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ALLEVIATION OF ADVERSE EFFECTS OF SALT STRESS ON GROWTH OF MAIZE (*ZEA MAYS* L.) BY SULFUR SUPPLEMENTATION

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Abstract

Sulfur has considerable importance in ameliorating the adverse effects of salinity by modulating different physiological and biochemical pathways in the plants. A study was conducted to assess the response of maize in improving maize growth by exogenous application of sulfur under salt stress conditions. Seeds of maize varieties were sown in plastic pots containing loamy soil and treatments of sulfur (40, 80 mM) and salinity (25, 75 mM) were applied. For the determination of classical growth analysis, two harvests were taken at 7 and 21 days of treatment application while the growth parameters, photosynthetic pigments and biomolecule contents were determined by harvesting 52 days old plants. Results showed that salt stress reduced shoot and root length, fresh and dry weight, leaf area, relative growth rate, leaf weight fraction, unit leaf rate, specific leaf area, leaf area ratio, root shoot allometry, chlorophyll *a*, chlorophyll *b*, chlorophyll *a/b* ratio, total chlorophyll, starch and carbohydrate contents in both maize cultivars. However, sulfur application (40 mM) not only improved all studied growth parameters, photosynthetic components and biomolecules but also developed salt tolerance in salt sensitive maize cultivar (Pak Afgoi 2003). In crux, sulfur at 40 mM is very effective in improving maize growth under salt stress condition.

Key words: Carbohydrates, Classical growth analysis, Chlorophyll, Maize, Sulfur, Salinity, Starch.

Introduction

Salt stress is seriously declining agricultural production every year. It seriously reduces crop growth and development that leads to decrease in plant yield. The reduction in plant growth due to salinity is attributed to reduction in plant water potential that increases osmotic potential in the plant cell. The high amount of salt in the plant cell causes specific ion toxicity and imbalance in nutritional contents (Lauchli & Epstein, 1990). Moreover, excessive salt concentration reduces the CO_2 availability that reduces photosynthetic pigments and ultimately the process of photosynthesis becomes hampered (Ashraf & Harris, 2013). The reduction in photosynthesis reduces the production of starch and carbohydrates. Among various hazardous effects of salinity on plant growth, the reduction in germination and seedling growth, suppression in leaf area expansion, limiting photosynthetic area and limited production of dry matter has significant importance (Riffat & Ahmad, 2016; Farooq *et al*., 2019). So, there is an urgent need to devise effective methods to combat the toxic effects of salinity.

Among various methods to counteract the damages caused by salt stress, nutrient management is very economical and easy approach. Earlier researches on various crops (rice, wheat, maize) have shown the development of salt tolerance by exogenous application of inorganic nutrients (Riffat, 2017: Riffat & Ahmad, 2018a). Among macronutrients, sulfur has considerable importance in improving growth by improving salt tolerance potential of plants. Sulfur is found in amino acids cysteine, methionine, thioredoxine and sulfolipids (Singh, 2003). Application of sulfur improves plant growth and development by increasing proteins, vitamins, starch, carbohydrates, glutathione and photosynthetic pigments that increase leaf area, height, fresh and dry weight of the plant. Moreover, sulfur is a constituent of ferridoxin (Fe-S protein) that transports free electrons in the process of photosynthesis (Spadaro *et al*., 2010; Sharma *et al*., 2011; Neelam & Nalini, 2013). Thus, application of sulfur improves photosynthetic efficiency which improves production of carbohydrates and starch and ultimately overall plant growth. Therefore, sulfur application is necessary for balanced crop nutrition and for counteracting the adverse effects of salinity.

Maize has considerable nutritional significance. It contains many types of vitamins, phytochemicals and nutrients that are an essential constituent of our diet. However, the quality and production of maize is seriously affected by salt stress due to its salt sensitive nature (Farooq *et al.*, 2015). Therefore, efficient methods should be devised to improve salt tolerance of maize for meeting the needs of growing population. This study highlights the role of sulfur in improving salt tolerance potential of maize cultivars by enhancing various growth parameters, photosynthetic pigments and biomolecules under severe condition of salt stress.

Materials and Methods

Plan of study: The study was conducted in the wire house of Department of Botany, University of Agriculture Faisalabad. The seeds of two maize varieties (Agatti 2003; Pak Afgoi 2003) were obtained from Maize and Millet Institute Sahiwal, Pakistan. Seeds were sown in plastic pots (Temperature=33/28±3ºC day/night; Relative humidity=68/85±2%) filled with 10 kg loamy soil (pH=9.06; EC=1210µS/cm). Two levels of salt (25, 75 mM) were applied by using NaCl. Sulfur (40, 80 mM) was applied by using K_2SO_4. One set of plants were kept as control (0 mM salt and 0 mM sulfur). Foliar spray of sulfur (40, 80 mM) was also applied after germination. Two harvests were taken after 7 and 21 days of treatment application for the determination of classical growth analysis. Then final harvest was taken after 52 days for determining various growth parameters, photosynthetic components and biomolecules.

Determination of growth parameters

Shoot and root length: Length of shoot and root was determined by using scale (in centimetre units). Then average length of four maize plants of every replicate was calculated.

Shoot and root fresh weight: For the determination of fresh weight of shoot and root, electrical balance was used. Mean value of fresh weight was determined by taking average of four plants of each replicate.

Shoot and root dry weight: Dry weight of shoot and root was determined by covering the plants in paper, labelled and put in oven at 65ºC for 72 hours. Then weighed with the help of electrical balance and average was calculated of four plants of every replicate.

Leaf area per plant: For leaf area per plant, leaf width and leaf length was calculated and following formula was used.

$$\text{Total leaf area} = \text{Leaf length} \times \text{Leaf width} \times \text{Correction factor}$$

where correction factor = 0.65

Classical growth analysis: Classical growth analysis (relative growth rate, leaf weight fraction, unit leaf rate, specific leaf area, leaf area ratio, root shoot allometry) were determined by using software proposed by Hunt *et al*., (2002).

Determination of photosynthetic attributes

Chlorophyll contents: Chlorophyll contents (*a*, *b* and total chlorophyll) were determined by following Arnon (1949). Fresh plant material (0.5 g) was homogenized with 80% acetone in darkness. The extract was filtered and volume was maintained to 10 mL by using 80% acetone. Then absorbance was noted at 645 nm (for chlorophyll *a*), and 663 nm (for chlorophyll *b*) by using spectrophotometer (UV-1100). Following formulas were used for calculation.

$$\text{Chl. } a \text{ (mg/g)} = \frac{12.7\,(OD663) - 2.69\,(OD645) \times V}{1000 \times W}$$

$$\text{Chl. } b \text{ (mg/g)} = \frac{22.9\,(OD645) - 4.68\,(OD663) \times V}{1000 \times W}$$

$$\text{Total Chl. (mg/g)} = \frac{20.2\,(OD645) + 8.02\,(OD663) \times V}{1000 \times W}$$

where V= Volume of acetone used in extract (mL)

W= Fresh weight of plant in g

Determination of biomolecules

Starch: For the determination of starch contents in plant sample, the method proposed by Malik & Srivastava (1985) was followed. Firstly, anthrone reagent was prepared by dissolving 1 g anthrone in 1 L of concentrated H_2SO_4. For the determination of starch contents, 0.5 g dried plant sample was extracted in methanol, oven dried and again extracted with 5 mL water and 52% HCl (1:1 v/v). The mixture was centrifuged at 7500 *g* for 10 minutes. To 0.5 mL of supernatant, anthrone reagent was added, placed in water bath at 100ºC for ½ hour and kept at room temperature. After cooling down, the absorbance was noted at 625 nm using spectrophotometer (UV-1100).

