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Mitigation of salt stress induced inhibition on reproductive growth of maize *(Zea mays* L.*)* by supplemental sulfur

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Key words: Sulfur, Salinity, Reproductive growth, Forage value, Nutrients.

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Abstract

Among various approaches devised to endure the adverse effects of salinity, the use of various nutrients is very economical and shot gun approach. In macronutrients, sulfur has considerable importance in inducing salt tolerance in plants. Sulfur metabolites become very high in salinity that increases the nutrient availability to plants and increase crop yield. For determining the role of sulfur in enhancing reproductive growth of maize plants under saline conditions, two maize varieties (Agaitti 2003, Pak Afgoi 2003) were treated with various levels of sulfur (40, 80 mM) and salinity (25,75 mm). At fully mature stage, plants were harvested for the determination of various yield related attributes. It was found that salinity lowered all yield related parameters studied in this experiment i-e. length and number of cob per plant, total number of cobs, grains per cob, total number of grains, 50 grain weight, harvest index, total yield per plant, ionic contents (K^+, Ca^{2+}, NO_3^-, PO_4^{3-}, SO_4^{2-}, K^+/Na^+, Ca^{2+}/Na^+) and forage value parameters (protein, starch, carbohydrate, fiber, ash) and increased the sodium (Na^+) contents in maize plants. Pak Afgoi 2003 has more Na^+ contents as compared to Agaitti 2003. However, sulfur at 40 mM level not only improved the salt tolerance in both maize varieties by improving yield related attributes, nutrient contents and forage value parameters but also lowered the Na^+ contents to reduce the toxic effects of salinity. In core, sulfur application (40 mM) improved the crop yield by developing salt tolerance in maize plants.

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Introduction

Salinity has reduced the agricultural productivity, particularly in the arid and semiarid regions of the world (Gorji *et al.*, 2015; Shrivastava and Kumar, 2015). Probably, 50% of the arable land would be affected by salinity in 2050 (Jamil *et al.*, 2011). Therefore, it is needed that crop production (wheat, rice, maize) should be increased to meet the demands of growing population (Godfray *et al.*, 2010). Various abiotic factors including salt stress has lowered the production of plants. Salt stress has negative impact on crop growth, development and other morphological, physiological, biochemical and molecular attributes (Munns, 2002; Khan, 2003; Tester, 2003; Paul, 2012).

It causes reduction in photosynthesis, ionic homeostasis (Na^+, K^+, Ca^{2+}, NO_3^-, PO_4^{3-}, SO_4^{2-}) and water relations, that reduces germination, vegetative growth and ultimately plant yield (Bano and Aziz, 2003; Ashraf and Harris, 2004; Sheeren *et al.*, 2005; Waheed *et al.*, 2006; Gurmani *et al.*, 2007). The negative impacts of salt stress delays and reduce the flowering and yield of crops including rice, wheat, maize, barley, bean and cotton (Gill, 1979; Keating and Fisher, 1985). The reduction in reproductive growth and plant yield has been reported in the previous studies (Linghe and Shannon, 2000; Kafi and Goldam, 2000; Gain *et al.*, 2004; Nahar and Hasanuzzaman, 2009).

Among various means to reduce the toxic effect effects of salinity on plants, the application of various inorganic salts is very cheap and easy approach. Among macronutrients, sulfur has significant contribution in lowering the effects of salt stress (Nocito *et al.*, 2006; Khan *et al.*, 2013).

The sulfur metabolism synthesize such compounds that are helpful in reducing the toxic effects of abiotic stress due to their property of scavenging the free radicals produced under stressful environment (Khan *et al.*, 2014). In addition sulphur makes the plant body proteins, lipids polysaccharides, iron- S clusters, biotin, thiamine, glutathione, phytochelatins and glucosinolates.

These compounds up-regulate the genes specific for tolerance to salinity also moldulate the physiological processes that lower the toxic effects of salinity (Khan, 2013). Thus, it is evident that metabolites of sulphur play a significant role in salt tolerance of the plant by alteration in physiological and biochemical processes in the plants (Khan *et al.*, 2014; Riffat and Ahmad, 2016). Wang and Cui (2003) reportd that sulfur application increased the biomass of wheat, corn, oil seed rape and clover by 5-32%, while grain yield was increased by 3-20%. They concluded that both biomass and yield for all the crops except clover had a positive correlation with the sulfur application. Maize is an important crop having many nutritional benefits. It contains many vitamins (C, E, K, B_1, B_2, B_3 B_5, B_6), nutrients (Na, K, Ca), secondary metabolites, antioxidants and various biomolecules (Luo *et al.*, 2011; Shah *et al.*, 2016).

Due to its nutritional importance its production has been increased in past ten years. However, salt stress has seriously reduced its production and quality all over the world (Farooq *et al.*, 2015; Abd Elgawad, 2016). Therefore, such methods are needed to devise that induce salt tolerance in maize. Among these, sulfur application not only induces salt tolerance but also enhances the quality and productivity of maize. Hence, this study focuses on the improvement in salt tolerance potential, yield and nutritional contents of maize by exogenous application of sulfur.

Materials and methods

Experimental plan

Seeds of maize cultivars (Agaitti 2003 and Pak Afgoi 2003) were got from Maize and Millet Institute Sahiwal, Pakistan. The seeds were sown in pots filled with 10 kg soil. Various levels of salinity (25, 75 mM NaCl) and sulfur (40, 80 mM K_2SO_4) were applied at sowing time. Control plants (without addition of salinity and sulfur) were also grown with the experimental plants.

Determination of yield and yield related attributes

The plants were grown up to fully mature stage and then harvested for the determination of various parameters at reproductive stage.

Harvest Index

Harvest index (%) was calculated by formula given by Beadle (1987) as followed.

$$\text{Harvest index} = \frac{\text{Grain yield}}{\text{Biological yield}} \text{ x } 100$$

Sodium, potassium, calcium (Na^+, K^+, Ca^{2+})

For the determination of ionic contents (Na^+, K^+, Ca^{2+}), acid digestion method was used (Wolf, 1982). To 0.5 g of dried grounded material, 5 ml of concentrated H_2SO_4 was incorporated and kept at room temperature overnight. The flasks containing sample material were put in the digestion block at 320 °C for 30 minutes. To each flask, 1 ml H_2O_2 was added repeatedly until the material became clear and colourless. To maintain 50 ml volume of the extract, distilled water was added and the bottles were labelled. The concentration of ions (Na^+, K^+, Ca^{2+}) in the plant material was measured by flame photometer (Jenway PFP-7). For the formation of standard curves, a graded series of standards (10, 20 to 100 ppm) were made. Then comparison was done between the values of Na^+ K^+ and Ca^{2+} from flame photometer and standard curve for actual values showed in mg/g of dry weight.

