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SULFUR: A MULTIFUNCTIONAL ELEMENT THAT IMPROVES NUTRITIONAL VALUE OF MAIZE GRAINS

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Abstract

Sulfur is an integral constituent of various proteins, vitamins and essential nutrients necessary for maintaining plant growth and development under saline conditions. An experiment was conducted to assess the role of sulfur in improving nutritional quality of maize grains at varying levels of sulfur (40, 80 mM) and salinity (25, 75 mM). Various vitamins, biomolecules and nutrient contents were analyzed. A significant reduction in vitamins (ascorbic acid, tocopherol, riboflavin, niacin, thiamine), biomolecules (protein, carbohydrate, starch) and nutrient (K^+, Ca^{2+}, NO_3^-, PO_4^{3-}, SO_4^{2-}, K^+/Na^+, Ca^{2+}/Na^+) contents was observed by imposition of salinity. However, salinity increased Na^+ contents at all applied salt levels. Application of 40 mM sulfur significantly improved ascorbic acid, tocopherol, riboflavin, niacin, thiamine, protein, carbohydrate, starch, K^+, Ca^{2+}, NO_3^-, PO_4^{3-}, SO_4^{2-}, K^+/Na^+, Ca^{2+}/Na^+, while Na^+ contents were reduced by sulfur application. Salt tolerant maize variety (Agatti 2003) responded well to sulfur fertilization in comparison to salt sensitive maize cultivar (Pak Afgoi 2003). In short, both levels of sulfur (40, 80 mM) effectively reduced toxic effects of salinity by improving vitamins, biomolecules and nutritional contents in maize grains. However, 40 mM sulfur was found more effective in improving salt tolerance potential of maize plants.

Key words: Biomolecules, Nutrients, Sulfur supplementation, Salinity, Vitamins.

Introduction

Salt stress has significant contribution in reducing agricultural productivity all over the world. According to an estimate, nearly 930 million ha soil has been affected due to primary salinization and 77 million ha due to secondary salinization in the world (Shahid *et al*., 2018). Salinity affects various physiological and biochemical processes in plants that ultimately reduces crop yield. Salt stress causes imbalance in nutritional composition, vitamin contents and ionic toxicity (Ratnakar & Rai, 2013; Riffat & Ahmad, 2018a). Various chemical methods are utilized for reclaiming salt affected soils. Among these, application of inorganic nutrients is very economical and shot gun approach.

Among macronutrients, sulfur is very necessary for plant growth and development. It is an important constituent of various types of amino acids, antioxidants, proteins, iron sulfur clusters, polysaccharides, lipids, vitamins, cofactor, peptides and sulfo-lipids (Reich *et al*., 2016). Sulfur plays significant contribution in improving rate of photosynthesis, production of various biomolecules, improving oil contents in cereals and enhancing nutritive quality of forage crops (Riffat & Ahmad, 2018b). According to an estimate, 0.1-0.5% sulfur is required for crop production on dry weight basis (Sutar *et al*., 2017). Application of sulfur improves nutrient use efficiency in plants under saline conditions. It may be due to reason that sulfur helps to eliminate excessive production of Na^+ ions. Earlier studies showed that sulfur fertilization improved seed yield and yield related parameters in maize, wheat and rice under stress conditions (Ali *et al*., 2012; Kubenkulov *et al*., 2013; Manesh *et al*., 2013). Moreover, various metabolites of sulfur play a key role in stress tolerance in plants. In short, sulfur application is considered an important amendment for reclaiming saline-sodic soils.

Maize is very important crop due to its nutritional significance. It constitutes starch (72%), proteins, (10%), fat (4%), vitamins, essential minerals and fibre. It is utilized in oil, beverages, glue, fuel, alcohol and ethanol. The annual maize production in the world is 717 million metric tons (Ranum *et al*., 2014). Due to its multipurpose uses, annual demand has been raised. However, production and quality of maize is reducing due to salt stress condition. It may be due to the reason that maize is salt sensitive crop (Farooq *et al*., 2015). Sulfur is necessary for improving maize yield because it plays significant role in improving chlorophyll pigments, plant nutrition and, synthesis and translocation of various biomolecules (Rasheed *et al*., 2014; Riffat, 2018). The aim of this study was to assess the role of sulfur in enhancing quality and productivity of maize grains by improving various types of vitamins, nutrients and biomolecules. Another objective of this study was to improve salt tolerance potential of maize by sulfur fertilization.

Materials and Methods

Plan of study: The experiment was conducted in the wire house of Old Botanical Garden University of Agriculture, Faisalabad Pakistan. Seeds of two maize varieties (Agatti, 2003; Pak Afgoi, 2003) were obtained from Maize and Millett Institute Sahiwal, Punjab Pakistan. Healthy and uniform seeds were sown in plastic pots filled with 10 kg loamy soil (pH=9.06; EC=1210µS/cm). Sulfur (40, 80 mM) treatment was applied by using potassium sulfate while sodium chloride (25, 75 mM) was used for salt treatment. The plants were grown up to fully mature stage and harvested after 45 days of treatment application. The grains were separated for the determination of various biochemical studies.

Determination of vitamin contents in maize grains

Ascorbic acid (vitamin C): Plant sample (0.25 g) was extracted with 10 mL of 6% trichloroacetic acid followed by addition of 2 mL of 2% dinitrophenyl hydrazine. After mixing one drop of 10% thiourea, the reaction mixture was boiled in water bath for 15 min. Then it was cooled down in ice bath and 5 mL of 80% H_2SO_4 was added to it. Then absorbance of the mixture was noted at 530 nm by using spectrophotometer (UV-1100) (Mukherjee & Choudhuri, 1983).

Tocopherol (vitamin E): Plant sample (2.5 g) was homogenized with 0.1 N sulphuric acid and volume of the mixture was maintained up to 50 mL by using 0.1 N sulphuric acid. After keeping overnight, the mixture was vortex and filtered. Its aliquot was used for tocopherol determination. In three centrifuge tubes, 1.5 mL plant extract, standard solution and water was added. After adding 1.5 mL each of xylene and ethanol, the tubes were closed, homogenized and centrifuged. A xylene layer was formed that was carefully separated. To 1 mL xylene layer, 1 mL of 2, 2′ dipyridyl reagent was added and vortex. The absorbance of the mixture was noted at 460 nm by using spectrophotometer (UV-1100) (Rosenberg, 1992).

Riboflavin (vitamin B_2): Extraction of plant sample (5 g) was done by using 100 mL of 50% ethanol and shaken for an h. After filtration of the mixture, 10 mL of extract, 10 mL of 50% potassium permanganate and 10 mL of 30% H_2O_2 were mixed and kept in water bath at 50°C for 30 min. After adding 2 mL of 40% sodium sulphate the volume of the mixture was maintained to 50 mL. The absorbance of the mixture was noted at 510 nm by using spectrophotometer (UV-1100) (Okwu & Josiah, 2006).