Carbohydrates: For the determination of carbohydrates, a procedure proposed by Hedge & Hofreiter (1962) was followed. Firstly some reagents were prepared. Anthrone reagent was prepared by dissolving 200 mg anthrone in 100 mL of chilled 95% H_2SO_4. Stock solution was prepared by dissolving 100 mg glucose in 100 mL distilled water. Working standard was prepared by diluting 10 mL of stock to 100 mL with distilled water and some drops of toluene were added and kept in refrigerator. For the determination of carbohydrate contents, 100 mg of plant sample was added in 5 mL of 2.5 N HCl and kept in water bath at 100ºC for 3 hours. The mixture was kept at room temperature for cooling down. Then neutralization of the mixture was done by adding sodium carbonate. The volume of the mixture was diluted to 100 mL with distilled water. By separating the supernatant, 0.5 mL aliquot was collected for analysis. The standard series of solution was prepared by adding 0, 0.2, 0.4, 0.6, 0.8 and 1 mL of the working standard in test tubes and volume was maintained to 1 mL with distilled water. To each test tube, 4 mL of anthrone reagent was put and heated in water bath at 100 ºC for 8 minutes. The mixture was cooled rapidly and dark green color was appeared. The absorbance of the mixture was noted at 630 nm by using spectrophotometer (UV-1100). Then a graph was plotted between concentration of standard on X-axis and absorbance of sample mixture on Y-axis. Following formula was used for determining amount of carbohydrate.

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

Statistical analysis: The experiment was arranged in completely randomized design (CRD) with three replicates. Analysis of variance (ANOVA) was performed by using Statistix 8.0. To find the difference in treatments, least significant difference (LSD) test was used (Steel *et al.*, 1996). Microsoft Excel was used for determining treatment variation and graphical representation of data. Classical growth analysis was determined by using software given by Hunt *et al*., (2002).

Results

Growth parameters: It was revealed that salinity has reduced the length, fresh and dry weight of shoot and root and leaf area of both studied maize cultivars (Agatti, 2003; Pak Afgoi 2003). Although all studied salt levels (25, 75 mM) caused reduction in growth parameters; maximum reduction in growth parameters was noted at 75 mM salt level (Figs. 1-4). Both varieties responded differently to salt application. Agatti 2003 (salt tolerant) showed less reduction in growth parameters in comparison to Pak Afgoi 2003 (salt sensitive). However, sulfur application improved salt tolerance potential in both maize varieties (Agatti, 2003, Pak Afgoi 2003). The application of sulfur increased plant growth in salt sensitive maize cultivar and reduced the toxic effects of salinity. A statistically significant V × S interaction for shoot length of maize plants supported the present findings (Table 1). Both levels of sulfur (40, 80 mM) improved plant growth at all studied salt levels (25, 75 mM). However, lower level of sulfur (40 mM) proved very effective in increasing growth of maize plants in comparison to higher sulfur level (80 mM). Overall, the application of sulfur not only increased leaf area, length, fresh and dry weight of shoot and root of both cultivars (Agatti, 2003; Pak Afgoi 2003); but also improved salt tolerance ability of maize plants by enduring high level of salinity (75 mM) (Figs. 1-4).

Results showed that salt stress decreased relative growth rate of both maize cultivars (Agatti, 2003; Pak Afgoi 2003). Maximum reduction in relative growth rate was observed at 75 mM salt applied. The application of sulfur improved relative growth rate at all studied salt levels (25, 75 mM). Although, both levels of sulfur (40, 80 mM) improved the relative growth rate; however, sulfur at 40 mM highly improved relative growth rate in comparison to higher sulfur level (80 mM) as evident from Fig. 5. Moreover, sulfur also improved salt tolerance in salt sensitive cultivar (Pak Afgoi 2003) by improving relative growth rate at seedling stage.

Leaf weight fraction was decreased by increasing salt levels. At 75 mM salt level, a higher reduction in leaf weight fraction was found. It was revealed from Fig. 5. By the application of sulfur (40, 80 mM) leaf weight fraction was highly improved in both maize varieties. Lower level of sulfur (40 mM) showed more improvement in leaf weight fraction as compared to higher sulfur level (80 mM). Agatti 2003 (salt tolerant) showed high leaf weight fraction as compared to Pak Afgoi 2003 (salt sensitive). However, sulfur improved salt tolerance in Pak Afgoi 2003 by improving leaf weight fraction at all levels of salinity (25, 75 mM) (Fig. 5).

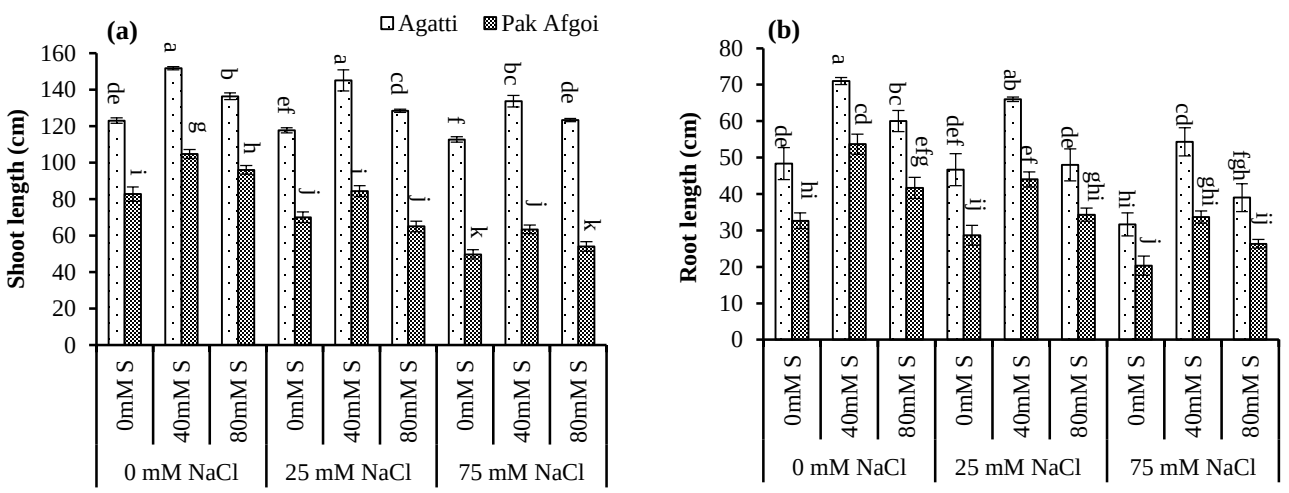

Fig. 1. Effect of different levels of sulfur (S) on shoot legth (a) root length (b) of maize (*Zea mays* L.) cultivars under saline conditions.