Phosphate (PO_4^{3-})

The phosphate contents were found by the procedure given by Yoshida (1976). Two reagents were prepared i-e Molybdate-vanadate solution and Nitric acid (2N). For Molybdate-vanadate solution, 25 g ammonium molybdate was put in 500 ml of water and 1.25 g of ammonium vanadate was dissolved in 1 N of HNO_3 separately. Then both solutions were mixed. For the determination of phosphate contents, 0.5 g dried grounded material was boiled in 5 ml of distilled water, filtered and 50 ml volume was prepared by using distilled water.1ml of the extract was taken and 2 ml of the 2 N HNO_3 was added and total volume was made up to 4 ml with distilled water, then 1 ml of molybdate-vanadate reagent was added and volume was maintained up to 10ml with distilled water. The mixture was vortexed and kept at room temperature for 20 minutes. Spectrophotometer (UV-1100) was used for analysing the color intensity at 420 nm, using water as blank.

For the construction of standard curve, 0.11 g monobasic phosphate (KH_2PO_4) was dissolved in 1 L water. Then standard series (2.5, 5, 7.5, 10, 12.5, and 15 mg/l PO_4^{3-}) was prepared by adding 1, 2, 3, 4, 5, and 6 ml of the stock solution in water to make 8 ml volume.

Nitrate (NO_3^-)

Nitrate contents were found by method given by Kowalwnko and Lowe (1973). Two reagents were prepared 0.01 % TCA and and 0.01 % working CTA. For the preparation of 0.01 % TCA, 0.247 g chromotropic acid disodium salt (CTA) was dissolved in 100ml of concentrated H_2SO_4. Then 10 ml of the stock was taken and diluted to 100 ml with H_2SO_4 for the preparation of 0.01 % CTA solution. For the determination of nitrate contents, 0.5 g dried ground material was boiled in 5 ml distilled water in water bath for one hour, filtered and 50 ml volume was prepared by adding distilled water. 3 ml of the extract and 7 ml of the working CTA solution was mixed, vortexed and yellow color intensity was measured at 430 nm using water as blank. 100 mg/l NO_3^- stock solution were prepared by dissolving 0.7216 g of pure dried KNO_3 in one liter water and 10, 20, 30, 40, 50 and 100 mg/l NO_3^- standard series was prepared by diluting the stock solution.

Sulfate (SO_4^{2-})

Tendon's (1993) procedure was used for sulphate determination in plants. Two reagents were prepared; barium chloride/polyvinyl alcohol and acid mixture. For the preparation of barium chloride/polyvinyl alcohol, 60 g of barium chloride was dissolved in 500 ml distilled water and 2 g polyvinyl alcohol was mixed in 400 ml of hot distilled water, allowed to cool and both solutions were mixed and volume was maintained to 1 L by adding distilled water. Acid mixture was prepared by mixing 800 ml of distilled water, 50 ml of glacial acetic acid, 20 ml of 85% orthophosphoric acid and 6 ml of concentrated sulfuric acid/water and final volume was maintained to 1 L by adding distilled water. For the determination of sulphate contents, a mixture of 5ml of the solution to be analysed, 5ml of the acid mixture and 5 ml of barium chloride/PVA was prepared, vortexed, and absorbance was noted on spectrophotometer (UV-1100) at 420 nm.

Total soluble proteins

Total soluble protein contents were found by using the method given by Bradford (1976). Phosphate buffer saline was prepared by mixing 2.7mM KCl, 10mM Na_2HPO_4, 1.37 mM NaCl and 2 mM KH_2PO_4 thoroughly and 7.2 pH was adjusted with HCl. Fresh plant material (0.5 g) was extracted with phosphate buffer saline and centrifuged. To the supernatant, stock was added in equal amount, vortexed, incubated for 30 minutes and absorbance was measured at 595 nm on spectrophotometer (UV-1100). The soluble proteins were measured by using bovine serum albumin (BSA) range (10 to 50 μg ml^{-1}) standard curve.

Carbohydrates

Carbohydrate contents were found by following the method proposed by Hedge and Hofreiter (1962). For the preparation of anthrone reagent, 200 mg anthrone was added in 100 ml of chilled 95 % H_2SO_4. Standard glucose stock was prepared by dissolving 100 mg glucose in 100ml water. From stock solution, 10 ml solution was taken and volume was maintained to 100 ml with distilled water. In this working standard solution, a few drops of toluene were also added. The solution was stored in a refrigerated after adding a few drops of toluene. For the determination of the carbohydrate contents, 100 mg of the sample material was boiled in 5ml of 2.5 N HCl and cooling was followed by the addition sodium carbonate crystals until effervescence ceases, 1 ml supernatant was collected for analysis. Absorbance was noted at 630nm on spectrophotometer (UV-1100). A graded series of standards were prepared by taking 0, 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard and volume was maintained to 1 ml by adding distilled water, followed by boiling in 4 ml anthrone reagent. Following formula was used for carbohydrate determination in plant sample.

$$\text{Carbohydrate/100mg of the sample} = \frac{\text{mg of glucose / volume of test sample}}{\text{volume of test sample}} \times 100$$

Starch

Starch contents were found by following the method proposed by Malik and Srivastava (1985). Anthrone reagent was prepared by adding 1 g anthrone in 1 L of conc. H_2SO_4.

For starch determination in plant material, 0.5 g dried plant material was extracted in methanol, dried in oven, and extracted again in 5ml of distilled water and 52 % HCl (1:1 v/v), centrifuged and 0.5ml of supernatant was added in anthrone reagent. The reaction mixture was incubated for 30 minutes, allowed to cool and absorbance of color was noted at 625nm using spectrophotometer (UV-1100).

