INNSPUB

International Journal of Agronomy and Agricultural Research (IJAAR)
ISSN: 2223-7054 (Print) 2225-3610 (Online)
http://www.innspub.net
Vol. 12, No. 5, p. 100-117, 2018

RESEARCH PAPER **OPEN ACCESS**

Improvement in nutrient contents of maize (*Zea mays* L.) by sulfur modulation under salt stress

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Article published on May 30, 2018

Key words: Sulfur, Salt tolerance, Maize, Growth, Nutrient.

Abstract

A key factor under saline conditions is the disturbance of osmotic potential that results in imbalance of nutrients in plants, while the application of sulfur not only improves the growth and nutrient status, but also improves the salt tolerance in plants. Thus, a study was carried out to determine the role of sulfur in salt tolerance of crop plants. The seeds of maize were sown in plastic pots filled with sand. Three levels of salinity (25, 50, 75 mM) and five levels of sulfur (20, 40, 60, 80, 100 mM) were applied at sowing time. Various growth parameters and nutrient contents were studied. The results showed that sulfur at 60 and 80 mM improved shoot and root length, fresh and dry weights, nutrient contents (K^+, Ca^{2+}, NO_3^-, PO_4^{3-}, SO_4^{2-} ,Ca^{2+}/Na^+, K^+/Na^+) and lowered Na^+ ions at all levels of salinity. For the determination of variation in salt tolerance potential, the phylogenetic tree was constructed by NTSys PC. Distance matrix showed that Agaitti 2003 and Pearl Basic showed high improvement in salt tolerance by sulfur application and showed more improvement in growth and nutrient contents in maize under salt stress conditions. While sulfur application has not much improved growth and nutrient contents in Pak Afgoi 2003 and Hybrid 1898 showing salt sensitivity as compared to other cultivars studied. In curx, sulfur application (60, 80 mM) has pronounced role in developing salt tolerance potential in maize cultivars by improving plant growth and nutrient contents.

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Introduction

Among other abiotic stresses, salt stress has significant harmful effects on agricultural crops (Rengasamy, 2006; Godfray *et al.*, 2010). In the world, salinity has affected 20 % of total cultivated area and 33 % of agricultural lands (Jamil *et al.*, 2011). Salt stress affects many physiological and biochemical processes in the plants. Excess of salts disturb the uptake of water (Kocheva and Georgiev, 2003), that leads to the damages to the cellular organs (Bewley and Black, 1994), reduction in chlorophyll synthesis (McDonald, 2000), and lipid peroxidation (Soeda *et al.*, 2005). The accumulation of salts in the soil horizon, replace and deplete the essential nutrients required for plant growth. As a result, plants become unable to tolerate salt stress (Epstein and Bloom, 2005). Jamil *et al.* (2011) reported that 50 % of arable land would be salinized in 2050. Therefore, such methods should be developed that induce salt tolerance in plants.

To overcome salt stress conditions, an economical way is to supply the essential nutrients to the plant, so the plant can properly carry out the physiological and biochemical processes inside the plant cell and tolerate the damages caused by salinity. Many inorganic nutrients, plant hormones and osmoprotectants are used for this purpose (Epstein and Bloom, 2005). Among the macronutrients, sulfur has significant contribution in salt tolerance as it helps the plants to uptake nutrients essential for growth and development (Fismes *et al.*, 2000). Therefore, sulfur can improve plant health and vigor by reducing the toxic effects of salt stress. Sulfur is also a very necessary component of proteins, pantothenic acid, vitamin B1, acetyl CoA and biotin (Taiz and Zeiger, 2006). For the proper growth, plants require sulfur in ample quantity. Moreover, sulfur fertilization is low cost and produce fine quality of crop yield (Fismes *et al.*, 2000).

Maize is very essential cereal all over the world after wheat and rice with respect to areas of cultivation and productivity (Sandhu *et al.*, 2007). Maize is enriched with vitamins (thiamine, niacin, riboflavin, pantothenic acid, pyridoxine), and nutrients essential for diet. It also contains oils, resin, salt, fibres and mucilage substances (Kumar and Jhariya, 2013). However, maize is very sensitive to salinity (Mass 1986). Therefore, its production has been severely affected due to salt stress. Maize responds to sulfur fertilization very fast (Ghosh *et al.,* 2000) because sulfur plays a pivotal role in developing salt tolerance in maize by regulating the plant metabolism. This study focuses on the improvement in salt tolerance in maize by sulfur application that not only regulates nutrient uptake and transport but also have significant role in growth and development of maize cultivars. Also, various maize cultivars are classified according to their salt tolerance potential based on growth and nutrient accumulation under saline conditions.

