Ann. For. Res. 66(1): 3357-3370, 2023
ISSN: 18448135, 20652445

ANNALS OF FOREST RESEARCH
www.e-afr.org

EFFECT OF FOLIAR APPLICATION OF ASCORBIC ACID AND *GIBBERELLIC* ACID ON MAIZE UNDER SALT STRESS

Hira Fatima[1], Ameer Khan[1,2], Muhammad Nadeem[3]

Addresses:

[1]Department of Botany, University of Sargodha, Sargodha, Pakistan

[2]Department of Botany, University of Education, Faisalabad, Pakistan

[3]Institute of Food Science and Nutrition, University of Sargodha, Sargodha, Pakistan

Corresponding Author: Hira Fatima (hiramakhdom@gmail.com)

Abstract

Maize is a vital cereal crop for mankind all over the world but its yields get affected by number of ecological stresses. Corn production is sensitive to abiotic issues; salinity reduces development of corn crops. This experiment was performed to check relation of salinity with maize and effect of foliar spray of ascorbic and gibberellic acid under saline and non-saline conditions. The highest value (193.11 µg g^{-1} F.Wt.) of proline was observed in Akbar at 110 mM NaCl stress and under foliar spray of ascorbic acid (240 ppm) and gibberellic acid (220 ppm) in combination, as the accumulation of proline help the plant against reactive oxygen species so this increase is helpful for plants to combat stress. The highest value (5.05 Unit g^{-1} F.Wt.) of ascorbate peroxidase was observed in Akbar at 110 mM NaCl stress and under foliar spray of ascorbic acid (120 ppm) and gibberellic acid (110 ppm) in combination and increase in levels by foliar sprays also play role in plants defence. The lowest value of total soluble protein (1.13 mg g^{-1} F.Wt.) was observed in Akbar at 110 mM NaCl without any exogenous spray as high salt stress break down proteins into amino acids and as a result concentration of amino acid increase due to salt stress. The exogenous application of ascorbic acid (240 ppm) and gibberellic acid (220 ppm) in combination was helpful for plants against the effects of salinity and improved the proline and ascorbate peroxidase in leaves of both maize varieties at vegetative stage under stress conditions.

Keywords: Maize; Ascorbic acid, salt stress, gibberellic acid

Introduction

Salt stress is one of the biggest barrier to the world for food production. In South Asia, the continued expansion of salt-contaminated soils is of greater concern due to the rising demand for food to rapidly growing population. Globally, there are approximately one billion hectares of salt-contaminated land (Harper et al. 2021). Physiological and biochemical process become alters under saline conditions ultimately reduced the growth of the plants (Khan et al. 2011). Saline condition disturb the physiological responses and alter the ion balance, stomatal behavior and photosynthetic ability and cause the growth decrease (Al-Taie et al. 2013). Ionic stress and osmotic stress can be caused in the plants due to salinity in soil. In the first phase plant suffer from osmotic stress which results in physiological drought of plants. In the second phase, sodium and chloride ions enter in the plant body and disturb the cell organelle (Ahmadizadeh et al. 2016). Different types of reactions are observed by different cereal plants in response to level and duration of salinity (Da-Silva et al. 2008).

As maize is a vital cereal crop for mankind throughout the world but its yields get affected by number of ecological stresses (Lesk et al. 2016). Corn production is sensitive to abiotic issues, dehydration, heat, salt concentration, aqueous soil, wind, and cold on a large scale (Hara et al. 2012). Maize show different response towards salinity according to its growth stages and period of salt exposure (Zhang et al. 2020). Salinity reduces development and lowers the yield of corn crops (Farooq et al. 2015).The physiological processes of plants are mutated by salinity stress (Negrão et al. 2017). Both sodium and chloride are main ions that form diverse physiological process (Tavakkoli et al. 2010).

Total soluble proteins are the most metabolically active proteins known in plants while sodium toxicity reduces proteins. Reduction in amount of total soluble proteins may be due to decrease in the amount of synthesis or due to increase in protein hydrolysis under salinity stress (Farhangi-Abriz & Ghassemi-Golezani, 2016). Ionic imbalance occur in plants facing salt stress so to maintain this ionic imbalance plants try to synthesize these proteins (Hasegawa, 2013). Decrease in protein concentration enhances the production of total free amino acids because of breakdown of proteins into amino acids under stress conditions (Ali et al. 2009).