Niacin (vitamin B_3): Plant sample (1 g) was shaken in 10 mL of 1 N H_2SO_4 for 30 min. The solution was filtered after adding three drops of ammonia. To 2 mL filtrate, 1 mL of 10% KCN (potassium cyanide) solution and 1 mL of 0.02 N H_2SO_4 was dissolved and absorbance of the mixture was noted at 470 nm using spectrophotometer (UV-1100) (Okwu & Josiah, 2006).

Thiamine (vitamin B_1): 2 mL of 1 M HCl and 1.4 mL of 0.4% sodium nitrite solution was added and cooled in ice bath for 5 min. To this mixture, 4 ml of 2% urea was added, vortex and left for 3 min. Plant extract (2 mL) was mixed in 5 mL of 0.5 M sulfanilic acid and 5 mL of 30% NaOH and optical density of the mixture was noted at 490 nm by using spectrophotometer (UV-1100) (Hassan & Azeez, 2005).

Determination of biomolecules in maize grains

Total soluble proteins: Extraction of plant sample (0.5 g) was done in phosphate buffer saline. The mixture was centrifuged and supernatant was collected. Equal amount of supernatant was mixed with stock, vortex and incubated for 30 min. The absorbance was noted at 595 nm using spectrophotometer (UV-1100) (Bradford, 1976).

Carbohydrates: To 100 mg plant sample, 5 mL of 2.5 N HCl was added and kept in water bath at 100°C for three hours. After the mixture cooled down, neutralization was done with sodium carbonate and volume was maintained to 100 mL with distilled water. To 1 mL supernatant, 4 mL anthrone reagent was added and kept in water bath for 8 min. The absorbance of the mixture was noted at 630 nm by using spectrophotometer (UV-1100) (Hodge & Hofreiter, 1962).

Starch: Extraction of plant sample (0.5 g) was done with methanol. The plant residue was kept in oven and re-extracted with 5 mL distilled water and 52% HCl (1:1 v/v). The mixture was centrifuged at 7500 g for 10 min. To 0.5 mL supernatant, anthrone reagent was added and kept in water bath at 100°C for 30 min. The optical density of the mixture was noted at 625 nm by using spectrophotometer (UV-1100) (Malik & Srivastava, 1985).

Determination of nutrient contents in maize grains

Sodium, potassium, calcium: For determining sodium, potassium and calcium ions, acid digestion was done. To dried plant sample (0.5 g), 5 mL of concentrated H_2SO_4 was added and kept overnight. The flasks containing the mixture were placed in digestion block and heated at 350°C for 30 min. After cooling down, 1 mL of H_2O_2 was added and flasks were placed in digestion block for 20 min. The previous steps were done again and again until plant sample was completely dissolved and colourless solution was appeared. The volume of plant extract was maintained to 50 mL with distilled water. The values of ionic contents (Na, K, Ca) were determined by using flame photometer (Jenway PFP-7). A series of standards (10, 20 to 100) was prepared, standard curve was drawn and the values of ionic contents (Na^+, K^+, Ca^{2+}) were compared with standard curve (Wolf, 1982).

Nitrate: Dried plant material (0.5 g) was boiled in 5 mL distilled water for 1 h. After filtration the volume was maintained to 50 mL with distilled water. Plant extract (3 mL) was mixed with 7 mL of working CTA solution and vortex. Colour intensity was noted at 430 nm by using spectrophotometer (UV-1100) (Kowalenko & Lowe, 1973).

Phosphate: Dried plant material (0.5 g) was boiled in distilled water for 1 h. After filtration the volume of the plant extract was maintained 50 mL with distilled water. Plant extract (1 mL) was mixed with 2 mL of 2N HNO_3 and volume was maintained to 4 mL with distilled water. To this solution 1 mL of molybdate-vanadate reagent was added and volume was maintained to 10 mL with distilled water. The mixture was vortex and cooled at room temperature for 20 min. Optical density was measured at 420 nm by using spectrophotometer (UV-1100) (Yoshida *et al*., 1976).

Sulfate: To 5 mL of plant extract, 5 mL of acid mixture and 5 mL of barium chloride/PVA solution was added and thoroughly shaken for 30 s. The aliquot was shifted to spectrophotometer cuvette and absorbance of the mixture was noted at 420 nm using spectrophotometer (UV-1100) (Tendon, 1993).

Statistical analysis

The experiment was designed in completely randomized pattern with three replicates. The data for all variables were subjected to analysis of variance technique (ANOVA) (Steel & Torrie, 1986) by using co-stat software (CoHort Software, 2003, Monterey, California). Statistix 8 was used for determining the difference in treatment means using Lease Significance Difference Test (LSD) (Steel *et al.*, 1996). Microsoft excel was used for graphical representation of the data.

Results

Vitamin contents in maize grains: Results revealed that salt stress significantly reduced ascorbic acid contents in both maize varieties. At 75 mM salt level, a high reduction in ascorbic acid contents was found. However, sulfur application (40, 80 mM) improved ascorbic acid contents at all studied salt levels (25, 75 mM) in both varieties (Fig. 1a) as seen by statistically significant V × S interaction (Table 1). Low level of sulfur (40 mM) was found more effective in improving ascorbic acid contents in maize plants (Fig. 1a). Moreover, sulfur application also reduced the toxic effects of salinity by improving the ascorbic acid contents. These findings are strengthened by statistically significant V × Sa × S interaction (Table 1). Overall, salt tolerant maize variety (Agatti, 2003) accumulated higher ascorbic acid contents in comparison to salt sensitive maize cultivar (Pak Afgoi, 2003).

Salinity reduced tocoperol contents in grains of both maize varieties as shown by statistically significant V × Sa interaction at 0.05 confidence interval (Table 1). However, application of sulfur significantly improved tocopherol contents in both maize varieties at all studied salt levels (25, 75 mM) as revealed from statistically significant Sa × S interaction at 0.05 confidence interval (Table 1). Sulfur at 40 mM proved very effective in improving tocopherol contents in comparison to higher level of sulfur (80 mM). Moreover, sulfur improved salt tolerance in maize plants by improving tocopherol contents in salt sensitive maize variety (Pak Afgoi, 2003) (Fig. 1b).

Statistical analysis revealed that salinity reduced the riboflavin contents in both maize cultivars as shown from statistically significant V × Sa interaction (Table 1). Higher salt level (75 mM) reduced riboflavin contents in both maize cultivars. While, application of sulfur (40, 80 mM) was found very effective in improving riboflavin contents in both maize varieties at all studied salt levels (25, 75 mM). These results are supported by statistically significant Sa × S interaction (Table 1). Agatti 2003 contained higher riboflavin contents in comparison to Pak Afgoi, 2003 (Fig. 1c).