Materials and methods

Plan of study

A pot experiment was conducted for determining the effect of sulfur in improving growth attributes and ionic contents under saline condition. The experiment was conducted in the environmental biology and plant ecology lab of the Department of Botany, University of Agriculture, Faisalabad. The seeds of maize cultivars (varieties: Sadaf, MMRI, Pearl Basic, Agaitti 2003, Saiwal 2002, Pak Afgoi 2003 and hybrids: Yusafwala Hybrid, Hybrid 1898) were acquired from Maize and Millete Institute Sahiwal, Pakistan. The seeds were sown in pots made up of plastic and filled with thoroughly washed sand.

Treatment application

For the application of sulfur potassium sulphate (20, 40, 60, 80, 100 mM) was applied and sodium chloride (25, 50, 75 mM) was used for salinity treatment at sowing time. One set was kept without any treatment named as control (0 mM NaCl, 0 mM S). Hogland's nutrient solution was applied for irrigation during the study. The harvest of plants was taken after 14 days of treatment application for determining the changes in growth and ion accumulation.

Shoot and root length

The length of shoot and root was determined with the help of scale and average length of shoot and root of four plants of every replicate was calculated.

Shoot and root fresh weight

For determining the shoot and root fresh weight, electrical balance was used. The mean value of four plants of every replicate was calculated and average was taken.

Shoot and root dry weight

Shoot and root material was packed in paper envelops and kept in oven at 65°C for nearly two days. By using electrical balance the mean value were calculated.

Ionic contents

0.5g dried plant material was weighed and kept in digestion flasks. To each flask 5 ml of concentrated sulfuric acid was incorporated and kept overnight at room temperature. Then flasks were kept in digestion block for heating at 350°C for a period of 30 minutes. For adding H_2O_2 all flasks were removed from incubation block. 1 ml of H_2O_2 was added in each flask and again placed in the incubation block. For complete digestion of the plant material, in the digestion flasks, H_2O_2 was added in same manner. The plant material became digested in the acid solution and clear solution was obtained and then cooled at room temperature. Then distilled water was used for maintaining 50 ml volume of the extract. Then filtered and filled in the bottles labelled with proper treatment. This solution was used for the determination of the ionic contents (Wolf, 1982). Flame photometer (Jenway PFP-7) was used for the determination of Na^+, K^+ and Ca^{2+} contents in plant material. The standards (from 10, 20 to 100 ppm) of Na^+ K^+ and Ca^{2+} ions were prepared and the curves were drawn. A comparison was done of the values of the standard curve and values from the flame photometer and real values were calculated in mg/g dry weight.

Phosphate

The phosphate contents were determined by following the procedure given by Yoshida (1976). First reagents were prepared. *Molybdate-vanadate* solution was prepared by dissolving 25g ammonium molybdate in 500 ml water. Separately 1.25g ammonium vanadate was dissolved in 500 ml of 1 N HNO_3. Then equal volumes of two solutions were mixed to get Molybdate-vanadate solution.

Nitric acid (2N) was prepared by taking 10 ml of HNO_3 and volume was made up to 100ml with distilled water. For determination of phosphate contents, 0.5g plant material was homogenised and boiled in 5ml distilled water for 1h. Following filtrated volume was maintained to 50ml with distilled water. 1ml of the extract, 2 ml of 2N HNO_3 and 1ml of distilled water were mixed, vortexed and cooled at room temperature for 20 minutes. The absorbance was noted at 420 nm on spectrophotometer (UV-1100) by using blank water. For the preparation of standards, stock solution of 25 mg/l PO_4^{3-} was prepared by dissolving 0.11g of monobasic phosphate (KH_2PO_4) in 1L water. Then a series of solution (2.5, 5, 7.5, 10, 12.5, and 15 mg/l PO_4^3) was prepared by adding1, 2, 3, 4, 5 and 6 ml of 25 mg/l PO_4^{3-} and volume was maintained to 8 ml.