Various scientists have employed various methods to lessen the harmful impacts of salinity in plants. Different chemicals like AsA, SA, and trehallose are used for resistance in plants against stress conditions (Hossain et al. 2014). Ascobin can also stimulate the growth of plants which was previously inhibited by salinity (Sadak et al. 2013). The harmful impacts of reactive oxygen species on vegetation developments can be alleviated by treating various modifications such as germination-promoting ions, amino groups, and antioxidants chemicals (George et al. 2012). Among various techniques exogenous applications of osmoprotectants and antioxidants like ascorbic acid is very powerful method to alleviate the salinity consequences. It is considered that foliar application of AsA can efficiently control antioxidative metabolism in crops. External application of AsA can be done by sprinkling, pre-treatment of seeds and application by rooting medium (Athar et al. 2009). GA_3 also has potential to enhance the performance of crops under normal conditions. Foliarly sprayed GA_3 also lowers the intensity of Na^+, so in this study the performance of ascorbic acid and gibbererelic acid is checked on maize plants with and without salt stress as well as with and without different foliar sprays.

Materials and methods

This study was conducted in department of botany, University of Sargodha, which is situated in Sargodha city of Pakistan. The seeds of two maize varieties Sahiwal-2002 (Tolerant) and Akbar (Sensitive) were collected from Maize and Millets Research Institute, Yusafwala, Sahiwal. Experiment was performed in plastic pots by creating two levels of Nacl salt treatments i.e. 55 and 110 mM. After 2 weeks of seeds germination foliar spray of ascorbic acid and gibberellic acid in separate as well as in combination forms was done on the plants. The concentration of ascorbic acid was kept 120 and 240 ppm and the concentration of gibberellic acid was kept 110 and 220ppm. A combination of these two foliar sprays was also used to check its effects on maize

The Bates's method (1973) was used to measure proline contents in maize. Leaves (0.5 g) were finely grated using 10 mL of 3% sulfosalicylic acid following a filteration by by Whatmam filter paper (WFP, No. 2). 2 mL of this filtrate was mixed with 2 mL of acid ninhydrin solution (This acid ninhydrin solution was prepared when 1.25 g ninhydrin was dissolved in 20 mL of 6 molar orthophosphoric acid and 30 mL of glacial acetic acid) and 2 mL of glacial acetic acid. This mixture was heated in water bath at 100 degree Celsius for 1hour. Then mixture was cooled by using ice bath and 10 mL toluene was added which forms a chromophore, air was passed continuously for 1 to 2 minutes so that aqueous phase can be separated from chromophore. This isolated colored phase was allowed to appear at room temperature for 2 to 3 minutes. By using spectrophotometer absorbance was recorded at 520 nm. In the experiment we used toluene for taking reading of blank.

Method decribed by Cakmak, (2002) was used for determining of ascorbate peroxidase activity. At 290nm decrease in ascorbic acid absorbance in 1 mL reaction mixture was noticed. Total soluble proteins were measured by following the method of Lowry *et al.* (1951). Hamilton & Van-Slyke's method (1943) was used to measure total free amino acid.Fresh leaves (~0.5 g) were taken for extraction using 0.2 molar phosphate buffer of 7.0 pH. Reaction mixture containing extract (1.0 mL), pyridine (1 mL) and ninhydrin (1 mL) was kept in water bath for heat and were diluted to 50 ml by distilled water. Spectrophotometer (Hitatchi, 220, Japan) was used to record readings at 570 nm. Leucine was used to develop standard curve for detection of total free amino acids in each sample.

Statistical analysis. For analysis of variance (ANOVA) along with least significant difference we used Statistix 8.1. (Steel et al. 1997).

Results

TABLE 1. Analysis of variance (ANOVA) for proline, ascorbate peroxidase, total soluble proteins content and total free amino acid contents of maize varieties Sahiwal 2002 and Akbar at vegetative stage, under different levels of foliar sprays of ascorbic acid and gibberellic acid when control and saline conditions were created.