Niacin contents were decreased by increasing salt level in both maize cultivars. It was evident from statistically significant V × Sa interaction (Table 1). Application of sulfur improved salt tolerance potential of maize varieties by improving niacin contents at all studied salt levels (25, 75 mM) as evident from statistically significant V × Sa × S interaction (Table 1). However, lower level of sulfur (40 mM) proved highly effective as compared to higher level of sullfur (80 mM) in both maize varieties in salinized and non-salinized medium. Agatti 2003 (salt tolerant) accumulated higher niacin content in comparison to Pak Afgoi, 2003 (salt sensitive) (Fig. 2a).

It was found that thiamine contents were decreased by salt treatment in both maize varieties as evident from statistically significant V × Sa interaction (Table 1). At 75 mM salt level, thiamine contents were highly reduced. However, application of sulfur (40, 80 mM) improved thiamine contents in both varieties at all studied salt levels as shown from statistically significant V × S interaction (Table 1). It was revealed that lower level of sulfur (40 mM) was found very effective in improving thiamine contents in comparison to higher level of sulfur (80 mM). Agatti 2003 (salt tolerant) accumulated higher thiamine contents in comparison to Pak Afgoi, 2003 (salt sensitive) (Fig. 2b).

Biomolecules in maize grains: Results revealed that salt stress decreased protein contents in both maize genotypes. At elevated salt concentration (75 mM), a high reduction in protein contents was found. Application of sulfur improved protein contents in both maize cultivars at all salt levels (25, 75 mM) as seen from statistically significant Sa × S interaction (Table 2). Low level of sulfur (40 mM) was found more effective in improving protein contents in comparison to higher sulfur concentration (80 mM) (Fig. 3a). Salt tolerant maize genotype (Agatti 2003) contained higher protein contents as compared to salt sensitive maize variety (Pak Afgoi, 2003) (Fig. 3a).

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

SOV	df	AA	Tocopherol	Riboflavin	Niacin	Thiamine
Variety (V)	1	0.027 ***	1.65e-6 ***	0.219 ***	2.03e-6 ***	47.29 ***
Salinity (Sa)	2	0.0086 ***	6.41e-7 ***	0.035 ***	1.14e-7 ***	10.98 ***
Sulfur (S)	2	0.0048 ***	5.52e-7 ***	0.041 ***	1.36e-7 ***	25.17 ***
V x Sa	2	1.85e-4 *	1.04e-38 ns	0.0027 *	7.47e-9 ns	0.87 *
V x S	2	4.38e-4 **	1.04e-38 ns	0.0028 *	7.29e-10 ns	0.89 *
Sa x S	4	4.98e-5 ns	1.25e-8 *	0.0035 **	2.56e-9 ns	0.36 ns
V x Sa x S	4	4.67e-4 ***	3.32e-38 ns	0.0037 **	1.79e-9 ns	0.15 ns
Error	36	5.54e-5	4.46E-09	8.083e-4	3.07E-09	0.19

*, **, *** = Significant at 0.05, 0.01 and 0.001 levels, respectively. ns = Non-significant
Abbreviations: Exponent (e), AA = Ascorbic acid

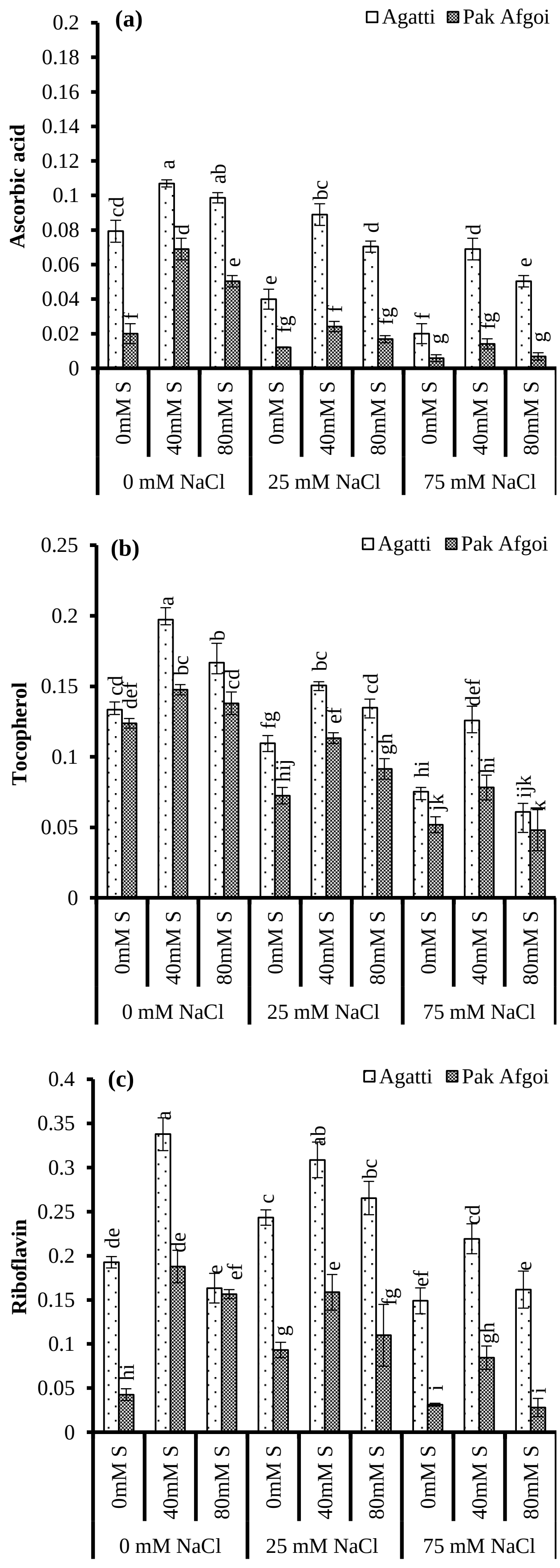

Fig. 1. Effect of different levels of sulfur (S) and NaCl on ascorbic acid (µg/g dry. wt.) (a) tocopherol (µg/g f. wt.) (b) riboflavin (µg/mL dry. wt.) (c) contents of maize (*Zea mays* L.) grains under saline conditions.