Nitrate

Nitrate contents in the plant tissue were determined by using the method given by Kowalenko and Lowe (1973). Firstly 0.01 % TCA was prepared. 0.247 g chromotropic acid dosodium salt (CTA) was mixed in 100 ml of conc. H_2SO_4. 0.01 % stock was prepared by maintaining 10 ml of CTA stock to 100 ml with H_2SO_4. For nitrate determination, 0.5g dried plant sample was boiled in 5 ml distilled water for 1 h, filtered and volume was maintained to 50 ml with distilled water. Then 3 ml extract was mixed in 7 ml working CTA solution and vortexed. The absorbance was measured at 430 nm on spectrophotometer (UV-1100) by using water as blank. For the preparation of standard solution, stock was prepared by dissolving 0.7216 g of KNO_3 in 1L water. Standard series (10, 20, 30, 40, 50 and 100 mg/l NO_3^-) was prepared by diluting the stock solution.

Sulfate

Sulfate contents were found by following the method of Tandon (1993). Two reagents were prepared; Barium chloride/Polyvinyl alcohol and acid mixture. Barium chloride/Polyvinyl alcohol was made by dissolving 60g of $BaCl_2.2H_2O$ in 500 ml distilled water. Separately, 2g of polyvinyl alcohol was mixed in 400 ml water and both solutions were mixed after cooling. Finally volume was made to 100 ml with distilled water.

Acid mixture was made by mixing 50 ml glacial acetic acid, 20 ml 85 % orthophosphoric acid and 6 ml concentrated H_2SO_4/H_2O (1:1000) and 800 ml distilled water. The volume was maintained to 1L using distilled water. For sulphate determination, 5 ml of tested solution, 5 ml of acid mixture, and 5 ml of Barium chloride/Polyvinyl alcohol were mixed thoroughly, vortexed, allowed to stand for 30 seconds and absorbance was noted at 420 nm using spectrophotometer (UV-1100).

Statistical analysis

The study was conducted in Completely Randomized Design (CRD) in three factor factorial with three replicates. Microsoft excel was used for the preparation of graphs. Co-stat software was used for the performance of Analysis of Variance (ANOVA). For the determination of the variation in the salt tolerance ability of cultivars ameliorated by sulfur, cluster analysis was done by NTSysPC software (v2.10m). Phylogenetic tree was constructed on the basis of distance coefficient by using SAHN (Sequential Agglomerative Hierarchic and Non-over lapping) procedure.

Results

Effect of sulfur on growth parameters under salinity

The use of sulfur revealed a significant enhancement in shoot and root length, fresh and dry weights of all studied maize cultivars (Fig. 1-6). It was revealed from statistically significant V × S interaction (Table 1). Generally, sulfur improved the maize growth by developing salt tolerance at all levels (20, 40, 60, 80, 100 mM). However, 100 mM sulfur was not much efficient in this respect in improving the maize growth but is significant as compared to control (Fig. 1-6). This result was evident from statistically significant Sa × S interaction (Table 1). The application of sulfur improved the maize growth by lowering the toxic effects of salt stress. This was evident from statistically significant V × Sa × S interaction (Table 1). Among maize cultivars used in this study, Agatti 2003 showed salt tolerance by reducing the harmful effects of higher levels of sulfur and salinity. In comparison Pak Afgoi 2003 proved salt sensitive as its growth was much lowered by higher levels of salinity and sulfur.