Ash content

For the determination of ash contents the procedure given in AOAC Official Methods, 2002 (Sullivan and Carpenter, 1993) was followed. 3 g plant sample was ignited in crucibles until it became black then poured out in muffle furnace at 550 °C for 5 hours. Following expression was used for ash content calculation.

$$\text{Ash}(\%) = \frac{\text{Weight of ash}}{\text{Weight of flour sample}} \times 100$$

Fiber

The procedure mentioned in AOAC Official Methods, 2002 (Sullivan and Carpenter, 1993) for crude fibre estimation was used. 3g of the sample material was digested in 1.25% H_2SO_4 and filtration was followed by digestion in 1.25% NaOH and filtration. Then sample material was ignited in muffle furnace at 550-650 °C for 4 hours until ash was made. Following formula was used for calculation of crude fiber contents.

$$\text{Crude fiber }(\%) = \frac{\text{Weight loss on ignition}}{\text{Weight of flour sample}} \times 100$$

Statistical analysis

The experiment was carried out in completely randomized design with three replicates. Microsoft excel was used for graphical representation of the various attributes. The analysis of variance (ANOVA) was calculated by using COSTAT computer package (CoHort Software, 2003, Monterey, California).

Results

A- Yield parameters

Cob length

Statistical analysis has shown that salinity (25, 75 mM) reduced the cob length of both studied maize varieties (Table 1). Moreover, Pak Afgoi 2003 (salt sensitive) had small cobs as compared to Agaitti 2003, which produced large and healthy cobs. Fig. 1 showed that sulfur application (40, 80 mM) improved the cob length at all levels of salinity (25, 75 mM).

Number of cobs per plant

Cobs per plant were reduced by increasing the level of salinity. It was evident from Fig. 1. Maximum reduction in number of cobs per plant was found at 75 mM salinity. However, sulfur at 40mM increased the number of cobs per plant in both maize cultivars (Agaitti 2003, Pak Afgoi 2003) at all levels of salinity (25, 75 mM). It was evident from statistically significant Sa x S interactive effect (Table 1).

Total number of cobs

Results showed that total number of cobs was significantly lowered by increasing the concentration of salinity. At higher salt level (75 mM), few cobs were produced. Pak Afgoi 2003 was much affected by higher salt level as compared to Agaitti 2003. However, sulfur application (40, 60 mM) improved the total number of cobs in both cultivars of maize at all levels of salinity (25, 75 mM) (Fig. 1). It was evident from statistically significant Sa x S interaction (Table 1).

Grains per cob

Salinity reduced the grains production in each cob in both varieties of maize. Table has shown a statistically significant V x Sa interactive effect (Table 1). By exogenous application of sulfur (40, 60 mM) the production of grains was increased in each cob of both cultivars (Fig. 1). Moreover, sulfur has also reduced the toxic effect of salinity by improving the grains in each cob in salt sensitive variety (Pak Afgoi 2003). It was shown by statistically significant Sa x S interaction (Table 1).

Total number of grains

Statistical analysis has shown that salinity reduced the production of grains. Salt tolerant variety (Agaitti 2003) produced more number of grains as compared to salt sensitive variety (Pak Afgoi 2003). A significant V x Sa interactive effect supports this finding (Table 1). Sulfur application (40, 60 mM) not only improved the total number of grains but also improved the salt tolerance in maize plants (Fig. 1).

Weight of 50 grains

It was revealed that salinity decreased the 50 grain weight. Both maize cultivars showed different response to salt application. Pak Afgoi 2003 produced lower weight of 50 grains as compared to Agaitti 2003. It was shown by statistically significant V x Sa interaction (Table 1). Nevertheless, sulfur application at 40mM, improved the weight of 50 grains under saline conditions in both maize cultivars (Pak Afgoi 2003, Agaitti, 2003) (Fig. 1). These results are drawn from statistically significant Sa x S interactive effect (Table 1).

Harvest index

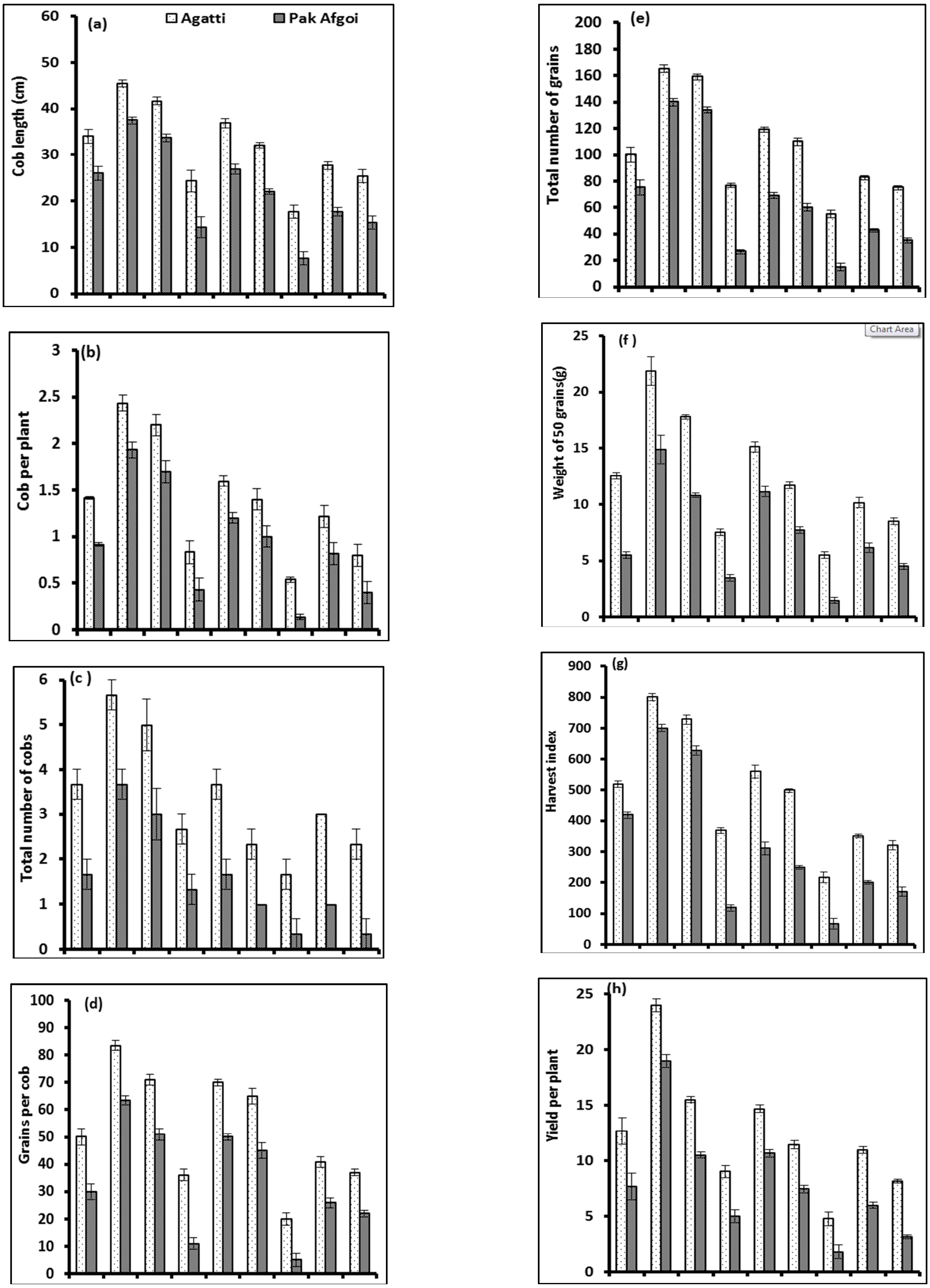
Salinity (25, 75 mM) also reduced the harvest index in both maize varieties. It was shown by statistically significant V x Sa interactive effect (Table 1). Harvest index was higher in Agaitti, 2003 in comparison to Pak Afgoi, 2003. Application of sulfur (40, 60 mM) improved the harvest index in both studied maize cultivars. However, its 40 mM was more effective in improving the harvest index (Fig. 1).