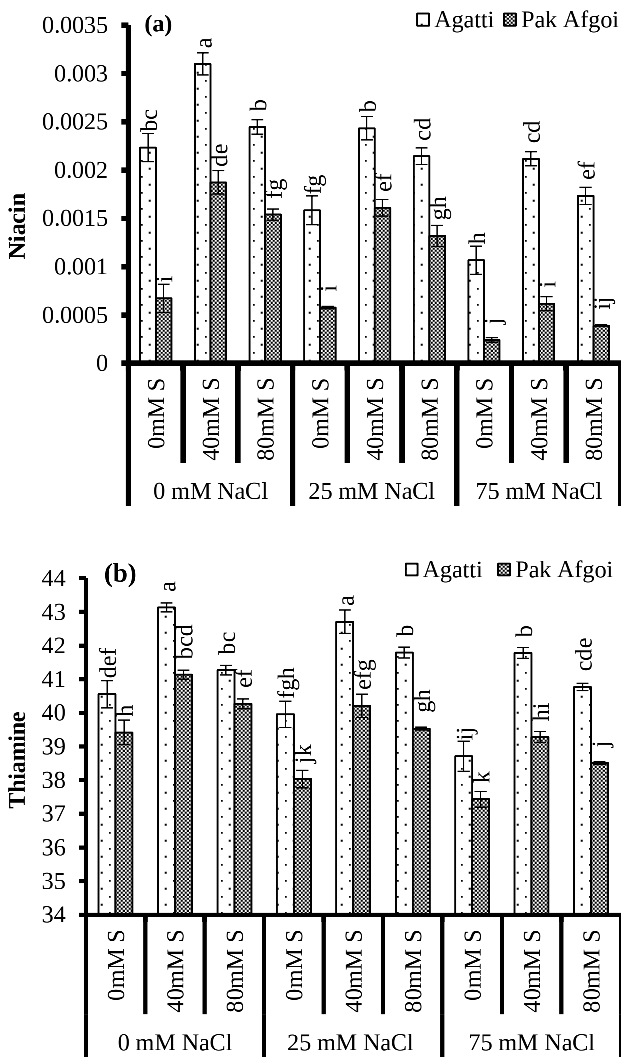

Fig. 2. Effect of different levels of sulfur (S) and NaCl on niacin (µg/g f. wt.) (a) and thaimine (µg/g dry. wt.) (b) contents of maize (*Zea mays* L.) grains under saline conditions.

Imposition of salt stress reduced carbohydrate concentration in maize variety. At 75 mM salt level, a high reduction in carbohydrate contents was found. However, application of sulfur highly improved carbohydrate contents in both maize varieties at all salt levels (25, 75 mM), as seen from statistically significant V × S interaction (Table 2). Moreover, sulfur at 40 mM highly improved carbohydrate contents in comparison to higher sulfur level (80 mM). Salt tolerant maize cultivar (Agatti 2003) accumulated higher carbohydrate contents in comparison to salt sensitive maize variety (Pak Afgoi, 2003) (Fig. 3b).

Imposition of salinity decreased starch contents in both maize cultivars. At 75 mM salt level, a high reduction in starch contents was found. However, application of sulfur improved starch contents in both maize varieties as revealed from statistically significant V × S interaction (Table 2). Moreover, sulfur also improved salt tolerance in salt sensitive maize variety (Pak Afgoi, 2003) by improving starch contents at all salt levels (25, 75 mM). Low level of sulfur (40 mM) highly improved starch contents in maize grains in comparison to higher sulfur concentration (80 mM) (Fig. 3c).

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

SOV	df	Protein	Starch	Carbohydrate
Variety (V)	1	0.014 ***	0.037 ***	14734.52 ***
Salinity (Sa)	2	0.0047 ***	0.042 ***	2043.09 ***
Sulfur (S)	2	0.0037 ***	0.026 ***	4610.68 ***
V x Sa	2	3.82e-5 ns	0.0031 ***	29.46 ns
V x S	2	1.71e-5 ns	0.0021 ***	806.018 ***
Sa x S	4	3.21e-4 **	9.65e-4 **	3.92 ns
V x Sa x S	4	9.28e-6 ns	2.70e-4 ns	12.46 ns
Error	36	7.37e-5	2.45e-4	14.51

*, **, *** = Significant at 0.05, 0.01 and 0.001 levels, respectively. ns = Non-significant
Abbreviations: Exponent (e)

Nutrient contents in maize grains: A marked increase in sodium (Na^+) contents was observed by imposition of salinity in both maize varieties. At 75 mM salt level, elevated concentration of sodium (Na^+) was found (Fig. 4a). However, application of sulfur reduced sodium (Na^+) accumulation in maize grains of both varieties as revealed from statistically significant V × S interaction (Table 3). Moreover, it was found that both varieties responded differently to sulfur application. Agatti 2003 (salt tolerant) accumulated lower concentration of sodium (Na^+) in comparison to Pak Afgoi 2003 (salt sensitive). These findings are supported by statistically significant V × Sa × S interaction (Table 3). Sulfur at 40 mM proved highly effective in reducing sodium (Na^+) contents in maize plants.

Statistical analysis has shown a significant reduction in potassium (K^+) contents by salt application in both studied genotypes as seen from statistically significant V × Sa interaction (Table 3). However, application of sulfur not only improved potassium (K^+) contents but also induced salt tolerance in salt sensitive maize plants. Moreover, sulfur at 40 mM highly improved potassium (K^+) contents in maize grains in comparison to higher sulfur level (80 mM). Salt tolerant maize cultivar (Agatti 2003) accumulated high potassium (K^+) contents as compared to salt sensitive maize variety (Pak Afgoi, 2003) (Fig. 4b).

Imposition of salt stress highly reduced calcium (Ca^{2+}) contents in grains of both maize varieties. Maximum reduction was noted at 75 mM salt level (Fig. 4c). These results are strengthened by statistically significant V × Sa interaction (Table 3). Application of sulfur highly reduced toxic effect of salinity by improving calcium (Ca^{2+}) contents in both maize cultivars. It was supported by statistically significant Sa × S interaction (Table 3). Lower level of sulfur (40 mM) was found highly effective in improving calcium (Ca^{2+}) contents in comparison to higher sulfur levels (80 mM). Moreover, Agatti 2003 accumulated high calcium contents and proved salt tolerant maize cultivar and Pak Afgoi 2003 accumulated lower calcium (Ca^{2+}) contents being salt sensitive maize variety (Fig. 4c).

Salinity caused reduction in nitrate (NO_3^-) contents in both maize varieties as seen from statistically significant V × Sa interaction (Table 3). At 75 mM salt level, a high reduction in nitrate (NO_3^-) contents was found. However, application of sulfur (40 mM) highly improved nitrate (NO_3^-) contents in maize plants to improve salt tolerance potential (Fig. 4d). These findings are supported by statistically significant V × S interaction (Table 3). Salt tolerant maize cultivar (Agatti, 2003) stored higher nitrate (NO_3^-) contents in comparison to salt sensitive maize genotype (Pak Afgoi, 2003) (Fig. 4d).