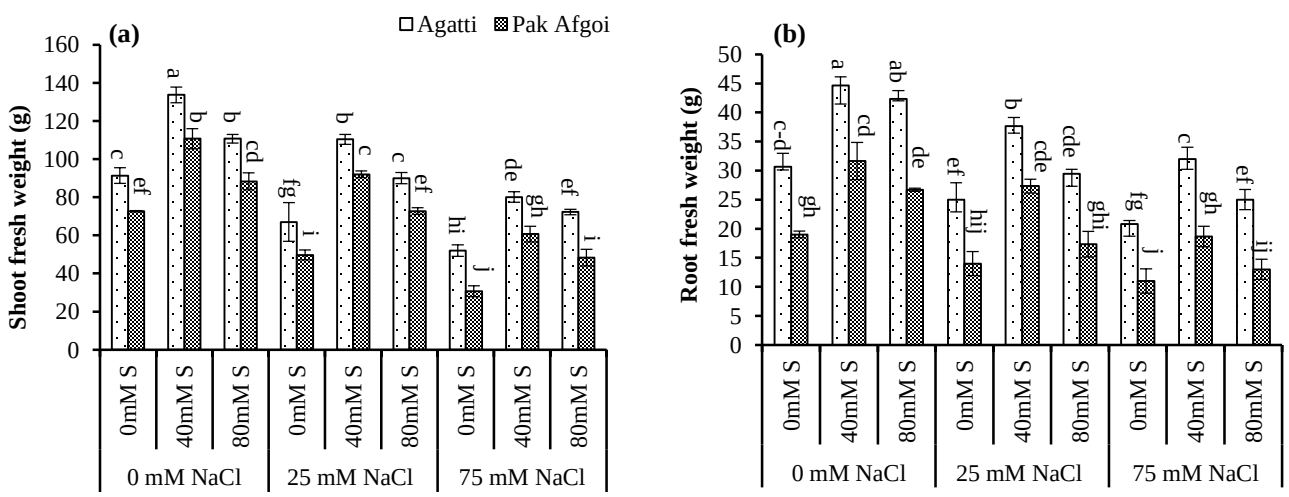

Fig. 2. Effect of different levels of sulfur (S) on shoot fresh weight (a) root fresh weight (b) of maize (*Zea mays* L.) cultivars under saline conditions.

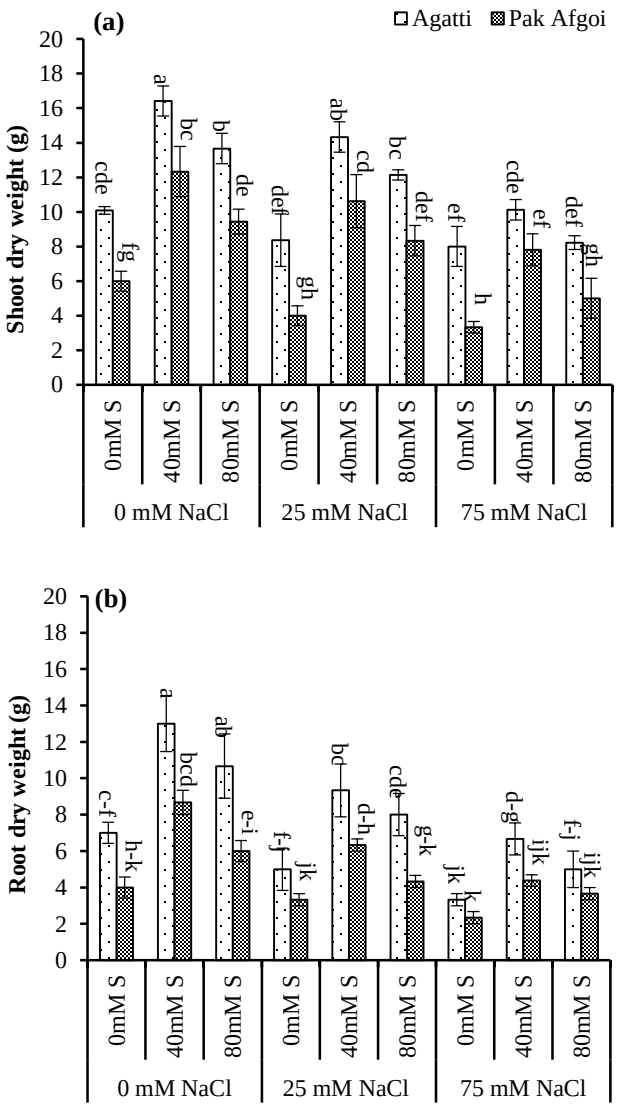

Fig. 3. Effect of different levels of sulfur (S) on shoot dry weight (a) root dry weight (b) root dry weight (f) of maize (*Zea mays* L.) cultivars under saline conditions.

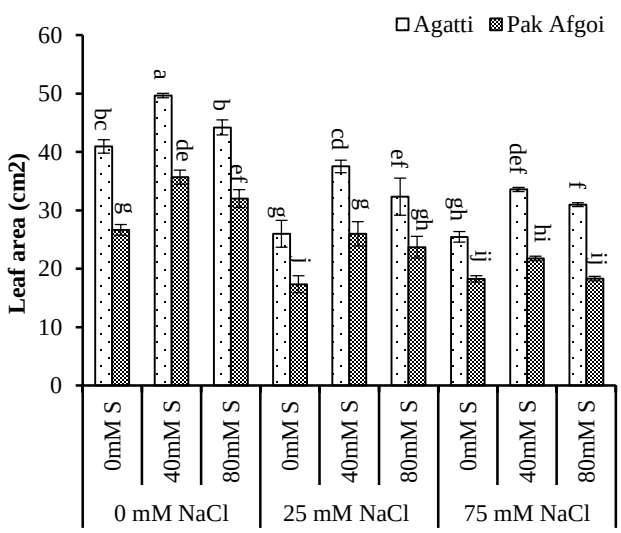

Fig. 4. Effect of different levels of sulfur (S) on leaf area of maize (*Zea mays* L.) cultivars under saline conditions.

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	SL	RL	SFW
Variety (V)	1	42000.66 ***	3733.35 ***	5500.46 ***
Salinity (Sa)	2	3111.79 ***	1321.91***	8674.05 ***
Sulfur (S)	2	2059.68 ***	1677.57 ***	6285.42 ***
V × Sa	2	710.16 ***	10.91 ns	21.46 ns
V × S	2	103.16 *	38.35 ns	4.87 ns
Sa × S	4	61.65 *	19.55 ns	105.01 ns
V × Sa × S	4	24.08 ns	14.32 ns	5.66 ns
Error	36	21.22	25.55	46.75
		RFW	**SDW**	**RDW**
Variety (V)	1	1976.53 ***	197.98 ***	103.76 ***
Salinity (Sa)	2	703.07 ***	81.94 ***	71.88 ***
Sulfur (S)	2	640.58 ***	127.25 ***	68.49 ***
V × Sa	2	6.34 ns	0.65 ns	6.81 ns
V × S	2	6.71 ns	1.15 ns	2.63 ns
Sa × S	4	25.0044 ns	5.87 ns	2.67 ns
V × Sa × S	4	2.71 ns	0.58 ns	0.37 ns
Error	36	9.92	2.55	2.36
		LA	**Chl *a***	**Chl *b***
Variety (V)	1	1700.10 ***	6.36e-4 ***	0.014 ***
Salinity (Sa)	2	927.45 ***	6.37e-4 ***	0.027 ***
Sulfur (S)	2	308.41 ***	2.59e-4 ***	0.0080 ***
V × Sa	2	18.35 ns	1.79e-5 ns	1.72e-4 ns
V × S	2	6.52 ns	2.21e-5 ns	5.63e-4 *
Sa × S	4	8.61 ns	7.52e-6 ns	6.16e-4 *
V × Sa × S	4	6.19 ns	3.99e-6 ns	2.62e-4 ns
Error	36	5.97	1.25e-5	1.70e-4
		Chl *a*/*b*	**Total Chl**	
Variety (V)	1	0.036 ***	0.021 ***	
Salinity (Sa)	2	0.024 ***	0.036 ***	
Sulfur (S)	2	0.0082 ***	0.011 ***	
V × Sa	2	4.17e-4 *	1.56e-4 ns	
V × S	2	2.66e-5 ns	7.94e-4 *	
Sa × S	4	1.99e-4 ns	5.21e-4 ns	
V × Sa × S	4	2.81e-4 *	2.25e-4 ns	
Error	36	8.95e-5	2.09e-4	