Source	df	Proline	Ascorbate peroxidase	Total soluble proteins content	Total free amino acid contents
Variety (V)	**1**	22910.5^{**}	26.3260^{**}	10.022^{**}	1054.50^{**}
Stress (S)	**2**	82773.7^{**}	71.3843^{**}	170.275^{**}	3723.85^{**}
Foliar Spray (F)	**6**	2363.4^{**}	1.6057^{**}	19.806^{**}	69.32^{**}
V*S	**2**	6473.1^{**}	4.8180^{**}	0.294^{NS}	336.94^{**}
V*F	**6**	53.9^{**}	0.0008^{NS}	0.043^{NS}	15.28^{**}
S*F	**12**	19.4^{NS}	0.0011^{NS}	0.154^{NS}	5.67^{NS}
V*S*F	**12**	30.2^{*}	0.001^{NS}	0.092^{NS}	10.88^{**}

Error	**84**	13.6	0.0065	0.117	4.85

Note: *=0.05, **=0.01 and NS=non-significant

Proline contents

Combined factorial analysis of variance (variety, salt stress and spray) for proline contents of two maize cultivars is presented in table 1. Proline contents were low without salt stress but enhancement in salinity level significantly enhanced the level of proline contents in both maize varieties without any exogenous spray (Figure 1). Moreover, Akbar performed better in regard to proline contents as compare to Sahiwal-2002 under saline conditions.

The maximum increase in proline contents was observed when ascorbic acid (240 ppm) and gibberellic acid (220 ppm) were applied in combination on both varieties while Akbar performed better as compared to Sahiwal-2002 with respect to proline contents under non saline conditions. Combine application of ascorbic acid (240 ppm) and gibberellic acid (220 ppm) as a foliar spray enhanced proline contents more in Akbar as compare to Sahiwal-2002 at salinity level 55 mM as compared to other treatments. In both varieties combine effect of ascorbic acid (240 ppm) and gibberellic acid (220 ppm) spray increased the level of proline contents, while Akbar performed better as compared to Sahiwal-2002 with respect to proline contents respectively at 110 mM NaCl which is the highest level of salinity (Figure 1).

The highest value (193.11 µg g^{-1} F.Wt.) of proline was observed in Akbar at 110 mM NaCl stress and under foliar spray of ascorbic acid (240 ppm) and gibberellic acid (220 ppm) in combination. The lowest value of proline (43.19 µg g^{-1} F.Wt.) was observed in Sahiwal-2002 at non saline condition without any exogenous spray. Overall, it is concluded that the combined foliar application of ascorbic acid (240 ppm) and gibberellic acid (220 ppm) improved the proline contents in leaves of both maize varieties at vegetative stage under stress conditions.