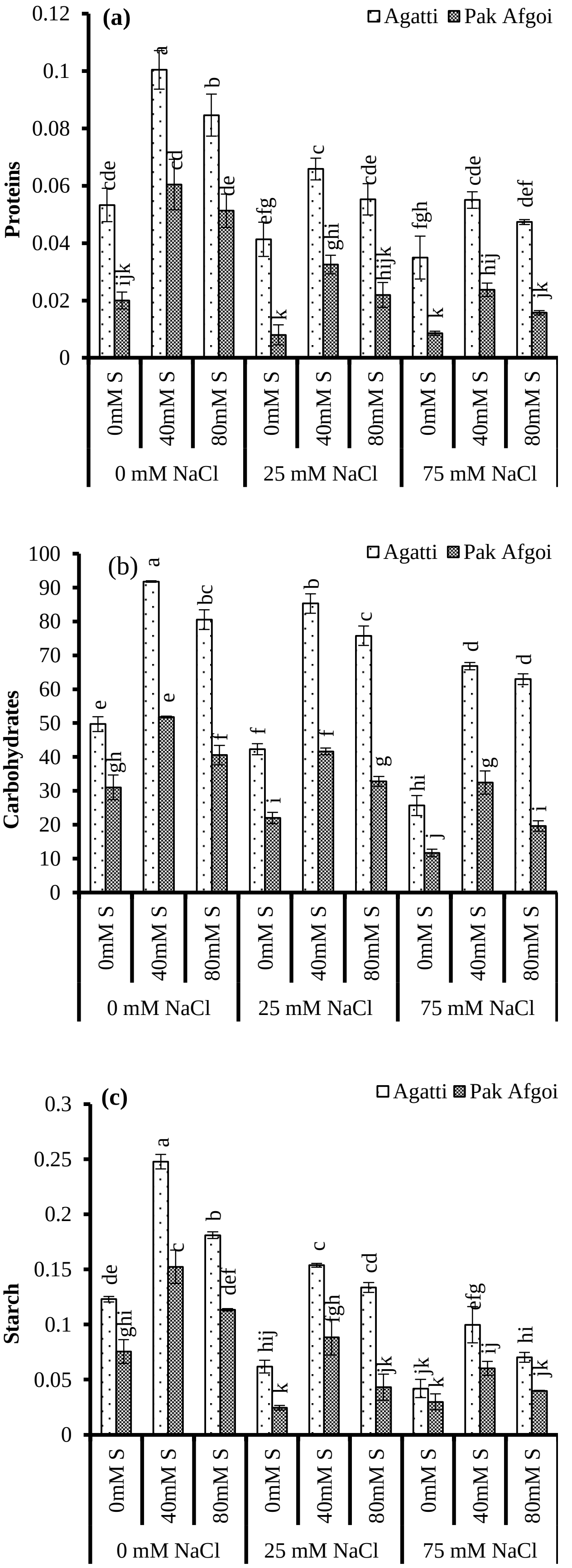

Fig. 3. Effect of different levels of sulfur (S) and NaCl on proteins (mg/g dry wt.) (a) carbohydrates (% dry wt.) (b) and starch (µmol/g dry wt.) (c) contents of maize (*Zea mays* L.) grains under saline conditions.

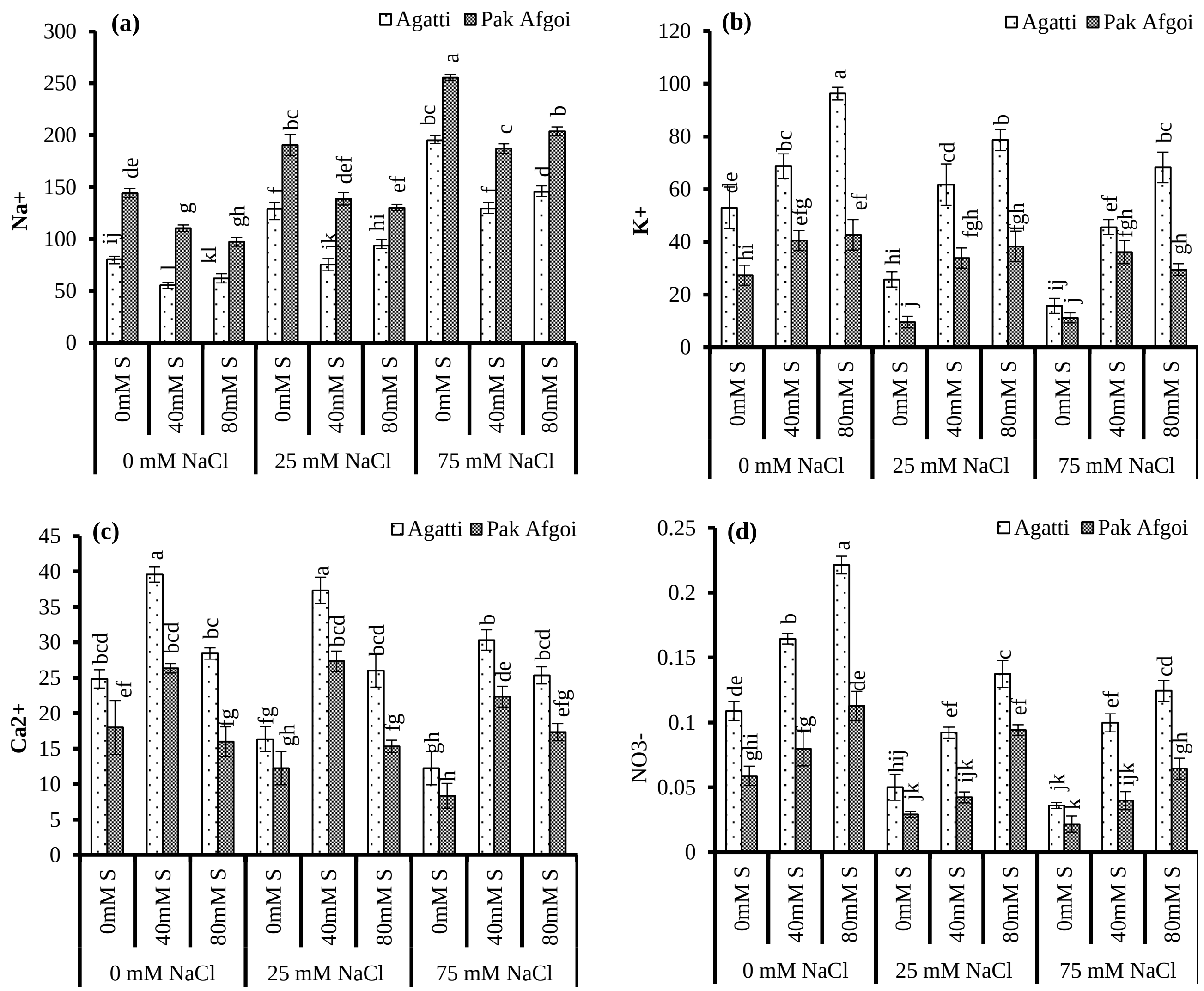

Fig. 4. Effect of different levels of sulfur (S) on sodium (Na^+) (mg/g dry wt.) (a) potassium (K^+) (mg/g dry wt.) (b) calcium (Ca^{2+}) (mg/g dry wt.) (c) and nitrate (NO_3^-) (mg/g dry wt.) (d) contents of maize (*Zea mays* L.) grains under saline conditions.