Table 1. Mean squares from analysis of variance (ANOVA) of the data for yield parameters of maize subjected to different levels of salinity and sulfur.

SOV	df	Cob length	Total number of cobs	Total number of grains	Harvest index
Variety (V)	1	11 ***	42.66 ***	19837.5 ***	37 ***
Salinity (Sa)	2	1442.97 ***	26 ***	28348.68 ***	797113.95 ***
Sulfur (S)	2	603.00074 ***	6.88 ***	10452.51 ***	197121.45 ***
V x Sa	2	6 ns	0.22 ns	712.5 ***	26 ***
V x S	2	1.45e-28 ns	0.22 ns	1.25e-27 ns	2.01e-26 ns
Sa x S	4	3.24 ns	1.22 *	748.96 ***	9065.57 ***
V x Sa x S	4	3.05e-28 ns	0.11 ns	2.13e-26 ns	1.87e-26 ns
Error	36	5.18	0.35	24.96	522.70
		Cob per plant	Grains per cob	Weight of 50 grains	Yield per plant
Variety (V)	1	2.53 ***	4816.66 ***	337.5 ***	266.66 ***
Salinity (Sa)	2	5.69 ***	5040.01 ***	278.58 ***	374.66 ***
Sulfur (S)	2	3.12 ***	4512.01 ***	235.85 ***	254.10 ***
V x Sa	2	0.015 ns	54.16 *	13.5 ***	1.16 ns

SOV	df	Cob length	Total number of cobs	Total number of grains	Harvest index
V x S	2	5.36e-31 ns	4.16 ns	9.11e-30 ns	0.66 ns
Sa x S	4	0.12 **	147.35 ***	8.41 ***	21.76 ***
V x Sa x S	4	4.24e-31 ns	4.16 ns	1.31e-29 ns	0.66 ns
Error	36	0.026	13.37	0.83	0.97

*, **,***=significant at 0.05, 0.01 and 0.001 levels, respectively. ns = non-significant *Abbreviation: Exponent (e).*



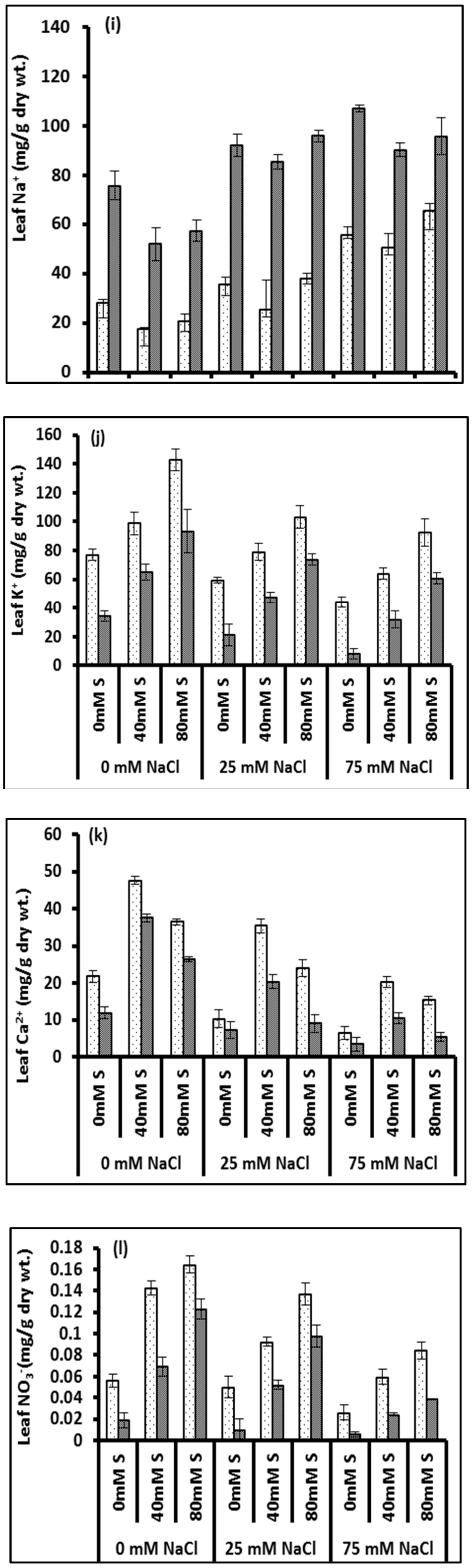


Fig. 1. Effect of different levels of sulfur (S) on cob length (a), cob per plant (b), total number of cobs (c), grains per cob (d), total number of grains (e), weight of 50 grains (f), harvest index (g), yield per plant (h), leaf Na^+ contents (i), leaf K^+ contents (j), leaf Ca^{2+} contents(k), leaf NO^{3-} contents (l) of different maize (*Zea mays* L.) cultivars under saline conditions.