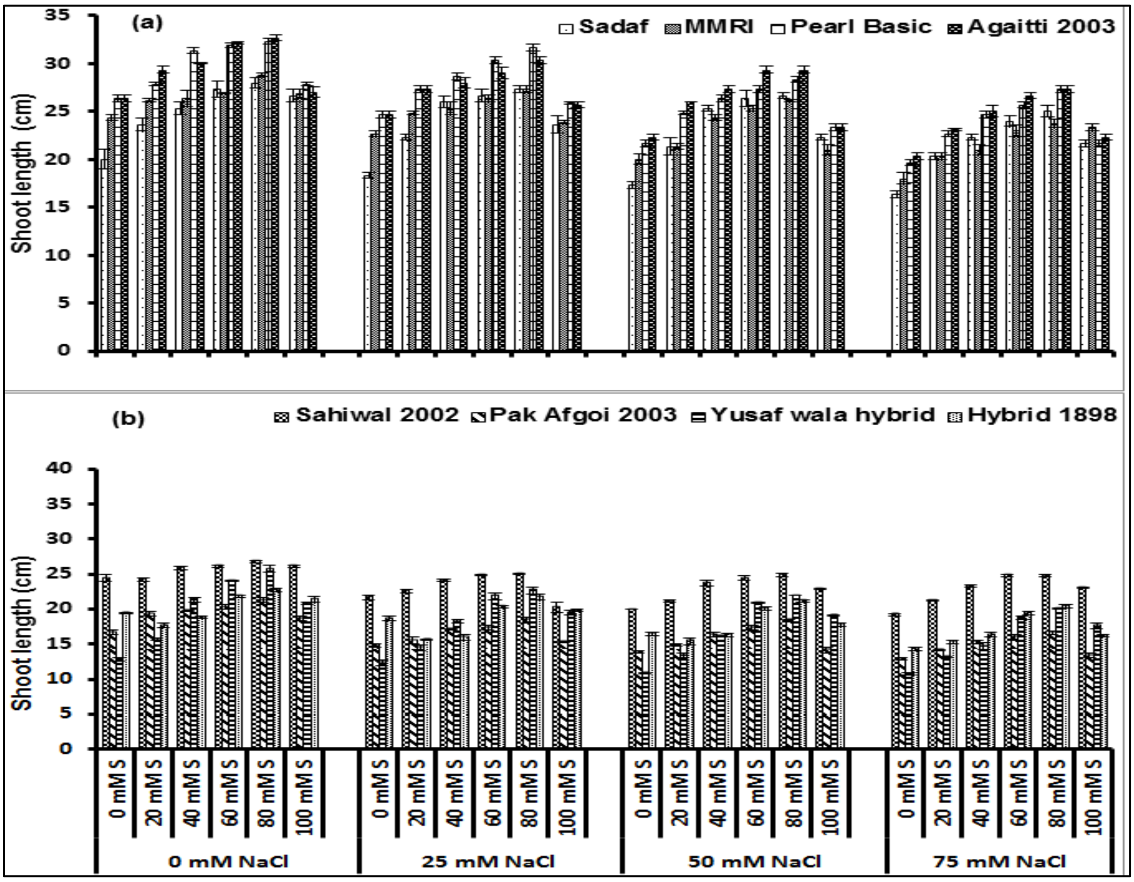

Fig. 1. Effect of different levels of sulfur (S) and NaCl on shoot length of different maize cultivars (*Zea mays* L.).