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

Abbreviations: Exponent (e), Shoot length (SL), Root length (RL), Shoot fresh weight (SFW), Root fresh weight (RFW), Shoot dry weight (SDW), Root dry weight (RDW), Leaf area (LA), Chlorophyll (Chl)

It was evident from findings of this study, that unit leaf rate was decreased at all levels of salinity (25, 75 mM). Moreover, salinity at 75 mM highly reduced unit leaf rate in both maize varieties. While application of sulfur improved unit leaf rate at all studied levels of salinity. Both studied sulfur levels (40, 80 mM) improved unit leaf rate. However, low level of sulfur (40 mM) highly improved unit leaf rate as compared to higher sulfur level (80 mM). Sulfur increased unit leaf rate in both varieties, however, Agatti 2003 well responded to sulfur fertilization as compared to Pak Afgoi 2003 (Fig. 6).

A marked reduction in specific leaf area was observed by salt application. Maximum reduction was noted at 75 mM salt level. The application of sulfur improved the specific leaf area at all salt levels (Fig. 6). Although both sulfur levels were proved effective in improving the specific leaf area, however, sulfur at 40 mM showed pronounced improvement in specific leaf area in both varieties. Agatti 2003 showed high improvement in specific leaf area by sulfur application in comparison to salt sensitive variety (Pak Afgoi 2003). However, sulfur application also improved the specific leaf area in Pak Afgoi 2003 to endure the harsh salt condition (Fig. 6).

It was revealed that a gradual decrease in leaf area ratio was observed by increasing the salt levels. Both varieties responded differently to salt application. Agatti 2003 showed less decrease in leaf area ratio as compared to salt sensitive maize cultivar (Pak Afgoi 2003). However, sulfur improved leaf area ratio in salt sensitive variety also. Sulfur at 40 mM showed high improvement in leaf area ratio in comparison to higher level (80 mM) (Fig. 7). Sulfur reduced the salt toxicity in Pak Afgoi 2003 by improving the leaf area ratio at all levels of salinity (25, 75 mM) (Fig. 7).

Root: shoot allometery was increased by increasing the salt levels in both studied maize cultivars. Maximum rise in root: shoot allometery was found at 75 mM salt level. Sulfur application worked synergistically to salt application. Both studied sulfur levels increased the root: shoot allometery in both varieties. However, sulfur at 40 mM highly increased the root: shoot allometery. While sulfur at higher level (80 mM) did not prove effective in rising the root: shoot allometery. Agatti 2003 showed high root: shoot allometery as compared to Pak Afgoi 2003 (Fig. 7).

Photosynthetic attributes: Results showed that salinity significantly reduced the chlorophyll *a* contents of both maize varieties (Table 1). The maximum reduction in chlorophyll *a* contents by salt application was noted at 75 mM salt level. However, sulfur application (40, 80 mM) improved chlorophyll *a* contents in salinized and non-salinized medium. Higher sulfur level (80 mM) was not proved very effective in improving chlorophyll *a* contents in both maize cultivars in comparison to low sulfur level (40 mM). Sulfur reduced the toxic effects of salinity by improving chlorophyll *a* contents in salt sensitive maize cultivar (Fig. 8).

Salt stress decreased chlorophyll *b* contents in both maize cultivars. At 75 mM salt level, the maximum reduction in chlorophyll *b* contents was found (Fig. 8). Sulfur application significantly improved chlorophyll *b* contents in both maize cultivars. The maximum improvement in chlorophyll *b* contents by sulfur application was found at 40 mM sulfur. Both varieties responded differently to sulfur application. It was shown by statistically significant V × S interaction (Table 1). Agatti 2003 (salt tolerant) highly improved chlorophyll *b* contents under salt stress conditions in comparison to Pak Afgoi 2003 (salt sensitive). In addition, sulfur significantly reduced harmful effects of salinity by improving chlorophyll *b* contents at all salt levels (25, 75 mM). It was evident from statistically significant Sa × S interaction (Table 1).

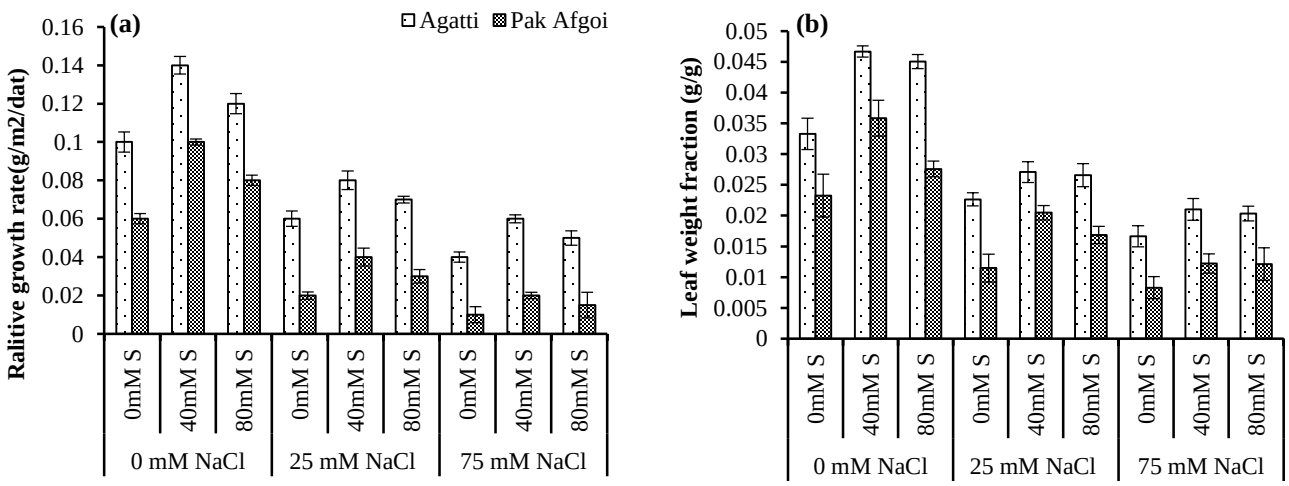

Fig. 5. Effect of different levels of sulfur (S) on relative growth rate (a) leaf weight fraction (b) of different maize (*Zea mays* L.) cultivars under saline conditions.