Yield per plant

It was observed that the yield per plant was reduced by application of salinity in maize plants. However, sulfur application improved the yield per plant. It was evident from statistically significant Sa x S interactive effect (Table 1). At 40 mM sulfur, maximum improvement in yield per plant was found. Agaitti 2003 produced more yield per plant as compared to Pak Afgoi, 2003 (Fig. 1).

B-Ionic contents

Sodium (Na^+)

A marked increase in Na^+ contents by salt application was found in both maize varieties as evident from statistically significant V x Sa interaction (Table 2). However, application of sulfur (40, 80 mM) lowered the Na^+ contents at all levels of salinity. At 40 mM sulfur maximum reduction in Na^+ contents was found in maize leaves. Pak Afgoi, 2003 had higher level of Na^+ as compared to Agaitti, 2003 (Fig. 1).

Potassium (K^+)

The exposure of maize plants to saline condition lowered the K^+ contents in maize plants. Agaitti, 2003 showed higher accumulation of K^+ contents as compared to Pak Afgoi, 2003. Sulfur at 40mM proved very efficient in improving the K^+ contents in maize plants while higher level of sulfur (80 mM) did not much improved the K^+ contents (Fig. 1).

Calcium (Ca^{2+})

Results have shown that salinity (25,75 mM) reduced the Ca^{2+} contents in both studied maize cultivars. Nevertheless, sulfur application improved the Ca^{2+} contents in maize leaves in both cultivars (Agaitti, 2003; Pak Afgoi, 2003). It was evident from statistically significant V x S interactive effect (Table 2). Agaitti, 2003 accumulated more Ca^{2+} contents as compared to Pak Afgoi, 2003 under saline conditions (Fig. 1).

Nitrate (NO_3^-)

A reduction in NO_3^- contents was found by salt application. Maximum reduction was reported at 75 mM salt level. A variation in both varieties to treatment application was noted. In Pak Afgoi, 2003, a little improvement in NO_3^- contents was noted by sulfur application as compared to Agaitti, 2003 in salt stress conditions. At 40 mM sulfur higher improvement in NO_3^- contents was found (Fig. 1).

Phosphate (PO_4^{3-})

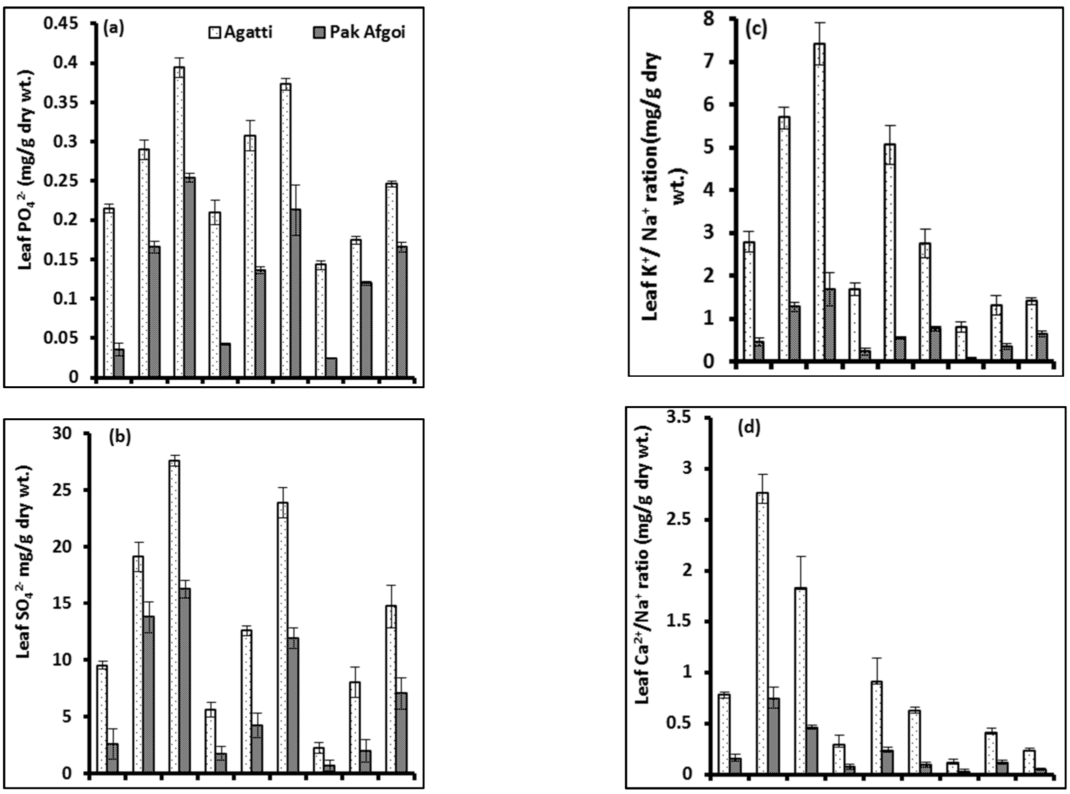
Statistical analysis has shown that a significant decrease in PO_4^{3-} contents was found in leaves of both maize cultivars. It was revealed from statistically significant V x Sa interaction (Table 2).

However, application of sulfur improved the PO_4^{3-} contents in both cultivars of maize. These findings were drawn from statistically significant V x S interactive effect (Table 2). Under salinized and non-salinized conditions, 40mM sulfur proved efficient in improving PO_4^{3-} contents in both maize cultivars. Moreover, higher level of sulfur (80 mM) showed a little improvement in PO_4^{3-} contents in Agaitti, 2003 and Pak Afgoi, 2003 (Fig. 2).

Table 2. Mean squares from analysis of variance (ANOVA) of the data for nutrient contents of maize subjected to different levels of salinity and sulfur.