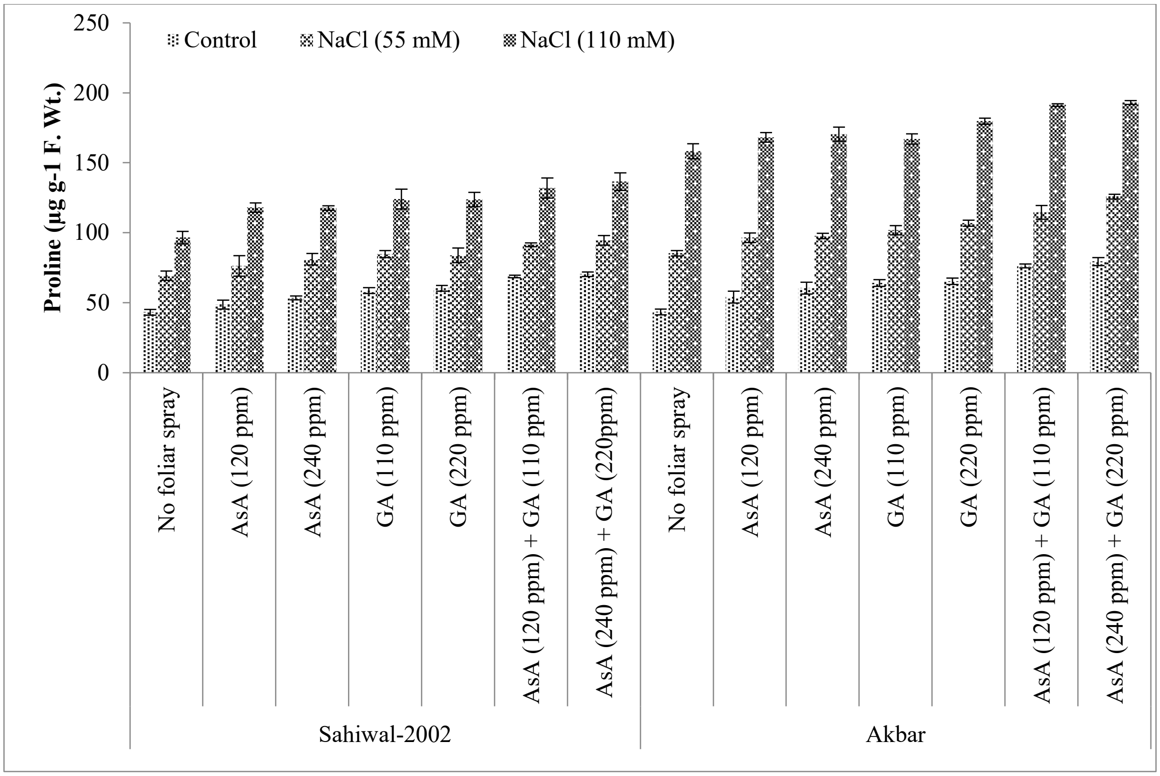

Figure 1. Effect of foliar applications of ascorbic acid and gibberellic acid on proline contents in leaves of maize varieties at vegetative stage under saline and non saline conditions

Ascorbate peroxidase activity

Combined factorial analysis of variance (variety, salt stress and spray) for ascorbate peroxidase contents of two maize cultivars is presented in table 1. Ascorbate peroxidase contents were low without stress but increase in salinity level significantly enhanced the level of ascorbate peroxidase contents in both maize varieties without any exogenous spray (Figure 2). Moreover, Akbar performed better in regard to ascorbate peroxidase contents as compare to Sahiwal-2002 under saline conditions.

The maximum increase in ascorbate peroxidase was observed when ascorbic acid (240 ppm) and gibberellic acid (220 ppm) were sprayed as combine effect in Sahiwal-2002 under non-saline conditions while combine effect of ascorbic acid (120 ppm) and gibberellic acid (110 ppm) spray showed the maximum ascorbate peroxidase increase in Akbar under non-saline conditions. The maximum increase in ascorbate peroxidase contents was observed when ascorbic acid (120 ppm) and gibberellic acid (110 ppm) were foliarly applied in combination while Akbar performed better then Sahiwal-2002 at 55 mM and 110 mM NaCl conditions (Figure 2).

The highest value (5.05 Unit g^{-1} F.Wt.) of ascorbate peroxidase was observed in Akbar at 110 mM NaCl stress and under foliar spray of ascorbic acid (120 ppm) and gibberellic acid (110 ppm) in combination. The lowest value of ascorbate peroxidases (0.64 Unit g^{-1} F.Wt.) was observed in Sahiwal-2002 at non saline condition without any exogenous spray. Overall, it is concluded that the combined foliar application of ascorbic acid (120 ppm) and gibberellic acid

(110 ppm) improved the activity of ascorbate peroxidase in leaves of both maize varieties at vegetative stage under stress conditions.

