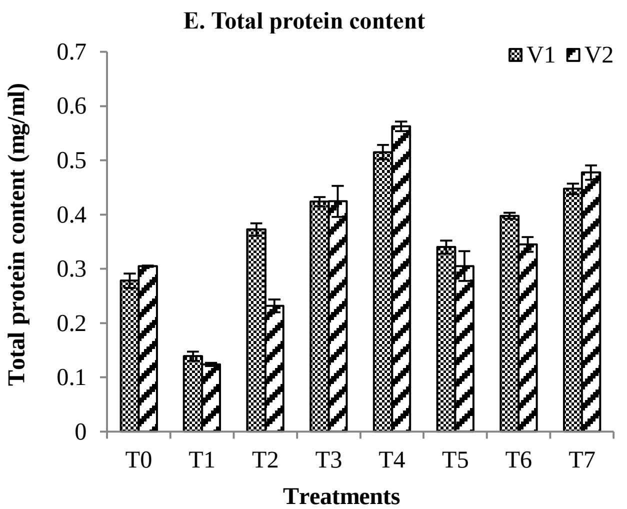

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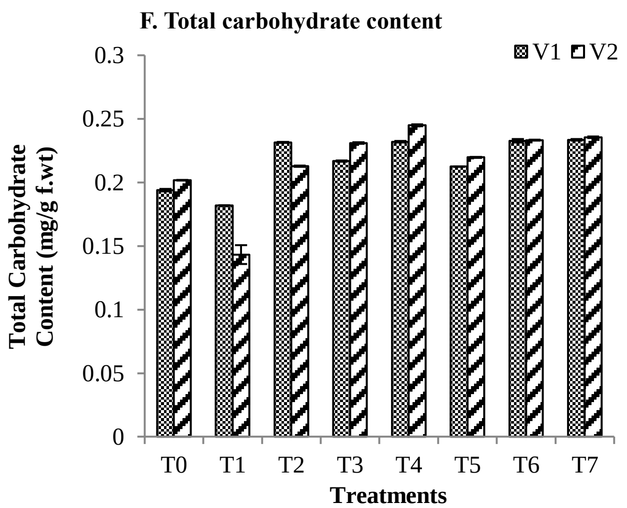

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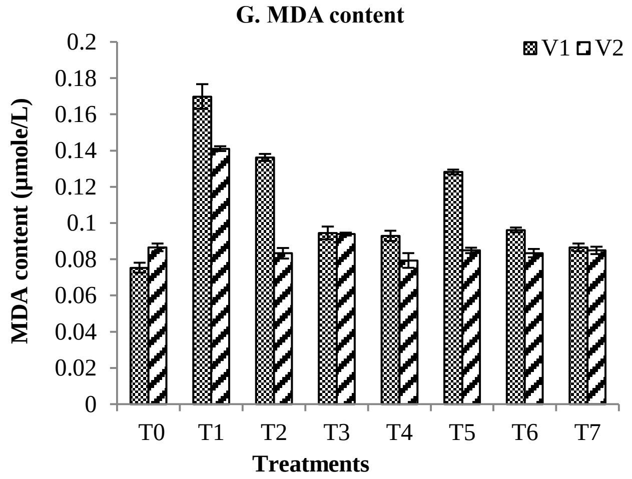

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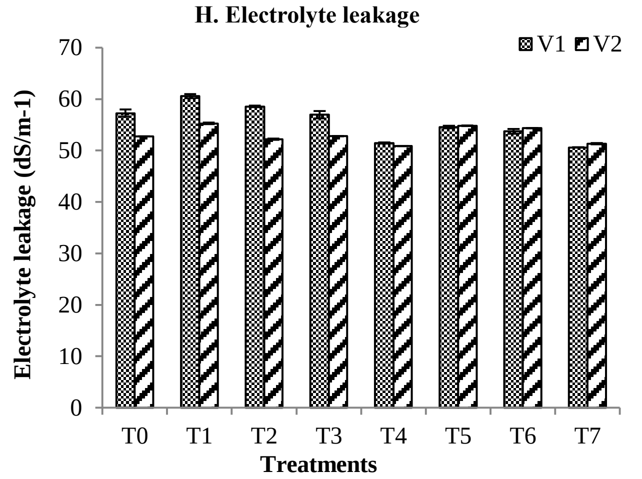