SOV	df	Leaf Na content	Leaf K content	Leaf Ca content	Leaf NO_3 content
Variety (V)	1	28694.02 ***	17625.84 ***	1232.66 ***	0.023 ***
Salinity (Sa)	2	5746.94 ***	5599.35 ***	1858.56 ***	0.014 ***
Sulfur (S)	2	715.72 ***	12999.42 ***	1530.91 ***	0.028 ***
V x Sa	2	492.11 **	114.98 ns	13.16 ns	3.33e-4 ns
V x S	2	122.91 ns	50.31 ns	60.16 **	3.35e-4 ns
Sa x S	4	102.78 ns	95.42 ns	93.73 ***	0.0015 ***
V x Sa x S	4	61.24 ns	39.52 ns	18.16 ns	2.51e-4 ns
Error	36	68.97	129.36	8.37	1.63e-4
		Leaf PO_4 content	Leaf SO_4 content	Leaf K/Na ratio	Leaf Ca/Na ratio
Variety (V)	1	0.23 ***	639.82 ***	94.92 ***	10.63 ***
Salinity (Sa)	2	0.033 ***	353.19 ***	25.93 ***	4.22 **
Sulfur (S)	2	0.12 ***	760.64 ***	13.41 *	4.26 **
V x Sa	2	0.0082 ***	11.55 *	12.35 *	1.69 ns
V x S	2	0.0017 *	39.56 ***	5.66 ns	2.17 *
Sa x S	4	0.0022 **	29.06 ***	4.43 ns	0.84 ns
V x Sa x S	4	5.094e-4 ns	5.15 ns	3.33 ns	0.61 ns
Error	36	3.93e-4	3.44	2.63	0.57

*, **,***=significant at 0.05, 0.01 and 0.001 levels, respectively. ns = non-significant *Abbreviation: Exponent (e).*



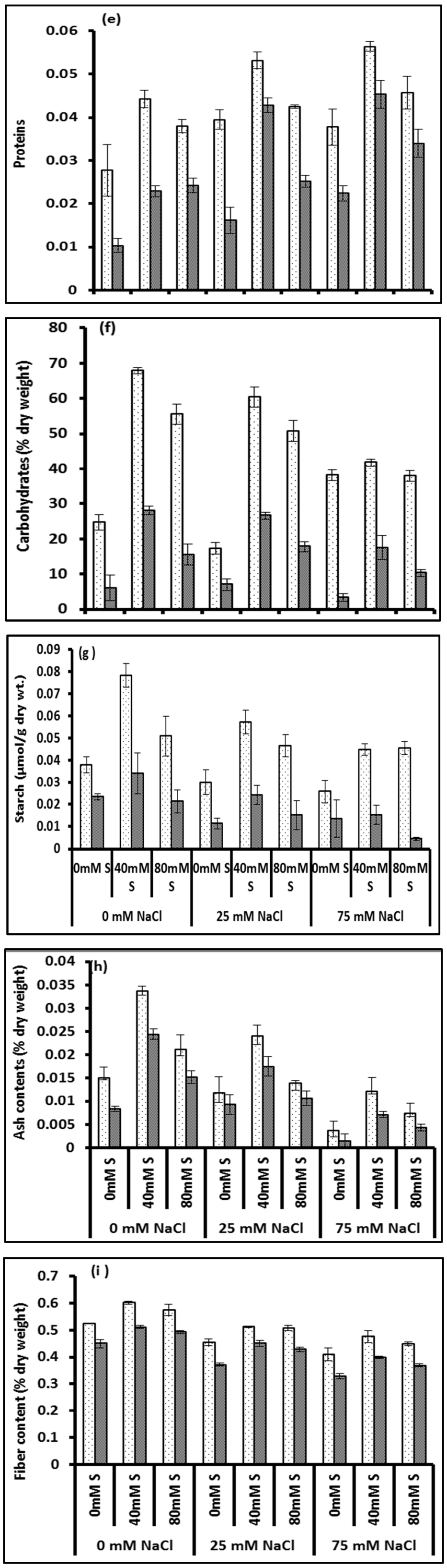


Fig. 2. Effect of different levels of sulfur (S) on leaf PO_4^{2-} (a) leaf SO_4^{2-} (b) leaf K^+/Na^+ ratio (c) leaf Ca^{2+}/Na^+ ratio (d) proteins (e) carbohydrates (f) starch (g) ash contents (h) and fiber contents (i) of different maize (*Zea mays* L.) cultivars under saline conditions.

Sulfate (SO_4^{2-})

The exposure of maize plants to salinity also reduced the SO_4^{2-} contents. Higher reduction was found at 75 mM salt level. The application of sulfur improved the SO_4^{2-} contents in both maize cultivars (salt tolerant, salt sensitive) as V x S interaction was statistically significant (Table 2). Both levels of sulfur (40, 80 mM) were proved beneficial in improving the SO_4^{2-}contents in leaves of maize plants. Agaitti 2003 responded well to sulfur application as compared to Pak Afgoi, 2003 in terms of SO_4^{2-} accumulation in leaves (Fig. 2).

K^+/Na^+ contents

Application of salinity decreased the K^+/Na^+ ratio in both maize cultivars which is evident from statistically significant V x Sa interactive effect (Table 2). However, application of sulfur at 40mM improved the K^+/Na^+ ratio in both varieties while higher level of sulfur (80 mM) showed a little improvement in K^+/Na^+ ratio in maize plants. Both varieties showed different response to sulfur application. Agaitti, 2003 had higher K^+/Na^+ ratio as compared to Pak Afgoi, 2003 at all levels of salinity (Fig. 2).

Ca^{2+}/Na^+ contents

It was found that salinity reduced the Ca^{2+}/Na^+ ratio in both studies maize cultivars (Agaitti, 2003; Pak Agdoi, 2003). At 75mM salt level, higher reduction in Ca^{2+}/Na^+ ratio was found (Fig. 2). However, sulfur application not only improved the Ca^{2+}/Na^+ ratio in both varieties of maize but also reduced the toxic effects of salinity in Pak Afgoi, 2003 (Fig. 2). These findings are drawn from Table showing statistically significant V x S interaction (Table 2).

C- Forage value parameters

Proteins

Salt stress caused a significant reduction in the protein contents of maize leaves at fully mature stage (Table). Pak Afgoi, 2003 (salt sensitive) accumulated low protein contents as compared to Agaitti, 2003 (salt tolerant) under saline conditions. However, sulfur application improved the protein contents in both maize cultivars. A statistically significant V x S interaction supports this finding (Table 3). Sulfur reduced the toxic effects of salinity by balancing the osmotic potential due to accumulation of proteins. It was evident from significant Sa x S interaction (Table 3). All levels of sulfur improved the protein contents, however, at 40 mM sulfur high protein contents were found (Fig. 2).

Carbohydrates

Statistical analysis showed that salinity decreased the carbohydrate contents in both studied maize cultivars. It was revealed from significant V x Sa interaction (Table 3). The application of sulphur not only improved the carbohydrate contents in maize leaves but also increased the maize tolerance to salt stress. It was shown by a significant V x S interactive effect in Table 3. Agaitti, 2003 showed high improvement in salt tolerance and accumulated more carbohydrate contents as compared to Pak Afgoi, 2003 (Fig. 2).