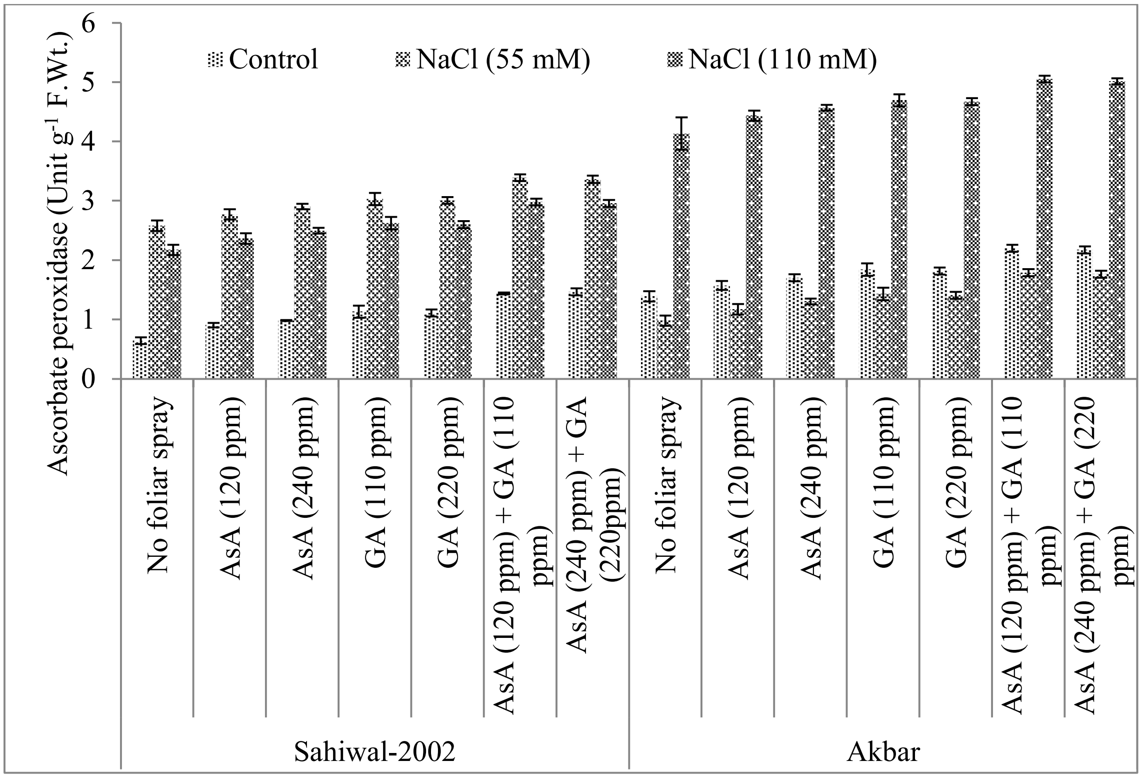

Figure 2. Effect of foliar applications of ascorbic acid and gibberellic acid on activity of ascorbate peroxidase in leaves of maize varieties at vegetative stage under saline and non saline conditions

Total soluble protein contents

Combined factorial analysis of variance (variety, salt stress and spray) for total soluble proteins content of two maize cultivars is presented in table 1. Total soluble protein contents were high in both maize varieties under non-saline conditions but increase in salinity level significantly decreased the level of total soluble protein contents in both maize varieties without any exogenous spray (Figure 3). Moreover, Sahiwal-2002 performed better in regard to total soluble protein contents as compare to Akbar under saline conditions.

The maximum increase in total soluble proteins was observed when ascorbic acid (120 ppm) and gibberellic acid (110 ppm) were foliarly applied as combination in Sahiwal-2002 under non-saline conditions while combine effect of ascorbic acid (240 ppm) and gibberellic acid (220 ppm) showed the maximum total soluble proteins content increase in Akbar under non-saline conditions. Combine application of ascorbic acid (240 ppm) and gibberellic acid (220 ppm) as a foliar spray enhanced total soluble protein contents more in Akbar as compare to Sahiwal-2002 at salinity level 55 mM as compare to other treatments. The maximum increase in total soluble protein contents was observed when gibberellic acid (110 ppm) was applied, while Akbar showed maximum increase at ascorbic acid (120 ppm) and gibberellic acid (110 ppm) in combination spray with respect to total soluble proteins content at 110 mM NaCl (Figure 3).

The highest value (8.55 mg g-1 F.Wt.) of total soluble proteins was observed in Sahiwal-2002 at non saline condition with foliar spray of ascorbic acid (120 ppm) and gibberellic acid (110 ppm) in combination. The lowest value of total soluble protein (1.13 mg g^{-1} F.Wt.) was observed in Akbar at 110 mM NaCl without any exogenous spray. Overall, it is concluded that the foliar application of gibberellic acid (110 ppm) mitigated the negative effects of salinity and improved the total soluble protein contents in leaves of both maize varieties at vegetative stage under stress conditions.