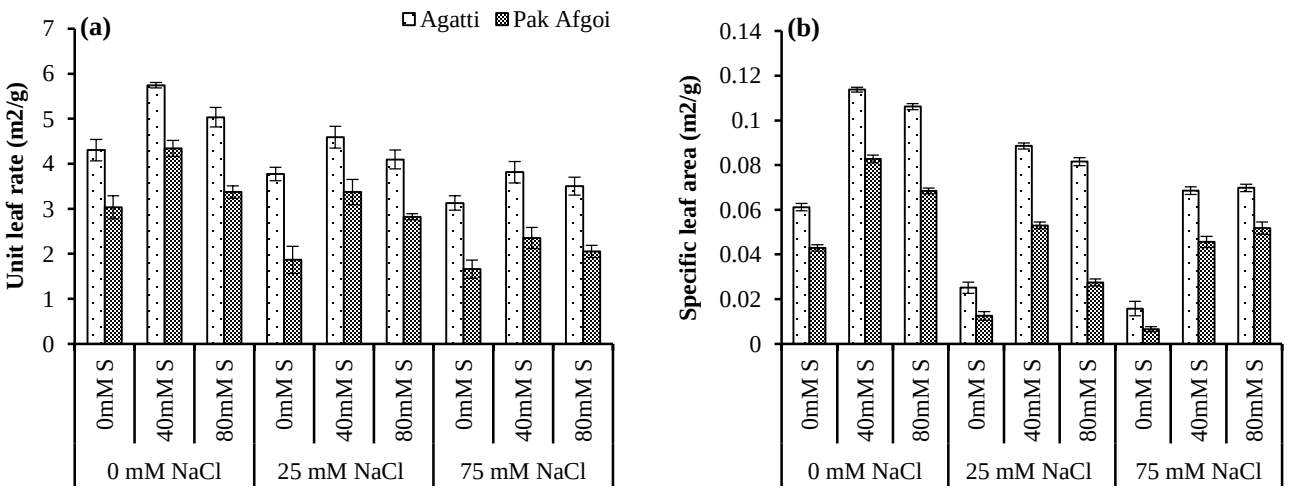

Fig. 6. Effect of different levels of sulfur (S) on unit leaf rate (a) specific leaf area (b) of different maize (*Zea mays* L.) cultivars under saline conditions.

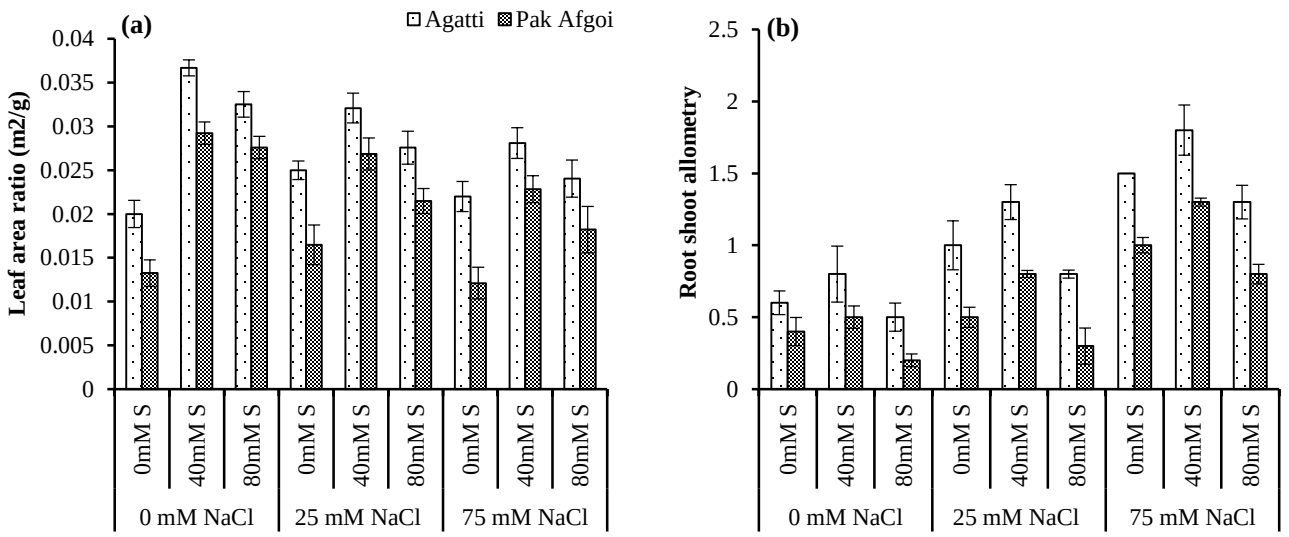

Fig. 7. Effect of different levels of sulfur (S) on leaf area rtio (a) root shoot allometry (b) of different maize (*Zea mays* L.) cultivars under saline conditions.