Table 3. Mean squares from analysis of variance (ANOVA) of the data for yield parameters of maize subjected to different levels of salinity and sulfur.

SOV	df	Protein	Carbohydrate	Starch	Ash content	Fiber content
Variety (V)	1	0.0053 ***	11451.33 ***	0.010 ***	3.36e-4 ***	0.083 ***
Salinity (Sa)	2	0.0015 ***	303.72 ***	0.0012 ***	8.56e-4 ***	0.065 ***
Sulfur (S)	2	0.0019 ***	2708.09 ***	0.0016 ***	6.24e-4 ***	0.022 ***
V x Sa	2	8.79e-5 *	57.017 *	4.93e-6 ns	1.95e-5 ns	5.39e-5 ns
V x S	2	1.67e-5 ns	209.72 ***	5.81e-4 **	1.41e-5 ns	2.63e-5 ns
Sa x S	4	1.07e-4 ***	295.53 ***	1.25e-4 ns	4.66e-5 **	1.18e-4 ns
V x Sa x S	4	5.55e-5 *	160.89 ***	7.68e-5 ns	5.11e-7 ns	1.45e-4 ns
Error	36	1.78e-5	13.68	8.61e-5	1.09e-5	4.09e-4

*, **,***=significant at 0.05, 0.01 and 0.001 levels, respectively. ns = non-significant *Abbreviation: Exponent (e).*

Starch

Starch contents were also reduced by salt application. Under salt stress conditions, improper supply of nutrients reduces the photoassimilates to the growing plant that ultimately reduce the starch contents. By the application of sulphur (40 mM, 80 mM) the starch contents were increased significantly. It was shown by statistically significant V x S interactive effect (Table 3). Salt tolerant cultivar (Agaitti, 2003) accumulated more starch contents as compared to salt sensitive cultivars (Pak Afgoi, 2003) (Fig. 2).

Ash content

Application of salt stress reduced the ash contents in both maize cultivars. The plants treated with sulfur showed more ash contents as compared to plants without sulfur treatment at all levels of salinity. It was evident from statistically significant Sa x S interaction. The maximum improvement was noted at 40 mM applied sulfur. It was revealed that Agaitti, 2003 showed more ash contents as compared to Pak Afgoi, 2003 (Fig. 2).

Fiber content

A marked reduction in fiber contents by salt application was revealed in this study. However, by the application of sulfur the fiber contents were improved in both maize cultivars. The maximum improvement in fiber contents was noted at 40 mM sulfur. Pak Afgoi, 2003 exhibited less fiber contents as compared to Agaitti, 2003 (Fig. 2).

Discussion

Different environmental and physiological factors affect the production and quality of crop plants. Among abiotic stresses, salt stress has significantly reduced the crop yield due to disturbance in physiological and biochemical parameters of plants (Flowers *et al.*, 2010; Ashraf *et al.*, 2010). In the current study, it was found that salinity reduced the crop yield in both maize cultivars. These findings are in accordance to the previous studies on different crops e.g. maize, sunflower, mungbean, wheat, and barley (Munns *et al.*, 2006; Raza *et al.*, 2007; Endris and Mohammed, 2007; Ahmed, 2009; Akram and Ashraf, 2011; Shahbaz *et al.*, 2011). However, sulfur containing fertilizers not only improve the quantity but also improve the quality of crop plants. Sulfur application improved the size, length, seed yield, 1000 seed weight and number of cobs per plant. It may be due to the reason that sulfur increased the photoassimilate supply to the growing crops which increased the seed yield and weight of 1000 seeds (Malhi *et al*., 2005). Khalid *et al.* (2009) found that the application of 40kg S ha^{-1} produced high biomass and seed yield. Also the sulfur contents of plants increased to cope the various environmental stresses. Malik *et al.* (2000) found that sulfur application has increased yield and yield components in cotton varieties. Gupta *et al.* (2004) reported that by applying sulfur to wheat crops the yield was increased. It may be due to the reason that sulfur application increased the Ca and S in the soil which increased the nutrient availability to the plants due to synergic effect. The increase in seed yield by exogenous application of sulfur has been reported in previous studies (Riley *et al*., 2000; Blake-Kalff *et al.,* 2000; Prasad, 2003; Ali *et al*., 2008). Harvest index is an important attribute for knowing the crop yield. An increase in harvest index shows that physiological mechanisms in plants are very efficient for mobilizing and transport of photosynthates to the young organs growing very fast (Jamal *et al.,* 2006; Malhi *et al*., 2007). In this study, it was found that by applying sulfur harvest index increased as compared to plwant with no sulfur application. This was due to the reason that sulfur metabolites help in the nutrient supply and transport to the young growing organs and induce salt tolerance under stress conditions. The increase in crop straw and grain yield by sulfur application in saline conditions may be due to the reason that sulfur application increase the availability of nutrient and decrease the toxic effect of salinity by reducing Na^+ contents in plants (Aslam *et al*., 2001).

Nutritional status in the plant is very much important for maintaining plant metabolism to work properly. The imbalance in the nutritional status in the plant makes it very sensitive to various environmental conditions. Various abiotic factors including salt stress, causes the improper supply of nutrients to the plant body which lead to disturbance in osmotic potential and ultimately lower down the metabolism of plants. The reduction in metabolic activities in plants causes the reduction in plant growth. In different crops, the reduction in plant growth due to ionic imbalance has been reported e.g. *Lycopersicon esculentum*, *Spinacia oleracea*, *Physalis peruviana* and *Zea mays* (Kaya *et al*., 2001; Al-Karaki *et al*., 2001; Miranda *et al*., 2010; Collado *et al*., 2010). This study showed that salt stress increased the Na^+ contents in the plant body while other nutrients (K^+, Ca^{2+}, NO_3^- and PO_4^{3-}, K^+/Na^+, Ca^{2+}/Na^+) has become reduced by application of salinity. This is supported by earlier researches (Fortmeier and Schubert, 1995). Na^+ ions present in salt not only lower the water potential of soil but also changes the permeability of root for Ca^{2+} ions (Meloni *et al.,* 2008). However, the presence of Ca^{2+} makes the plasma membrane more permeable for transport of ions (K^+, NO_3^-, PO_4^{3-}). High concentration of Na^+ ions also slow down the uptake and transport of NO_3^- and PO_4^{3-} in the plant. In the previous studies it has been reported that salt tolerant plants compartmentalize the Na^+ ions that improve the uptake of Ca^{2+} and K^+, hence, K^+/Na^+ and Ca^{2+}/Na^+ ratio become high (Shirazi *et al*., 2005; Song *et al*., 2009). These earlier findings has been supported by current study, where, Agaitti, 2003 has high concentration of K^+, Ca^{2+}, NO_3^-, PO_4^{3-} and lower concentration of Na^+ at all levels of salinity applied as compared to salt sensitive variety Pak Afgoi, 2003.