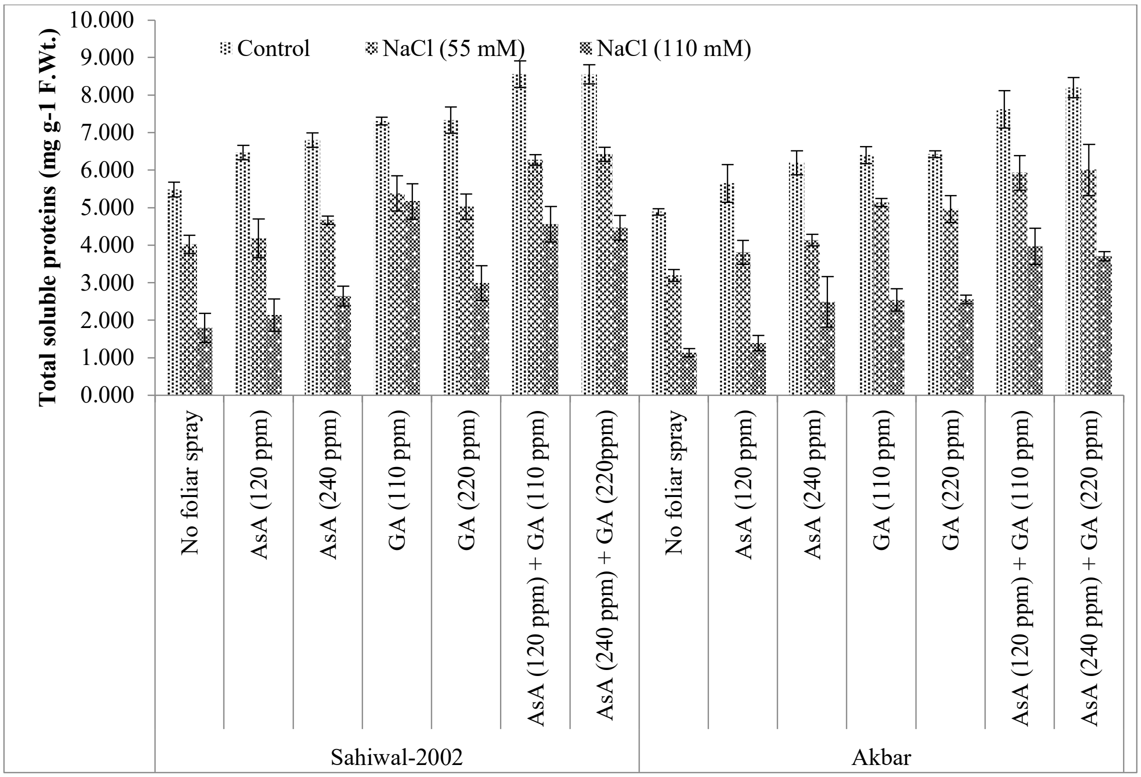

Figure 3. Effect of foliar applications of ascorbic acid and gibberellic acid on total soluble proteins contents in leaves of maize varieties at vegetative stage under saline and non saline conditions

Total free amino acid contents

Combined factorial analysis of variance (variety, salt stress and spray) for total free amino acid contents of two maize cultivars is presented in table 1. Total free amino acid contents were low in both maize varieties under non-saline conditions but increase in salinity level significantly enhanced the level of total free amino acid contents in both maize varieties without any exogenous spray (Figure 4). Moreover, Akbar performed better in regard to total free amino acid contents as compare to Sahiwal-2002 under saline conditions.

The maximum increase in total free amino acids was observed when ascorbic acid (120 ppm) and gibberellic acid (110 ppm) were applied as combine effect in Sahiwal-2002 under non-saline conditions while combination of ascorbic acid (240 ppm) and gibberellic acid (220 ppm) showed the maximum total free amino acid increase in Akbar under non-saline conditions.

Application of ascorbic acid (120 ppm) as a foliar spray increased total free amino acid contents in Sahiwal-2002, while in Akbar ascorbic acid (240 ppm) and gibberellic acid (220 ppm) increased total free amino acid content at salinity level 55 mM as compare to other treatments. In Sahiwal-2002 combine effect of ascorbic acid (240 ppm) and gibberellic acid (220 ppm) increased the level of total free amino acids contents while in Akbar combination of ascorbic acid (120 ppm) and gibberellic acid (110 ppm) improved total free amino acids contents respectively at 110 mM NaCl (Figure 4).

The highest value (49.57 mg g-1 F. Wt.) of total free amino acids was observed in Akbar at 110 mM NaCl stress and under foliar spray of ascorbic acid (120 ppm) and gibberellic acid (110 ppm) in combination. The lowest value of total free amino acids (17.24 mg g-1 F. Wt.) was observed in Sahiwal-2002 at non saline condition without any exogenous spray. Overall, it is concluded that the combined foliar application of ascorbic acid (240 ppm) and gibberellic acid (220 ppm) mitigated the negative effects of salinity and improved the total free amino acid contents in leaves of both maize varieties at vegetative stage under stress conditions.