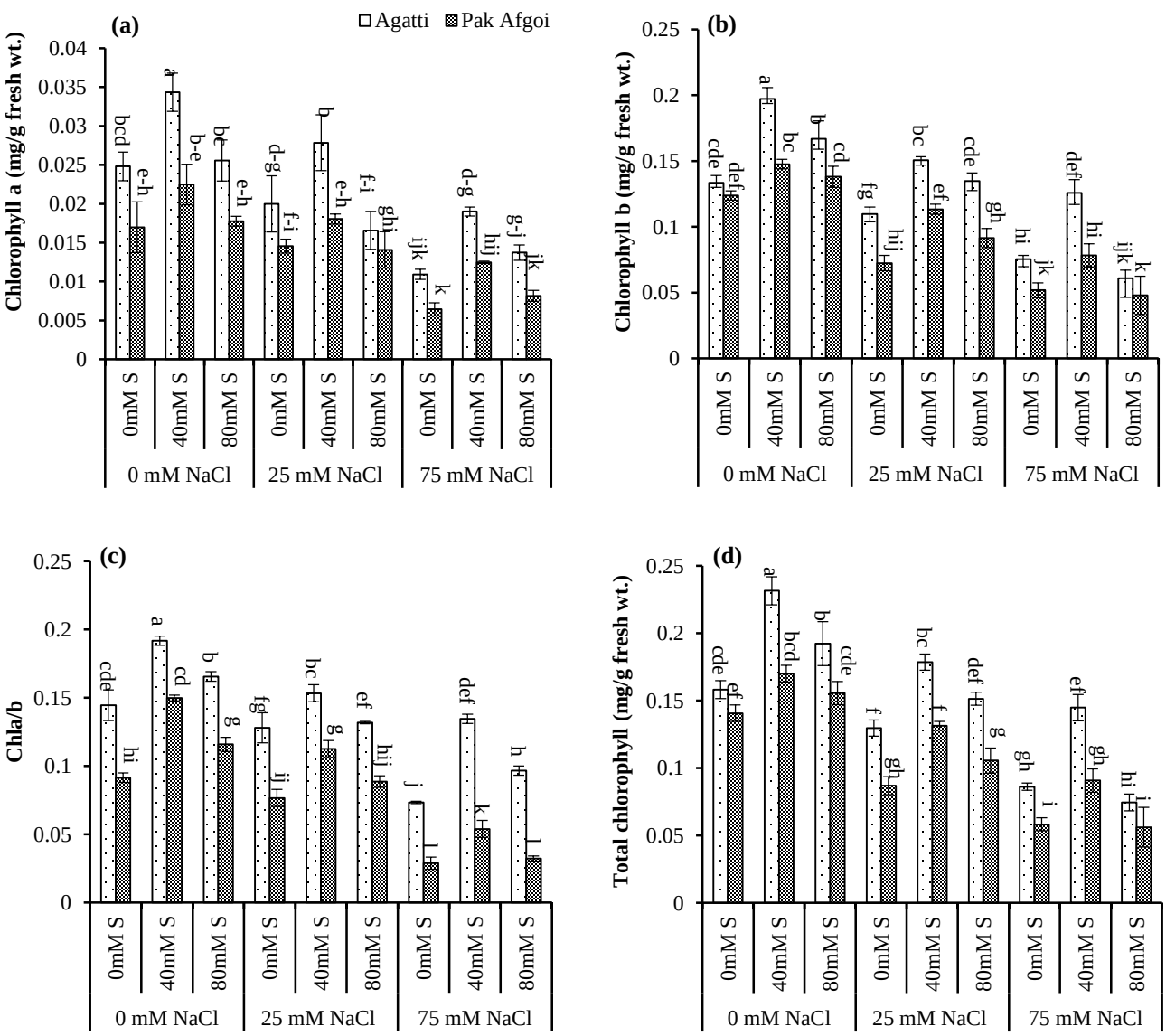

Fig. 8. Effect of different levels of sulfur (S) on chlorophyll *a* (a) chlorophyll *b* (b) chl*a*/chl*b* (c) and total chlorophyll (d) of different maize (*Zea mays* L.) cultivars under saline conditions.

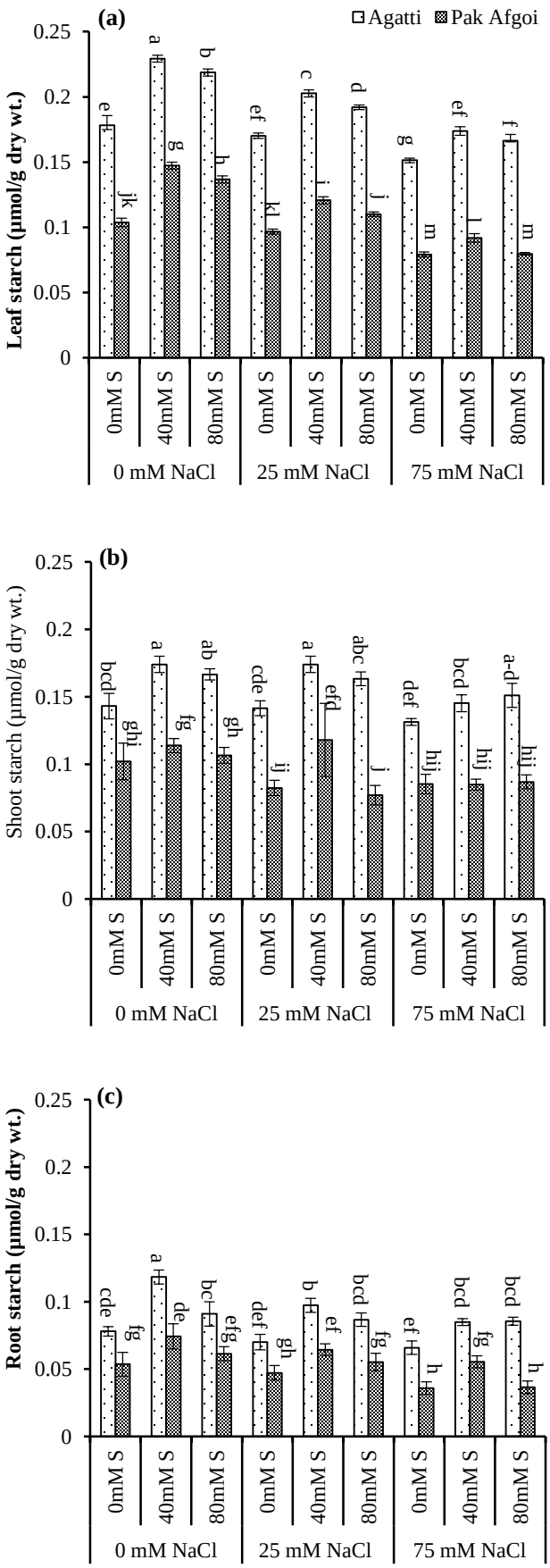

Fig. 9. Effect of different levels of sulfur (S) on starch content in leaf (a) shoot (b) and root(c) of different maize (*Zea mays* L.) cultivars under saline conditions.

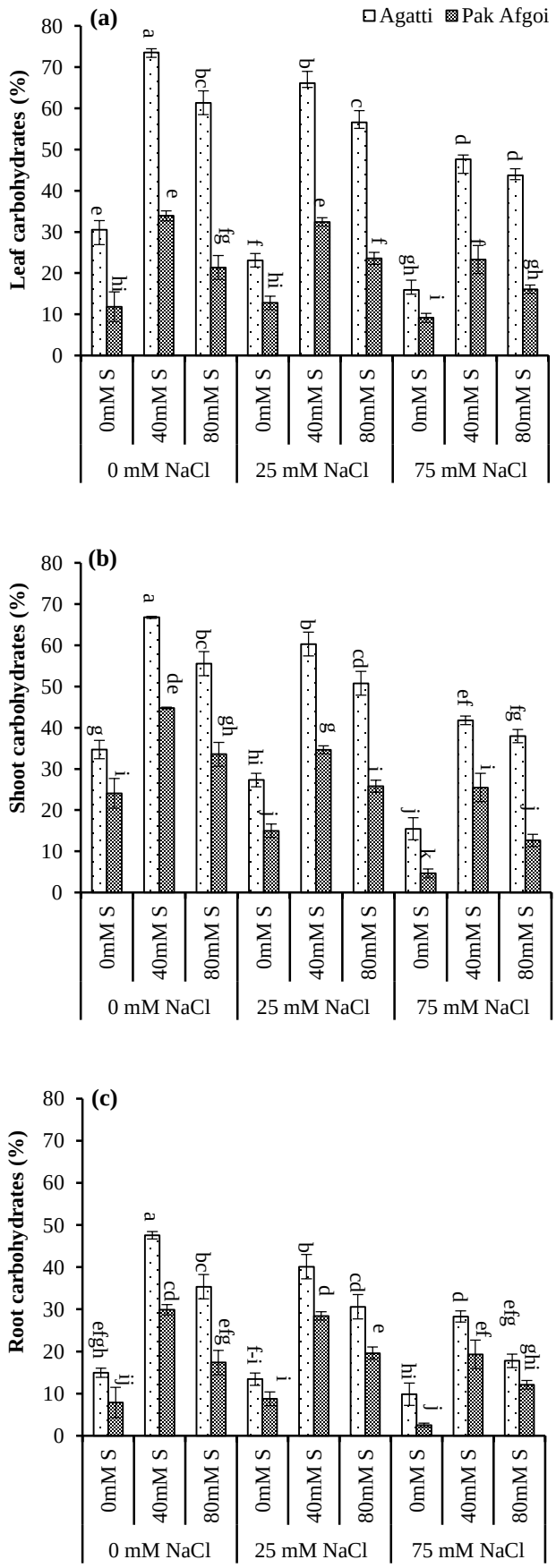

Fig. 10. Effect of different levels of sulfur (S) on carbohydrate content in leaf (a) shoot (b) and root(c) of different maize (*Zea mays* L.) cultivars under saline conditions.