The results showed that sulfur application not only improved the beneficial nutrients in the plant but also lowered the Na^+ contents as compared to plants with no sulfur applied. Sulfur has significant contribution in ionic homeostasis in the plant (Singh *et al*., 2011; Riffat, 2017). It not only reduces the accumulation of toxic ions in the plants that improve the productivity and quality of crop plants but also maintains the soil condition for production of healthy crops (Zhang *et al.,* 1999). Sulfur application improves the K^+/Na^+ and Ca^{2+}/Na^+ ratio that decrease the toxic effects of salinity (Badr *et al*., 2002; Prasad, 2003). The application of sulfur also improves the nitrate reductase activity in the plant that enhance the uptake of NO_3^- in the plant (Jamal *et al*., 2006). Sulfur application also enhances the PO_4^{3-} contents in the plant. Sulfur decomposes the phosphorous and activates the microorganism to provide the phosphorous in the form of phosphate to the plants (Glubiak *et al*., 2014).

The improvement in crop growth under stress condition due to sulfur supplementation has been reported in different crop plants *e.g. Lycopersicon esculentum*, *Spinacia oleracea*, *Physalis peruviana* and *Zea mays* (Al-Karaki *et al.*, 2001; Kaya *et al.*, 2001; Miranda *et al.*, 2010; Collado *et al.*, 2010).

In this study, salinity increased the protein concentration in the plant. Under saline conditions, total soluble proteins become accumulated in the cytoplasm that store nitrogen. Moreover, proteins also play significant role in the osmotic adjustment in the plant cell (Ashraf and Harris, 2004) that are defensive mechanism to cope the toxic effects of salinity. The results of this study support the earlier findings. Chao *et al.* (1999) evaluated that salt stress causes the increase in protein contents in *Lycopersicon esculentum* L.

In various plants (maize, sunflower, barley, rice, mung bean) the accumulation of total soluble proteins has been reported under saline conditions (Khosravinejad *et al.*, 2009; Kapoor and Srivastava, 2010). Results of present study showed that sulfur application improved the protein contents in both varieties of maize plants. These findings are in complete agreement to the previous studies. Jamal *et al.* (2006) reported that sulfur application enhance the protein production in the groundnut. The structure of proteins is protected by various metabolites of sulfur i.e. cytein has thiol (sulfhydryl) group that form a disulfide bond between two cystein residue and maintain the structure of protein (Leustek and Saito, 1999; Malhi and Leach, 2000). Hence sulfur application stabilizes the structural and functional integrity of protein in salt stress conditions.

Carbohydrates produced by photosynthesis are very essential for plant productivity. High concentrations of salt retard the plant growth by slowing down the photosynthetic rate that reduce the carbohydrate production (Parida and Das, 2005; Zobayed *et al.,* 2007). These findings are related to current study that showed a significant reduction in carbohydrate concentration by salt application while sulfur application improved the carbohydrate contents at all levels of salinity. Sulfur plays a significant role in catalytic and electrochemical functions of carbohydrates (Saito, 2004). Also a reduction in sulfur contents lower down the carbohydrate production in the plants (Neelam and Nalini, 2013).

Salt stress also reduces the starch contents in plants. Rajakumar (2013) evaluated that starch contents were decreased due to imposition of salinity. In salt tolerant plants the starch contents were high as compared to salt sensitive plants (Dubey and Singh, 1999; Kafi *et al.*, 2003). These studies are in complete agreement to current investigation where Agaitti 2003 (salt tolerant) has high starch contents as compared to Pak Afgoi 2003 (salt sensitive). Moreover, sulfur application has improved the starch contents in both varieties of maize at all levels of salinity. These findings are supported to previous studies (Kumar *et al.*, 2007; Wang *et al.*, 2008; Klikocka, 2010; Sharma *et al.*, 2011).

Results revealed that salinity reduced the ash contents in both varieties of maize. Accumulation of abundant salts due to salinity causes reduce uptake of nutrients and water contents that results in poor production of fresh and dry weights of plants. Ultimately reproductive capacity of plant becomes reduced. This results in reduction in forage value parameters, including ash contents of plants. Previous studies support the present findings. By increasing salinity ash contents decreased significantly (Zhuo *et al.* 2015). However, application of sulfur has significant effect on improving the reproductive capacity of the plant. It was revealed that sulfur application improved the ash contents at all levels of sulfur applied under salt stress conditions.

Salinity has significantly decreased the fiber contents in shoots of both studied maize varieties. These findings are related to previous studies. Singh *et al.* (2014) found a significant decrease in fiber contents by imposition of salinity. El-Saidi and Hawash (1971) showed 50% reduction in fiber contents in *H. sabdariffa* in comparison to control due to salt stress conditions.

However, sulfur application has not only reduced the toxic effects of salt stress but also improved reproductive capacity of the maize plant by improving fiber contents. This finding is related to the redults of Maqsood *et al.* (2008) who showed that application of potassium sulphate enhance the fiber contents in maize plants.

Conclusion and recommendations

It has been concluded that salt stress has negative impact on production and quality of crop plant. However the application of sulfur induced salt tolerance in maize plants and improved all yield related parameters. The maximum improvement in reproductive capacity of maize by sulfur application was found at 40mM sulfur while higher level of sulfur was not much effective in improving salt tolerance in maize plants. Both maize varieties (Agaitti, 2003 and Pak Afgoi, 2003) showed differential response to sulfur application. Agaitti, 2003 (salt tolerant) showed improved nutrient contents, forage value parameters (protein, carbohydrates, starch, fiber, ash) and ultimaltey yield and yield related components. However, sulfur has also improved the plant yield of salt sensitive variety as compared to the plants with no sulfur application. Hence, it is recommended that to enhance the reproductive capacity of the maize plants under saline conditions, sulfur at 40 mM should be applied.

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