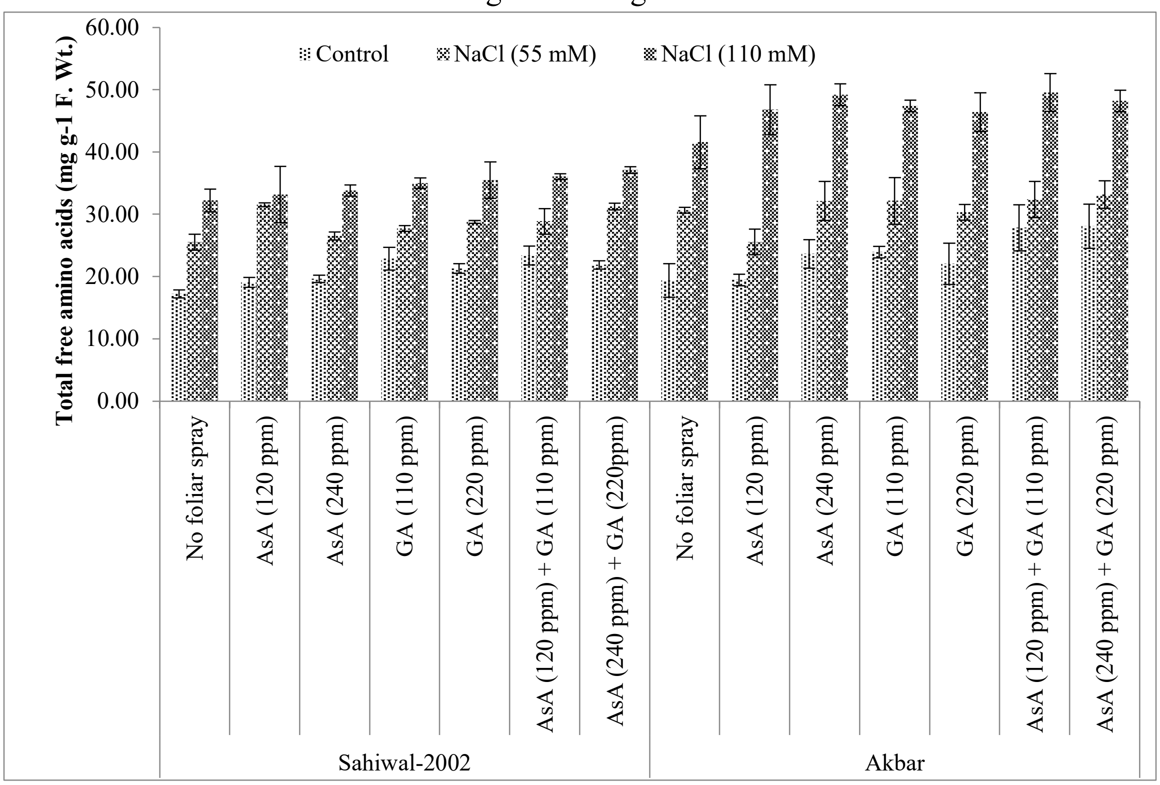

Figure 4. Effect of foliar applications of ascorbic acid and gibberellic acid on total free amino acid contents in leaves of maize varieties at vegetative stage under saline and non-saline conditions

Discussion:

Proline contents

Proline is one of the most important universal non-enzymatic antioxidants which play a substantial role not only in scavenging of ROS but also modulates a number of fundamental mechanisms in plants and ameliorates the damaging effects of both biotic and abiotic stresses (Akram et al. 2017). It was revealed in present study that total concentration of proline was enhanced under salt stress (55mM and 110 mM of NaCl) in both maize varieties at vegetative growth stage (Figure 1). Present study has further revealed that exogenous foliar application of ascorbic acid in combination with gibberellic acid (240 ppm + 220 ppm) ameliorated the negative effects of salt stress (55 mM and 110 mM of NaCl) by improving the endogenous concentration of osmoprotectants (proline contents) in both maize varieties at vegetative stage. Increased concentration of both ameliorants was found to be effective with increasing level of salt stress, Similar findings were reported by Athar et al. (2009) in wheat for salt stress amelioration by exogenous application of ascorbic acid. Shalata & Neumann (2001) also suggested that exogenous foliar application of ascorbic acid and gibberellic acid ameliorates the drastic effects of salt stress on maize growth and endogenous constituents required for sustainable production of maize grains.