Table 3. Mean squares from analysis of variance (ANOVA) of the data for nutrient contents in grains of maize subjected to different levels of salinity and sulfur.

SOV	df	Na^+	K^+	Ca^{2+}	NO_3^-
Variety (V)	1	40292.99 ***	9990.01 ***	992.44 ***	0.041 ***
Salinity (Sa)	2	41078.29 ***	1924.17 ***	173.60 ***	0.018 ***
Sulfur (S)	2	13295.01 ***	5819.41 ***	1055.21 ***	0.025 ***
V x Sa	2	66.52 ns	377.19 **	20.18 ns	0.0024 ***
V x S	2	438.54 **	1028.22 ***	44.46 *	0.0023 ***
Sa x S	4	551.33 ***	114.98 ns	50.51 **	2.93e-4 ns
V x Sa x S	4	108.38 ns	30.58 ns	0.88 ns	1.67e-4 ns
Error	36	78.64	61.48	9.72	1.67e-4
SOV	**df**	**PO_4^{3-}**	**SO_4^{2-}**	**K^+/Na^+**	**Ca^{2+}/Na^+**
Variety (V)	1	0.16 ***	305.48 ***	3.075 ***	0.49 ***
Salinity (Sa)	2	0.148 ***	203.95 ***	1.31 ***	0.21 ***
Sulfur (S)	2	0.140 ***	617.72 ***	0.90 ***	0.21 ***
V x Sa	2	0.001 ns	16.71 ***	0.49 ***	0.06 ***
V x S	2	6.11e-4 ns	22.85 ***	0.24 ***	0.049 ***
Sa x S	4	0.0042 *	16.29 ***	0.049 *	0.009 **
V x Sa x S	4	5.57e-4 ns	1.25 ***	0.021 ns	0.0048 *
Error	36	0.0013	1.49	0.013	0.0018

*, **, *** = Significant at 0.05, 0.01 and 0.001 levels, respectively. ns = Non-significant.

Abbreviations: Exponent (e), Na^+ = Sodium, K^+ = Potassium, Ca^{2+} = Calcium, NO_3^- = Nitrate, PO_4^{3-} = Phosphate, SO_4^{2-} = Sulfate

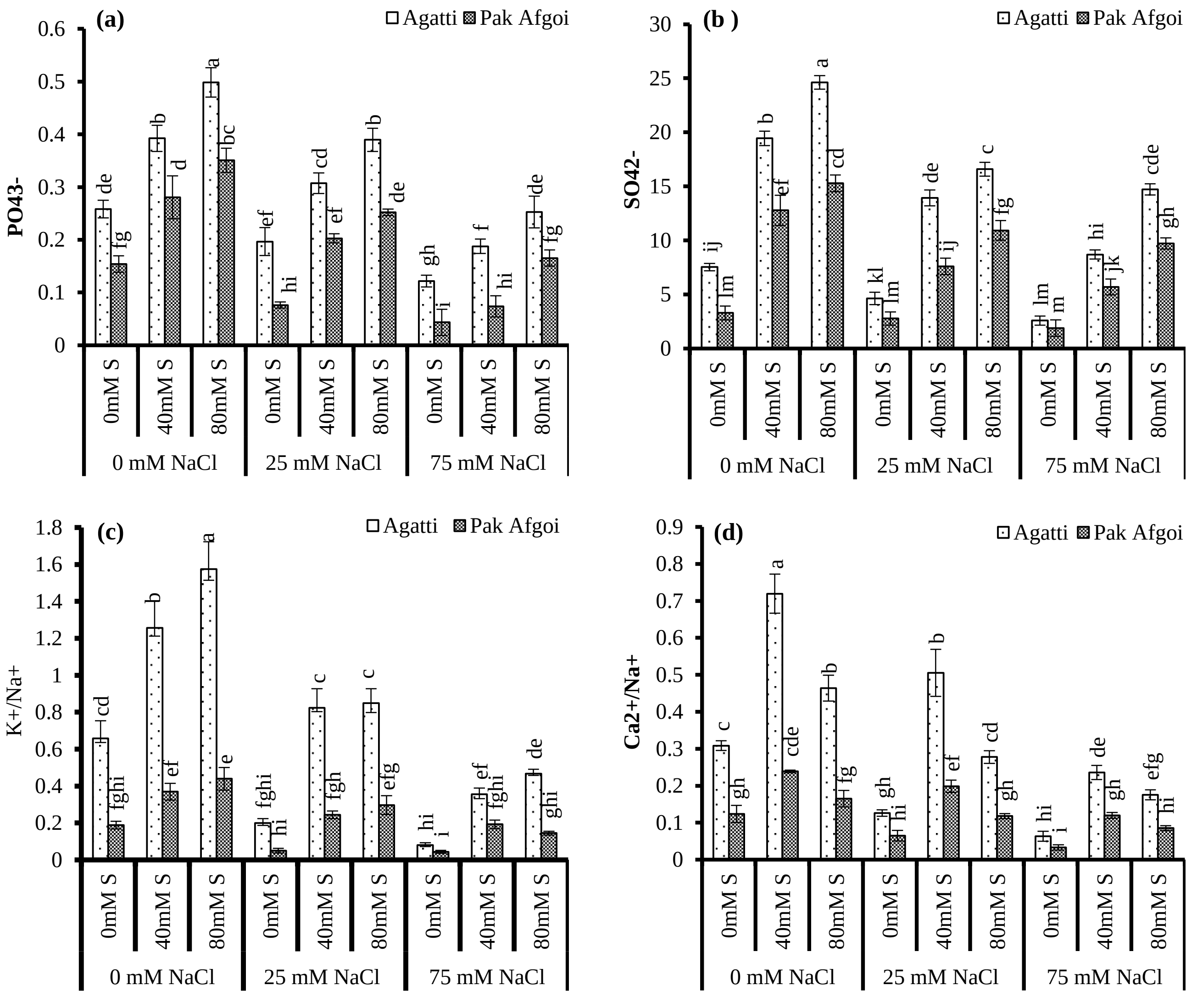

Fig. 5. Effect of different levels of sulfur (S) on phosphate (PO_4^{3-}) (mg/g dry wt.) (a) sulfate (SO_4^{2-}) (mg/g dry wt.) (b) potassium/sodium (K^+/Na^+) (mg/g dry wt.) (c) calcium/sodium (Ca^{2+}/Na^+) (mg/g dry wt.) (d) contents of maize (*Zea mays* L.) grains under saline conditions.

Table 4. Composition of maize grains per 100 g of edible portion.