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

SOV	df	Leaf starch	Shoot starch	Root starch
Variety (V)	1	0.085 ***	0.047***	0.014 ***
Salinity (Sa)	2	0.0093***	0.0018**	0.0015 ***
Sulfur (S)	2	0.0045 ***	0.0019**	0.0025 ***
V × Sa	2	1.75e-6 ns	2.22e-4 ns	5.80e-5 ns
V × S	2	1.33e-4 *	5.18e-4 ns	1.63e-4 ns
Sa × S	4	4.48e-4***	4.19e-4 ns	7.23e-5 ns
V × Sa × S	4	5.52e-6 ns	1.07e-4 ns	1.12e-4 ns
Error	36	2.94e-5	2.51e-4	9.82e-5
		Leaf carbohydrates	**Shoot carbohydrates**	**Root carbohydrates**
Variety (V)	1	9135.52 ***	4825.02 ***	1413.59 ***
Salinity (Sa)	2	805.34 ***	1881.002 ***	563.78 ***
Sulfur (S)	2	3944.92 ***	2971.13 ***	2331.23 ***
V × Sa	2	195.87 ***	15.41 ns	58.056 *
V × S	2	670.58 ***	205.36 ***	52.16 *
Sa × S	4	45.22 *	6.015 ns	38.83*
V × Sa × S	4	3.42 ns	12.046 ns	16.13 ns
Error	36	14.14	14.28	13.64

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

Abbreviations*:* Exponent (e)

Statistical analysis has shown that salt stress reduced chl *a*/chl *b* ratio in both studied maize varieties. This was evident from statistically significant V × Sa interaction (Table 1). A drastic decrease in chl *a*/chl *b* ratio was noted at75 mM salt level (Fig. 8). The application of sulfur improved chl *a*/chl *b* ratio in both maize cultivars (Agatti, 2003, Pak Afgoi 2003). Both varieties responded well to sulfur application. Although Pak Afgoi 2003 is salt sensitive, however, sulfur application increased chl *a*/chl *b* ratio in this cultivar to endure harsh condition due to salt stress. Thus, sulfur improved salt tolerance in Pak Afgoi 2003. The effectiveness of sulfur was noted at 40 mM sulfur level. These results were affirmed by statistically significant V × Sa × S interaction (Table 1).

Total chlorophyll contents were reduced by high level of salt stress (75 mM). Salt stress highly reduced total chlorophyll contents in salt sensitive maize cultivar (Pak Afgoi 2003). However, by applying sulfur at 40 mM, total chlorophyll contents were improved in Pak Afgoi 2003. While higher level of sulfur (80 mM) did not much improve total chlorophyll contents in both maize cultivars (Fig. 8). Overall, sulfur improved salt tolerance potential of both varieties by improving total chlorophyll contents. It was shown by statistically significant V × S interaction (Table 1).

Biomolecule contents: Results revealed that salt stress decreased starch contents in all studied organs (leaf, shoot, root) of both maize cultivars (Agatti 2003; Pak Afgoi 2003). A significant reduction in starch contents were noted at 75 mM NaCl. Application of sulfur (40, 80 mM), rose starch contents. However, low level of sulfur (40 mM) showed pronounced influence in improving starch contents (Fig. 9). Both varieties showed significant improvement in starch contents by sulfur application, however, Agatti 2003 accumulated more starch contents in comparison to Pak Afgoi 2003. It was shown by statistically significant V × S interaction for leaf (Table 2). While shoot and root showed non-significant V × S interaction. Sulfur ameliorated toxic effects of salinity in salt sensitive maize cultivar (Pak Afgoi 2003). This is evident from statistically significant Sa × S interaction for maize leaves (Table 2). Various organs of maize accumulated starch contents in different proportion. The order of starch accumulation was leaf > shoot > root (Fig. 9).

Statistical analysis has shown that salt stress reduced carbohydrate contents in both studies maize varieties. This was noted from statistically significant V × Sa interaction for leaf and root. However, in shoot, V × Sa interaction was found non-significant (Table 2). At 75 mM salt concentration, a high reduction in carbohydrate contents was observed. The application of sulfur (40, 80 mM) improved carbohydrate contents at all studied salt levels (25, 75 mM). However, sulfur at 40 mM improved carbohydrates very much in comparison to 80 mM sulfur level (Fig. 10). Moreover, sulfur also proved very effective in reducing the harmful effects of salinity by improving carbohydrate contents. It was shown by highly significant Sa × S interaction for leaf and root, while shoot showed non-significant Sa × S interaction (Table 2). Moreover, leaves accumulated higher concentration of carbohydrates in comparison to shoot and root (Fig. 10).

Discussion

In this study, salt stress caused a marked reduction in shoot and root length. The findings of this study are supported by previous study of Asaadi (2009). It may be due to the reason that salt stress causes the lowering of water potential in the growth medium that reduces cell turgidity that retards cell division, expansion, elongation and differentiation, which ultimately reduces plant biomass (Mazher *et al.*, 2007; Riffat & Ahmad, 2020). The application of sulfur significantly improved shoot and root length of all studied maize varieties. Various reports have shown that sulfur application increased length of shoot and root of various crops. Gilbert & Robson (1984) in an experiment found that sulfur application at 64 mg S/pot increased plant height in comparison to control were no sulfur was applied. However, present study revealed that high concentration of sulfur was not proved much effective in improving shoot and root length. Previous findings are supported by these results. Cerda *et al*., (1984) found that if sulfate contents were increased more than 45 mg dm^{-3} the shoot and root length was decreased. Therefore, low concentration of sulfur is more effective in improving shoot and root length in maize plants. Plants have complete regulatory mechanism for balancing sulfur at appropriate concentration. Various metabolites of sulfur play role in sulfur regulation. If high concentration of sulfur is present in root media, plant synthesizes glutathione which is transported from leaves to root and controls uptake and transport of sulfur appropriately (Herschbach & Ronnenberg, 1994).

Results revealed that salt stress decreased leaf area, and fresh and dry weight of maize plants. The reduction in leaf area may be due to the reason that salt stress causes reduction in photosynthetic rate that ultimately reduces chlorophyll contents and leaf growth which reduces the

leaf area (Ebert *et al.,* 2002). The reduction in fresh and dry biomass by high levels of salinity is due to the reason that salt stress reduces photosynthetic rate that lower production of assimilates which reduces plant growth and ultimately fresh and dry biomass (Netondo *et al*., 2004). Results from the present study revealed that sulfur application significantly improved leaf area and, fresh and dry weight of shoot and root. It may be due to the reason that sulfur application mainly focuses on improving root health. The healthier root efficiently uptake nutrients and water in plants that increase leaf area, fresh and dry biomass of the plants (Diepenbrock, 2000). Moreover, sulfur application also increases cell division in the meristematic zone which helps in healthier root and shoots production (Chandel *et al.,* 2002).