Activity of stress responsive enzyme

Salt stress induces the oxidative stress in plants by overproduction of reactive oxygen species. Plants have a well-defined, complex antioxidative defense system comprising non-enzymic and enzymic constituents to overcome the salinity induced oxidative stress of ROS a complex. The overproduction of reactive oxygen species have suppressed the production of antioxidant stress responsive enzymes (Mates, 2000). Peroxidases are heme containing glycoproteins which play role in number of physiological processes including biosynthesis of lignin and ethylene, defense system against abiotic and biotic stress response (Kidwai et al. 2020). This class of enzymes catalysis the dehydrogenation of diverse group of phenolic and endiolic substrates by scavenging H_2O_2 and protects the plant cells from various kind of injurious and destructive effects of reactive oxygen species (Sharova et al. 2020). It was also reported that plants with high levels of antioxidants, either constitutive or induced, have greater resistance to oxidative damage induced by heat, drought or salinity stresses (Elkelish et al. 2020). These findings are in conformity with findings of Meloni et al. (2003). These negative impacts of salt stress and salinity induced oxidative stress on plant growth, development and defense system of plants were successfully ameliorated by use of various compounds like ascorbic acid, giberellic acid, proline, salicylic acid, melatonin etc in various crops including maize (Baki *et al*., 2000; Flores *et al*., 2000; De Lacerda et al. 2003; Carillo et al. 2005 El-Esawi *et al*., 2018). Present study has revealed that combine foliar application of ascorbic acid and giberellic acid (240 ppm and 220 ppm) was most effective to ameliorate the effects of salt stress on accumulation and activity of stress responsive enzyme in both maize varieties during vegetative stage.

Total soluble proteins and free amino acids

Proteins and amino acids are nitrogen containing organic compounds which play vital role in primary growth and development of plants. They help to alleviate the drastic impacts of abiotic stress including salinity. These are readily available source of nitrogen during high level of salt stress, when plant is unable to up take nitrogen from soil (Azevedo et al. 2009). Previous studies revealed that level of soluble proteins and free amino acids suddenly increases in plants when they perceive stress stimulus (Amini & Ehsanpour, 2005) but the quantitative and qualitative pattern of these organic compounds changes in plants with prolonging stress period and severity (Wimmer et al. 2003). Furthermore, it has also been reported that salt tolerant varieties accumulates higher free amino acids and protein contents while salt sensitive varieties have low level of these compatible solutes. Therefore, quantity of these important nitrogenous organic compounds was taken under consideration in present study to evaluate the response of maize varieties under salinity (NaCl) stress and its alleviation of growth hormone (gibberellic acid) and antioxidant (ascorbic acid).

In present study, variety Sahiwal-2002 had relatively higher values (mg per g of leaf tissues) for total soluble protein contents than variety Akbar (mg per g of leaf tissues) under non-saline conditions. Exposure of 55mM and 110 mM of NaCl salt stress reduced the level of total soluble proteins in both maize varieties at vegetative growth stage (Figure 3 and 4). The reduction in level of soluble proteins in leaf tissues of both maize varieties in response to exposure of salt stress is might be attributed to their degradation into basic elements such as carbon and nitrogen for growth and basic phenomena necessary for survival of plants under stress conditions (Bolton et al. 2019). Similar trend for free amino acids and soluble proteins findings were reported in maize seedlings under water stress (Ranieri et al. 2009; Chrystal et al. 2020) and salt stress (Neto et al. 2021). Present study also confirmed that salt tolerant variety (Sahiwal-2002) showed less reduction in level of total soluble proteins and total free amino acids as compared to salt sensitive variety (Akbar) of maize. These findings are in conformity with findings of De Lacerda et al. (2003), Voigt et al. (2009) and El-Esawi et al. (2018) in sorghum, cashew and maize respectively. These negative impacts of salt stress on maize plants of both varieties were mitigated by foliar application of combination of ascorbic acid and gibberellic acid.

Conclusion: Salt stress decreased the amount of total soluble proteins but Ascorbic acid and gibberellic were effective in counteracting the adverse effect of salinity stress through increase in total soluble protein contents. The number of total free amino acids increased with the increase in salinity. However exogenous treatment of Ascorbic acid and gibberellic acid significantly improved all parameters of maize under salinity. Combine application of ascorbic acid and gibberellic acid protected the plants from oxidative damage and improved protein, ascorbate peroxidase and proline content of the plant. These contents are increased to protect the plants from different damages caused by salinity in many forms such as production of reactive oxygen species.

Acknowledgement: This research work is a part of PhD thesis, performed by PhD scholar Hira Fatima under the supervision of Dr. Ameer Khan and Co-supervision of Dr. Muhammad Nadeem, we are thankful to Higher Education Commission of Pakistan.

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