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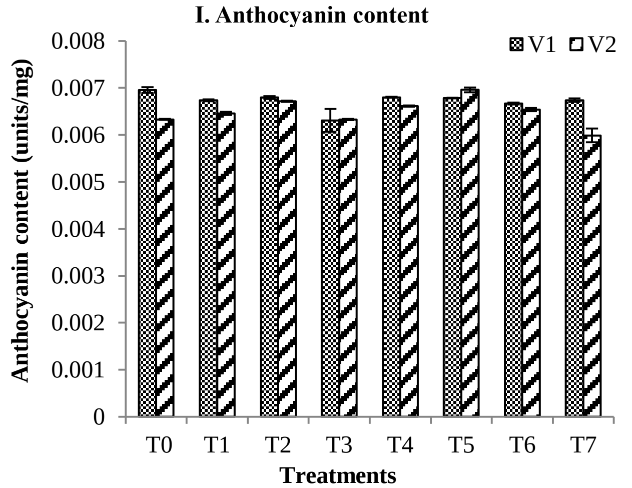

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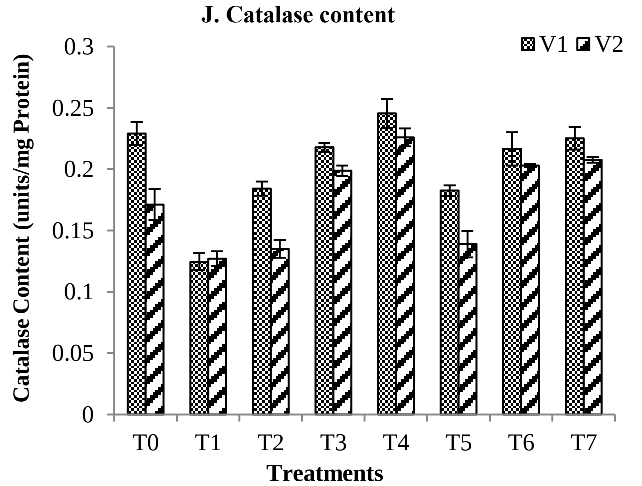

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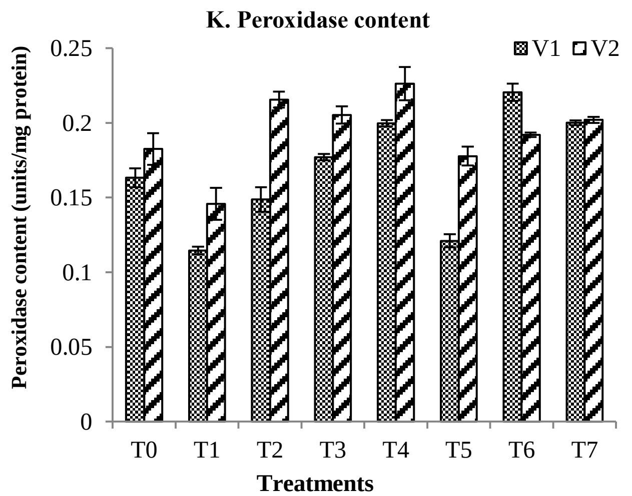