Composition	Per 100 g of edible portion
Riboflavin	0.10mg
Thiamine	0.42 mg
Ascorbic acid	0.12 mg
Carbohydrate	71.88 g
Protein	8.84 g
Minerals	1.5 g
Sodium	15.9 mg
Potassium	286 mg
Calcium	10 mg
Sulfur	114 mg
Phosphorus	348 mg

Source: (Shah *et al*., 2015)

Statistical analysis has shown high reduction in phosphate contents under salt stress conditions. Maximum reduction in phosphate contents was noted at 75 mM salt level (Fig. 5a). Application of sulfur found helpful in improving phosphate (PO_4^{3-}) contents in grains of maize plants at all salt levels as evident from statistically significant Sa × S interaction (Table 3). Sulfur application at low level (40 mM) highly improved phosphate (PO_4^{3-}) contents in maize grains in comparison to higher sulfur level (80 mM). Moreover, Agatti 2003 (salt tolerant) accumulated higher phosphate (PO_4^{3-}) contents as compared to Pak Afgoi 2003 (salt sensitive) (Fig. 5a).

Results revealed that salt stress caused significant reduction in sulfate (SO_4^{2-}) contents in both maize cultivars as revealed from statistically significant V × Sa interaction (Table 3). Maximum reduction in sulfate (SO_4^{2-}) contents was found at 75 mM salt level (Fig. 5b). However, application of sulfur highly improved sulfate (SO_4^{2-}) contents in both maize cultivars at all salt levels. These findings are strengthened by statistically significant V × S interaction. Moreover, sulfur also reduced toxic effects of salinity by improving sulfate (SO_4^{2-}) contents in both maize cultivars as evident from statistically significant Sa × S interaction (Table 3). It was found that salt tolerant maize variety (Agatti, 2003) accumulated high sulfate (SO_4^{2-}) contents in comparison to salt sensitive variety (Pak Afgoi, 2003) (Fig. 5b).

Imposition of salt stress reduced K^+/Na^+ ratio in grains of both maize cultivars. These findings are supported by statistically significant V × Sa interaction (Table 3). While, application of sulfur highly improved K^+/Na^+ ratio in both maize varieties at all studied salt levels as evident from statistically significant V × S interaction (Table 3). Moreover, low level of sulfur (40 mM) highly improved K^+/Na^+ ratio in comparison to higher sulfur concentration (80 mM). Agatti (salt tolerant) showed high K^+/Na^+ ratio as compared to Pak Afgoi 2003 (salt sensitive) (Fig. 5c).

Results revealed that salt stress caused a significant reduction in Ca^{2+}/Na^+ ratio in both maize varieties. At 75 mM salt level, Ca^{2+}/Na^+ ratio was highly reduced (Fig. 5d). However, application of sulfur (40 mM) highly improved Ca^{2+}/Na^+ ratio under salt stress conditions. A statistically significant V × S interaction supported the present findings (Table 3). Salt tolerant maize variety (Agatti, 2003) contained high Ca^{2+}/Na^+ ratio in comparison to salt sensitive maize cultivar (Pak Afgoi 2003) (Fig. 5d).

Discussion

Salt stress caused a marked reduction in quality and productivity of crop plants. Among various means to encounter salt toxicity, exogenous application of various inorganic fertilizers is very economical and shot gun approach. Sulfur has significant importance in developing salinity tolerance by regulating various physiological and biochemical attributes in plants (Riffat & Ahmad, 2020a, b). Application of sulfur enhances grain quality of maize by improving nutritional composition, seed life, germination ability and developing salinity tolerance. As a result high quality maize grains produce that fulfill most of human nutrition. Maize grains contain multivitamins (ascorbic acid, riboflavin, niacin, thiamine, tocopherol, pantothenic acid, pyridoxin), nutrients, folic acid, tryptophan, phytochemicals and various biomolecules (Table 4) (Shah *et al*., 2015). As maize responds quickly to sulfur fertilization so application of sulfur has proved very much helpful in improving nutritional value of maize grain under saline conditions.

Vitamins are considered key growth regulators that improve plant growth and development by regulating plant metabolism to encounter salt stress. Maize grains constituents a high proportion of vitamins. Sulfur has significant contribution in improving vitamin contents that induce salt tolerance in maize grains. The current study revealed that salt stress reduced ascorbic acid contents in grains of both maize genotypes (Agatti, 2003; Pak Afgoi, 2003). It has been reported that ascorbic acid concentration was decreased by increasing salt level (Mittal *et al.*, 2018). Ascorbic acid regulates cell division, cell expansion, photosynthesis, biosynthesis of various hormones and antioxidants in plants (Pastori *et al*., 2003). In this study, application of sulfur has improved ascorbic acid contents in maize grains at all applied salt levels (25, 75 mM). Previous studies revealed that ascorbic acid concentration was increased by increasing sulfur supply (Chandra & Pandey, 2014).

Current study showed a reduction in tocopherol contents in maize grains due to salt toxicity. It has been reported that tocopherol contents in rice were reduced by high level of salinity (Turan & Tripathy, 2013). Tocopherol is involved in scavenging reactive oxygen species, membrane stability and protects photosynthetic machinery (Munne-Bosche & Falk, 2004). In this study, application of sulfur enhanced tocopherol contents in maize grains. It may be due to the reason that glutathione a sulfur containing compound protects cell membrane by maintaining tocopherol in reduced form (Tausz *et al.*, 2004).

Results revealed that salt stress reduced riboflavin contents in grains of maize plants. Previous studies have shown that riboflavin concentration was decreased by higher level of salinity (Ratnakar & Rai, 2013). Riboflavin is a part of different coenzymes regulating redox reactions and plant metabolism. It also plays an important role in abiotic stress tolerance in crop plants (Deng *et al*., 2014). It was found that application of sulfur fertilizer improved riboflavin contents in grains of maize genotypes.

Salt stress reduced niacin contents in grains of both maize cultivars. These findings are related to previous studies (Magdi *et al*., 2013) reporting a reduction in niacin contents by imposition of salinity in *Faba bean* plants. Niacin is involved in redox reactions and regulates secondary metabolites and defense related response of plants (Berglund, 1994). Hence, appropriate concentration of niacin is necessary for normal metabolic reactions in plants. The present study have shown that application of sulfur improved niacin contents in maize grains and induced salinity tolerance in plants.

Imposition of salt stress also reduced thiamine contents in maize grains. This finding is in accordance to earlier study which reported a significant reduction in thiamine contents by increasing salt level (Ratnakar & Rai, 2013). Thiamine is an important vitamin that acts as cofactor in regulation of various metabolic processes in plants. It also acts an antioxidant under abiotic stress conditions. Moreover, an iron-sulfur cluster protein is necessary for thiamine biosynthesis (Raschke *et al*., 2007). Hence sulfur is an essential constituent of thiamine (Leustek, 2002). Previous literature supported present study which revealed a marked increase in thiamine contents by sulfur application in grains of maize genotypes.