In the present work, results revealed that classical growth analysis (relative growth rate, leaf weight fraction, unit leaf rate, specific leaf area, leaf area ratio, root shoot allometry) were greatly influenced by salt application. The findings of this study are supported by earlier reports. Karlberg *et al.,* (2006) reported that salt stress reduced relative growth rate in plants. It might be due to the reason that salt stress causes the accumulation of salts in the root medium that reduces osmotic potential in plants causing lowering of water uptake and elevating the respiration rate leading to reduction in plant growth. Lambers *et al*., (1998) reported that leaf weight fraction was reduced by high level of salinity. Leaf weight fraction is the total biomass of plant distributed to leaves. The reduction in leaf weight fraction may be due to the reason that salt stress causes improper distribution of plant dry matter. During stress conditions plant dry matter is distributed to other structures of plants other than leaves that reduce leaf weight fraction (Miranda, 2010). Karlberg (2006) found a reduction in unit leaf rate by imposition of salt stress. He attributed this reduction to rise in respiration and reduction in photosynthesis. Ulloa *et al*. (2006) observed that by imposition of 120 mM NaCl, in *Physalis* 51% and in cucumber 8-13% reduction in unit leaf rate was found. This study has shown that specific leaf area was also reduced by high concentration of salts in the rooting medium. The results are related to previous work of Li *et al*. (2005). Specific leaf area measures leaf areas relative to leaf dry weight (Hunt, 1990). A reduction in specific leaf area indicates alteration in leaf structure or high concentration of Na^+ and Cl^- in the leaf (Miranda, 2010). It may be due to the reason that salinity reduces the assimilatory surface that reduces total rate of photosynthesis that increase energy required for osmotic adjustment (Karlberg *et al*., 2006). Leaf area ratio was reduced by application of salt stress which is an important growth determinant (Bresinsky *et al.,* 2008). It is function of specific leaf area and leaf weight ratio (Lambers *et al*., 1998). The reduction in leaf area by application of salinity may be due to the reason that high salt concentration in growth medium reduces photosynthetic area that lowers leaf elongation and increases width of palisade and spongy parenchyma (Bosabalidis & Kofidis, 2002). Results showed that root: shoot allometry was increased by application of salinity. Salt stress caused inequality in morphology and physiology of maize plants. It is related to study of Mendez-Alonzo *et al*. (2012) who reported that salt stress caused inequality in size and allometry of the plants. In the current study, the application of sulfur improved all studied classical growth analysis at all levels of salinity. The improvement in classical growth analysis by sulfur application may be due to the reason that sulfur increases carbohydrate utilization for the formation of protoplasm. The cells formed in such manner have large size and thin wall that causes increase in specific leaf area and leaf area ratio. The increase in leaf area index and relative growth rate indicates good leaf area expansion that helps in efficient light interception which increases dry matter in leaves and shoot helping in improving root shoot allometry (Ahmad *et al*., 2007).

Study showed that salinity reduced the photosynthetic pigments (chlorophyll *a, b, a/b* and total chlorophyll) in maize plants. Among different reasons of reduction in photosynthetic pigments by salt application, accumulation of Na^+ and Cl^- ions in the chloroplast is reported that block thylakoid membrane photophosphorylation and electron transport system (Ashraf *et al.,* 2010; Riffat, 2018; Riffat & Ahmad, 2018b). This causes reduction in synthesis of photosynthetic pigments that reduces photosynthetic rate (Noreen *et al.,* 2010). Another reason is that salt stress reduces the concentration of magnesium that is necessary for chlorophyll production in leaf. Moreover salinity reduces stomatal conductance and ultimately transpiration rate. This may be due to the reason that salt stress increases the osmotic potential and decreases the water potential. The reduction in water potential limits stomatal opening and closing causing imbalance in gaseous exchange and disturbance in photosynthetic apparatus (Ashraf *et al.,* 2010). However, application of sulfur limits the toxic effects of salinity by improving photosynthetic pigments under salt stress condition. It may be due to the reason that sulfur helps to increase the activity of glutathione reductase and glutathione peroxidase (Khan, 2014). These enzymes improve photosynthetic pigments in plants. Lunde *et al*., (2008) evaluated that reduction in sulfur reduced chlorophyll in the plants. It was due to the reason that absence of sulfur reduces PSI, PSII, Rubisco and light harvesting antenna complex. This creates imbalance in PSI and PSII. Photosynthesis and carbon fixation rate is also disturbed that causes reduction in electron carriers produced during photosynthesis. Moreover, current study revealed that sulfur at low level (40 mM) was proved beneficial in improving photosynthetic pigments as compared to high level (80 mM). This finding was confirmed by earlier study by Kobayashi *et al*., (2015) who described its reason that, high sulfur level represses more than 90% of photosynthetic process due to destabilization of structure that causes inactivation of PSII resulting in reduction of growth and development of plants.

It was found that salt stress reduced starch contents in the plants. These findings are supported by earlier studies. Rajakumar (2013) found that by increasing salt stress (from 50-300 mM NaCl) the starch contents were reduced. In the current study it was found that salt tolerant maize genotype (Agatti 2003) accumulated higher concentration of starch in comparison to salt sensitive maize cultivar (Pak Afgoi 2003). Previous studies supported the present findings. Kafi *et al*., (2000) evaluated that salt tolerant wheat variety accumulated higher starch contents in comparison to salt sensitive

cultivar. The application of sulfur improved starch contents to endure the toxic effects of salinity. Various studies support the present finding (Klikocka, 2010; Sharma *et al.*, 2011).

The current study revealed that salt stress reduced carbohydrates in maize plants. It may be due to the reason that salt stress reduces rate of photosynthesis that lowers down the production of carbohydrate (Zobayed *et al.*, 2007). However, the appropriate concentration of carbohydrates is needed for meeting the energy requirement in the plant cell. In this study, results revealed that sulfur application improved the carbohydrate concentration in the plant cell even at higher level of salinity, which proved that sulfur improves carbohydrate concentration in the plant cell under saline conditions. Moreover, it helps to maintain the balance between other biomolecules to endure the toxic effects of salinity (Saito, 2004). While under absence of sulfur limitation condition, the carbohydrate contents become reduced (Neelam & Nalini, 2013).

Conclusion and Recommendations

Salt stress has drastic effect on photosynthetic attributes of plants resulting in reduction in biomolecule and ultimately plant growth. Among various methods to counteract the adverse effects of salinity, use of inorganic nutrients is very economical. Among macronutrients, sulfur has considerable significance in improving photosynthetic attributes and concentration of biomolecules that improves plant growth and development. Moreover, sulfur has also helped plants to overcome the toxic effects of salinity by developing salt tolerance in maize cultivars. In this study, it was found that application of sulfur at 40 mM level has significantly improved all studied growth parameters, photosynthetic components and biomolecule concentration at all applied salt levels (25, 75 mM). Sulfur has also improved the salt tolerance potential of salt sensitive maize cultivar (Pak Afgoi 2003). Hence, it is recommended that for improving photosynthetic attributes and concentration of biomolecules that ultimately increases plant growth, the application of sulfur at 40 mM is very much helpful.

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