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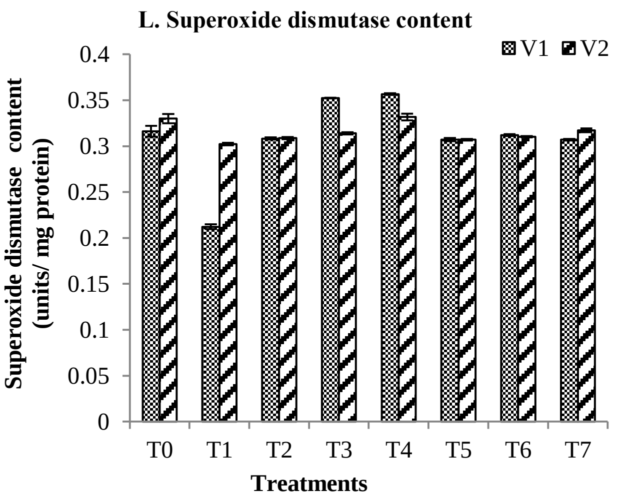

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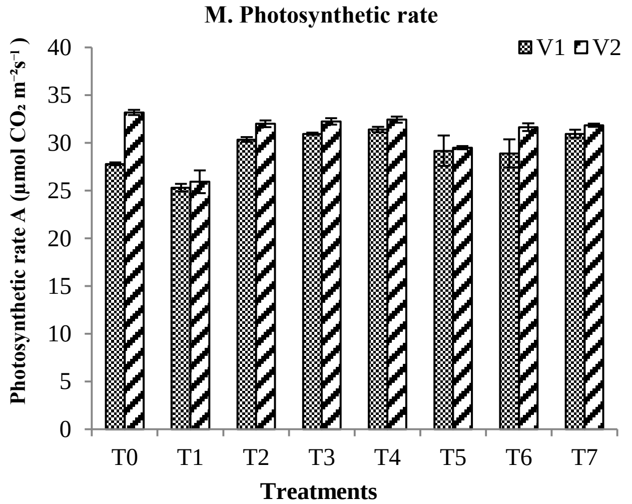
Fig. 1. Effect of various treatments of salt and mannitol on biochemical parameters of maize.



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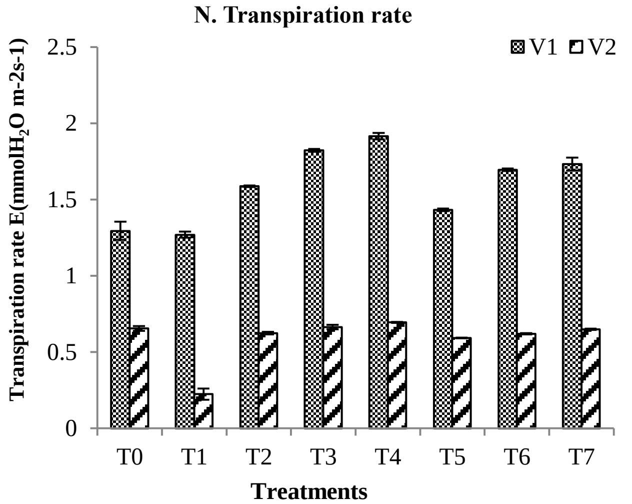

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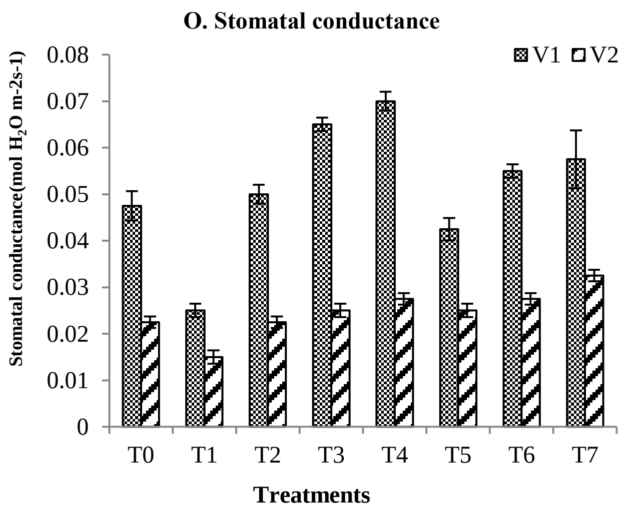