Biomolecules are building blocks of plant body. They include proteins, carbohydrates, lipids and nucleic acid. However, salt stress caused a serious reduction in biomolecule contents in grains of crop plants. This study has shown a marked reduction in protein contents in maize grains due to application of salinity. Previous studies have revealed that protein concentration was decreased by increasing salt levels in *P. imperialis* in comparison to control (Ayala-Astorga & Alcaraz-Meléndez, 2010). However, application of sulfur improved protein contents in maize plants. Sulfur has significant contribution in increasing quality of proteins present in forage and grain crops. It improves seed quality by providing adequate quantity of proteins necessary for high production rates (Hawkesford & De Kok, 2006). It has been reported that application of sulfur fertilizers highly increased seed protein in canola seeds (Ahmad *et al.*, 2000).

In current study, it was revealed that salinity also reduced starch contents in grains of both maize genotypes. These findings are related to previous studies which reported that salt stress (160 mol m^{-3} NaCl) significantly reduced starch contents in pea plants (Hernandez *et al.*, 1999). However, application of sulfur increased starch contents in maize grains under salinized and non-salinized medium. Previous studies supported the current results which evaluated that sulfur application (45 kg ha^{-1}) highly increased dry matter content, specific gravity, sugar and starch contents in potato plants (Sharma *et al*., 2011).

Results revealed that salt stress caused a significant reduction in carbohydrate contents in grains of both maize varieties. It was related to previous studies that shown application of salinity (30, 60, 90 mM NaCl) highly reduced carbohydrate contents in sweet sorghum (Almodares *et al.*, 2008). While application of sulfur proved very beneficial in improving carbohydrate contents in grains of maize plants. It may be due to the reason that sulfur application improved carbohydrate utilization for the synthesis of protoplasm. Moreover, sulfur deficiency highly reduced carbohydrate contents (Neelam & Nalini, 2013).

Salt stress causes imbalance in nutrient transport, ion toxicity and osmotic stress. Mineral nutrients have favorable potential in reducing salinity problem. Sulfur has significant potential in reducing salt toxicity by improving seed nutrition. In the present study, salt stress enhanced sodium (Na) contents in grains of both maize genotypes. This finding is relevant to previous studies which reported that salinity (due to NaCl) highly increased sodium (Na) contents (Sadak & Abd Elhamid, 2013). However, Application of sulfur reduced sodium (Na) accumulation in maize grains of both varieties. These results are in accordance to previous studies which revealed that sulfur application significantly reduced sodium contents in maize plants (Riffat & Ahmad, 2018a).

Results revealed that salinity reduced potassium (K) contents in maize grinas which are related to previous findings that shown a high reduction in potassium contents by salt application in *Triticum aestivum* (L.) (Rady & Mohamed, 2018). However, application of sulfur improved potassium (K) contents in maize grains under salinized and non-salinized medium. Previous studies supported the present findings which revealed that sulfur has positive correlation with potassium in increasing canola yield (Govahi & Saffari, 2006). Moreover potassium acts as counter cation for sulfate transport during the process of xylem loading and storage in vacuole (Reich *et al.*, 2016).

Imposition of salinity reduced calcium (Ca) contents in grians of both maize cultivars (Agatti, 2003; Pak Afgoi, 2003). These results are related to previous studies which revealed that salinity due to NaCl decreased calcium (Ca) in soybean (Arshi *et al.*, 2010). Calcium is very important for stabilizing cell wall, regulating ionic transport and ion exchange and controls activities of various enzymes (Hadi & Karimi, 2012). In this study, application of sulfur reduced toxic effect of salinity by improving calcium contents in grains of both maize cultivars. This may be due to the reason that calcium decreases toxic effects of salinity (Jaleel *et al*., 2007). It has been found that under saline conditions, sulfur application increased action of calcium ions in spinach and red oarch (Wilson *et al.*, 2000).

Results of this study revealed a reduction in nitrate (NO_3^-) contents by salt application. It may be due to the reason that, salt stress seriously affect nitrate (NO_3^-) uptake in plants due to interaction between nitrate (NO_3) and chloride (Cl^-) ions (Schmidhalter, 2005). Nitrogen is an important plant nutrient that has positive effects on growth and development of plants (Crawford *et al.*, 2000). Thus, its adequate quantity is necessary to improve plant growth under stress conditions. Sulfur has positive correlation with nitrogen fertilizers. Moreover, it also helps to improve nitrogen use efficiency (Fazili *et al.*, 2008).

In the current study, it was found that salt stress decreased phosphate (PO_4^{3-}) contents, however, application of sulfur improved phosphate (PO_4^{3-}) contents in grains of both maize cultivars. Phosphorous has may roles in metabolic processes of plants including glycolysis, respiration, activation of various enzymes, signaling process, nitrogen fixation and oxidation reduction reactions (Vance *et al*., 2003). Sulfur has synergistic relationship with phosphorous in improving the nutrition in soybean (Paliwal *et al.*, 2009). However, sulfur has positive interaction with phosphorous at lower nutrient rate and negative interaction at higher rate of nutrients (Islam *et al*., 2011).

Salinity also reduced sulfate (SO_4^{2-}) contents while application of sulfur improved the sulfate (SO_4^{2-}) contents in grains of maize plants. These results are supported by previous findings which evaluated that salt stress lowered sulfate (SO_4^{2-}) contents however, application of sulfur improved sulfate (SO_4^{2-}) contents in maize plants (Riffat, 2017).

In the present study, it was observed that salt stress reduced K^+/Na^+ ratio while application of sulfur improved K^+/Na^+ ratio in maize grains. These findings are related to previous studies which reported that salinity reduced K^+/Na^+ ratio in maize plants (Riffat & Ahmad, 2018a). However, sulfur application increased K^+/Na^+ ratio in maize plants (Reich *et al*., 2016). Results of this study revealed a reduction in Ca^{2+}/Na^+ ratio by salt application however, sulfur application improved Ca^{2+}/Na^+ ratio in grains of maize plants. These results are in accordance to previous studies which reported that sulfur helps to improve Ca^{2+}/Na^+ ratio in plants to avoid salt toxicity (Aslam *et al.*, 2001).

Conclusion and Recommendations

From the above discussion it is amply clear that salt stress has seriously reduced vitamins, biomolecules and concentration of beneficial nutrients in the grains of both maize genotypes. However, low level of sulfur (40 mM) proved very helpful in improving vitamins, biomolecules and nutritional contents in maize grains at all levels of salinity. Sulfur application also enhanced salt tolerance potential of salt sensitive maize cultivar (Pak Afgoi, 2003) by regulating nutrient uptake and transport, and providing vitamins, proteins, carbohydrate and starch contents that can encounter salt toxicity problems. Hence, it is recommended that application of 40 mM sulfur is very effective in improving grain quality in terms of vitamins, biomolecules and nutritional contents and ultimately crop productivity under salt stress conditions.

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