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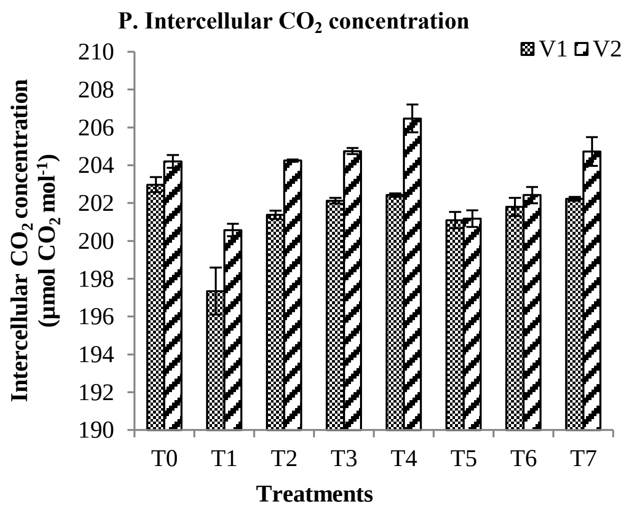

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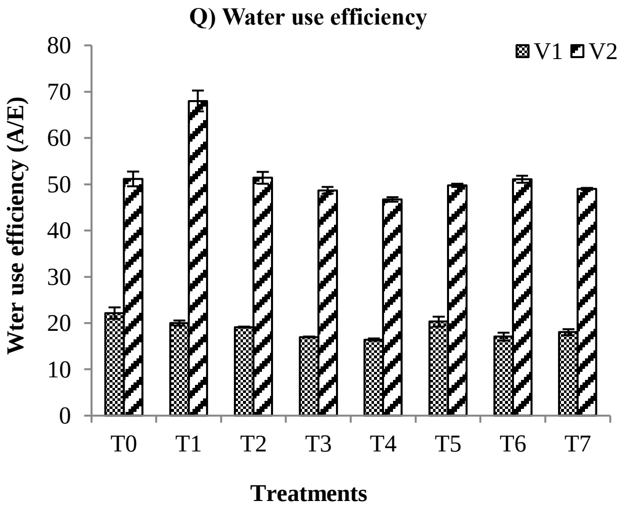

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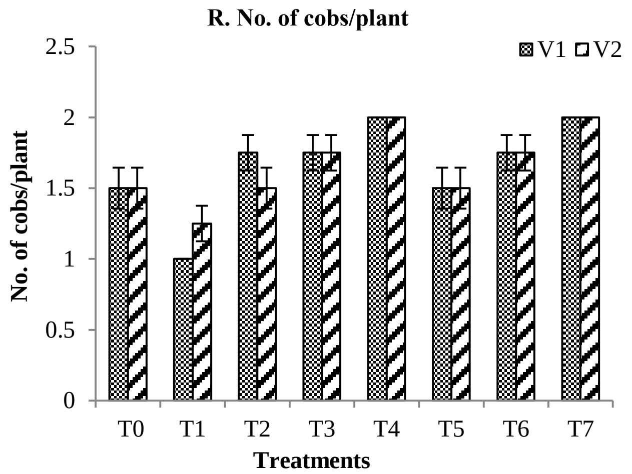
Fig. 2. Effect of various treatments of salt and mannitol on physiological parameters of maize.



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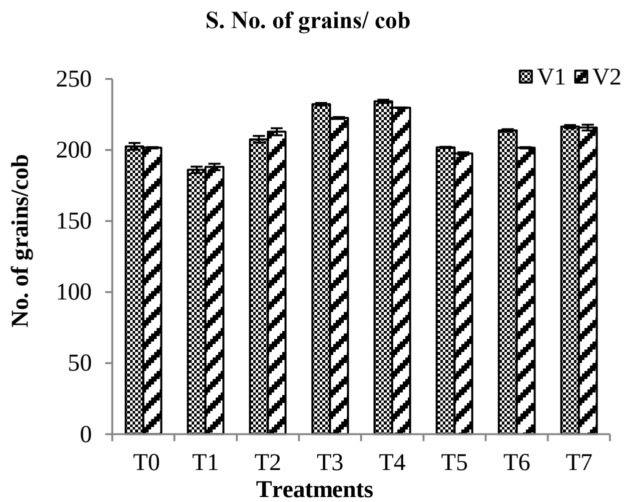

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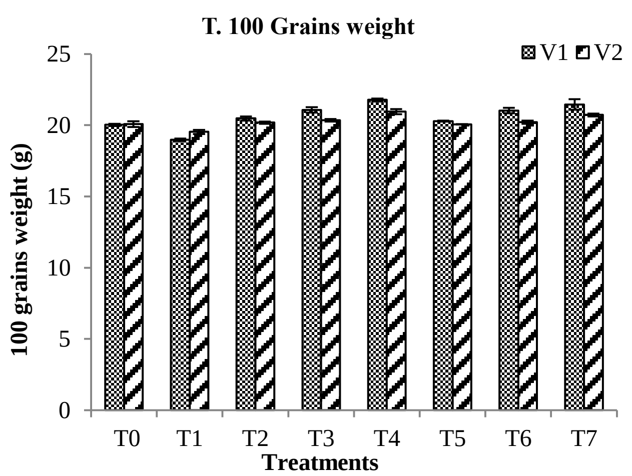

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Fig. 3. Effect of various treatments of salt and mannitol on yield parameters of maize varieties.

Discussion

Results obtained from our pot experiment clearly showed that salt stress had adverse effects on the growth and yield of Maize (*Zea mays* L.) plants and these harmful effects of salinity were ameliorated by the foliar application of mannitol. Application of mannitol was found effective for both varieties of maize (*Zea mays* L.) i.e. Salaarand LS-787 (V2) used in this experiment. The findings of our experiment were correlated with the work of El-Sayed (2011) who stated that in maize plants grown in sandy soils and are under salinity stress, there was a decrease in photosynthesis and yield of straw along with grains of maize per pot. The advantageous effects of mannitol on maize varieties under normal as well as stress conditions were obvious in our experiment.In case of biochemical parameters of both varieties of maize, there was a remarkable decrease by the application of NaCl stress. By the application of mannitol as foliar spray, these adverse effects of NaCl stress were ameliorated to great extent. There was an increase in the Glycine betaine content of the maize plants in response to the applied salt that depicted the activation of salt stress mechanism in those plants. The findings of our research are correlated with results of Wang *et al*., (2003) that showed that the elevation of accumulation of glycine betaine contents in plants which are salt stress tolerant as compared to salt sensitive plants. Similar results were obtained by the studies done on mulberry, sorghum, wheat and *Haloxylonrecurvum.*In our experiment when NaCl stress was induced to the plants, there was a substantial increase in the production of bioactive substances mostly Carotenoids. V_2 showed more positive response to the application ofoliar spray of mannitol to elevate the amount of carotenoids. This depicted the improved stress tolerance ability in maize plants by the application of mannitol. Similar to our experiment, when we correlated the studies of Kim *et al*., (2008) with our results they indicated that under NaCl-irrigated water there was an increase in the bioactive compounds mostly carotenoids and among them the β-carotene content increased to 37% in Romaine lettuce showing the improved defense system of plants against salt stress. The application of mannitol spray during salt stress also enhanced the chlorophyll pigments of maize plants. Both Chlorophyll ‘a’ and ‘b’ amounts were elevated by the foliar spray of mannitol, thus ameliorating the adverse effects of induced salt stress in maize plants. This ultimately increased the growth of plants. The results of our experiment are correlated with the results of Habiba *et al*., (2019) which showed that the foliar spray of mannitol on maize cultivars remarkably improved the chlorophyll pigments and carotenoids as compared to those that were not sprayed with mannitol under Cr-stressed plants. Electrolyte leakage of maize plants was higher in the NaCl stress as compared to the plants without the stress. The applied foliar spray of mannitol significantly decreased the electrolyte leakage of the plants of both maize varieties used in our research. Correlated with Kaya *et al*., (2013) work that showed that the Electrolyte leakage of NaCl stressed plants was distinctly high as compared to the plants that are not under NaCl stress. The foliarly applied mannitol and thiourea to the salt stressed maize plants decreased the electrolyte leakage in the leaves as compared to the plants that are not given the mannitol or thiourea treatment during salt stress. The application of mannitol showed significant improvement in the Peroxidase (POD), superoxide dismutase (SOD) and catalase (CAT) activity in both maize varieties. There was an elevation in the CAT, POD and SOD amounts which showed the activation of the defense mechanism in maize plants against salt stress. The results were comparable with the studies of Seckin *et al*., (2009) that showed that there was an improvement in the activities of antioxidant enzymes including SOD, POX, CAT, APX and GR in wheat plants with pretreatment of mannitol during salt stress. Mannitol also improved the physiological parameters of plants during salt stress as well as in normal conditions. Our results showed significant elevation in stomatal conductance (Gs) and transpiration rate (E) of both varieites of maize by the foliar spray of mannitol. The effect of salt stress was reduced remarkably by the applied different levels of mannitol. Our results were matched with the results of work done by El-katony *et al*., (2019) which depicted that transpiration (E) and Stomatal conductance (Gs) were comparable in the two hybrids of maize. Salt stress effectively decreased the Gs and E in both maize hybrids. With the applied salt stress of 100mM NaCl on both varieties of maize in our research there was a significant increase in the MDA content of plants under salt stress. It was seen that there was a noticeable decline in the MDA content of plants under salt stress which are foliarly sprayed with mannitol. This proved that amelioration of the effects of induced salt stress on maize plants.

Ceccarini *et al*., (2019) also elaborated in there research work that the remarkable reduction in MDA showed the strongest amelioration of the effects of salt stress in maize plants. These results are matched with the findings of our experiments. The results of our research depicted that the accumulation of soluble proteins and carbohydrate was also effectively reduced by 100mM NaCl stress. However, the application of mannitol improved the soluble proteins and carbohydrate contents. Our research clearly proved the positive effects of mannitol on maize plants under induced salt stress. The results are same as obtained by Zorb *et al*., (2004) in which it was concluded that the maize plants grown under less salt stress of 25mM gone through the shoot and root proteins differential regulation by 31 and 45% respectively but in case of high salt stress of 100mM NaCl, it went cross 80% of uncontrolled change in total protein content. Mannitol also gives benefits to the maize plants in term of increase in the yield of both varieties under normal as well as salt stress conditions. Results of our research showed that the applied salt stress of 100mM NaCl decreased the number of cobs/plant, number of grains/cob and 100 grains weight in both varieties of maize used in our experiment. This analysis was correlated by the experiments performed by Farooq *et al*., (2015) that showed the reduced grain weight and grain number under salt stress was responsible for poor yield of maize plants.

Conclusion

It is concluded that salt stress harmfully affected the growth of two maize varieties. The increase in the growth of plants was observed by the foliar application of mannitol under both normal and salt stress conditions. This indicated the activation of defense mechanism in these plants against salt stress. Foliar application of mannitol not only activated the salt stress mechanism in maize plants but also improved the growth and yield of plants. V1 was more adversely affected by applied salt stress of 100mM of NaCl as compared to V2. Although all treatments of mannitol (25mM, 50mM and 75mM) had significant positive impact on growth of maize plants, but the most effective treatment under normal conditions as well as salt stress conditions for significant improvement in plant growth and yield was foliar application of 75mM mannitol. LS-787 (V2) was more capable of tolerating the applied salt stress and it showed more positive results after foliar application of mannitol. By the analysis of results obtained by present research work it is concluded that the foliar application of 75mM mannitol is highly effective to ameliorate the harmful effects of salt stress. In areas where the salt stress is common, and there is continuous reduction in yield of maize, mannitol foliar spray is recommended for the improvement of biomass and yield.

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(Received for publication 6 July 2021)