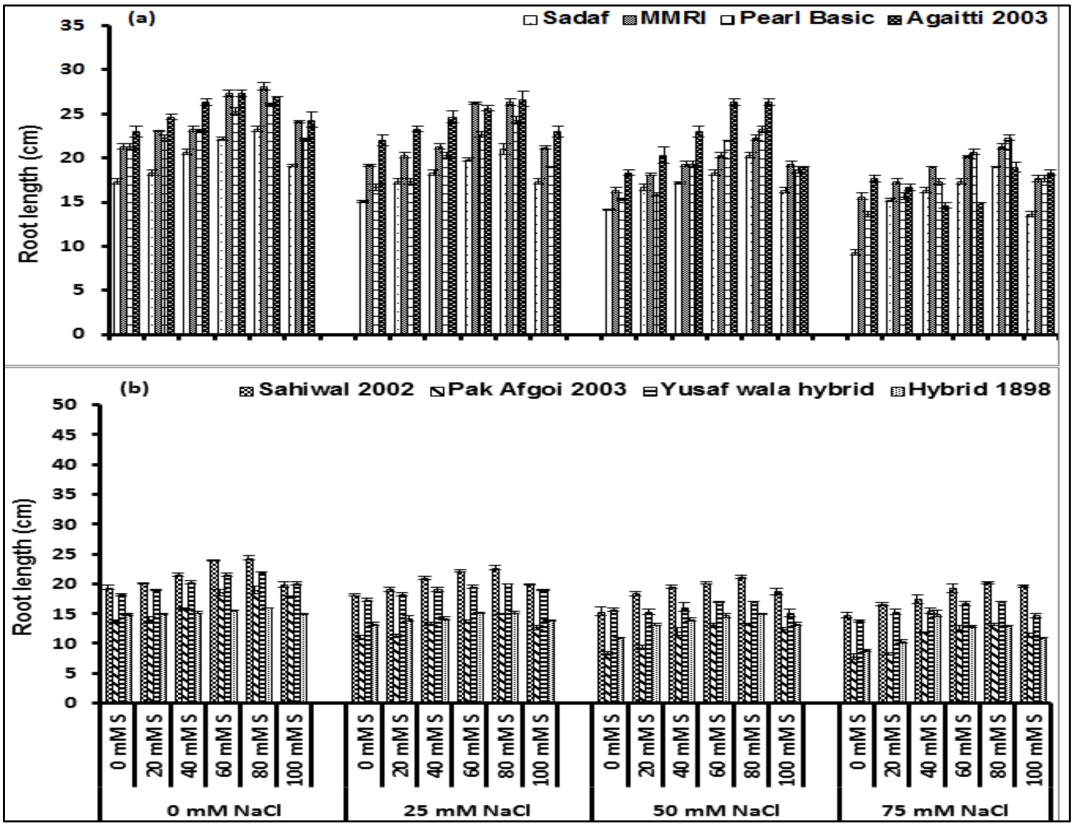

Fig. 2. Effect of different levels of sulfur (S) and NaCl on root length of different maize cultivars (*Zea mays* L.).

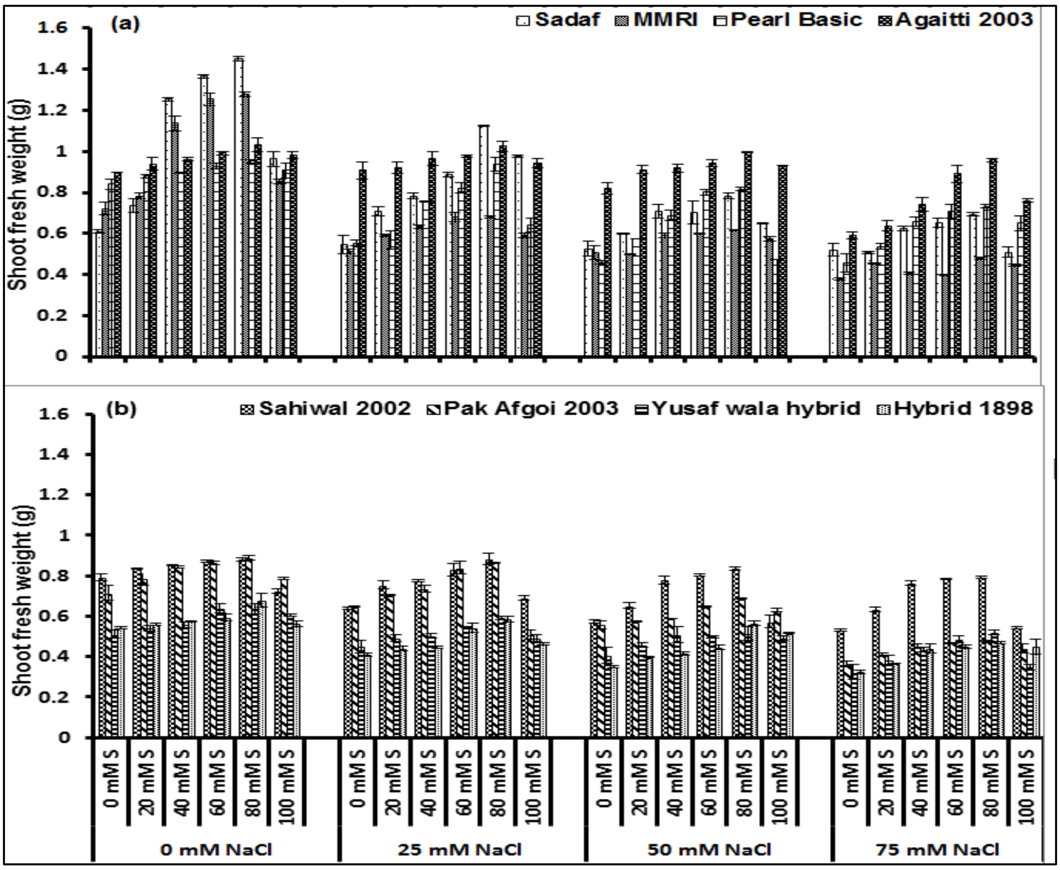

Fig. 3. Effect of different levels of sulfur (S) and NaCl on shoot fresh weight of different maize cultivars (*Zea mays* L.).

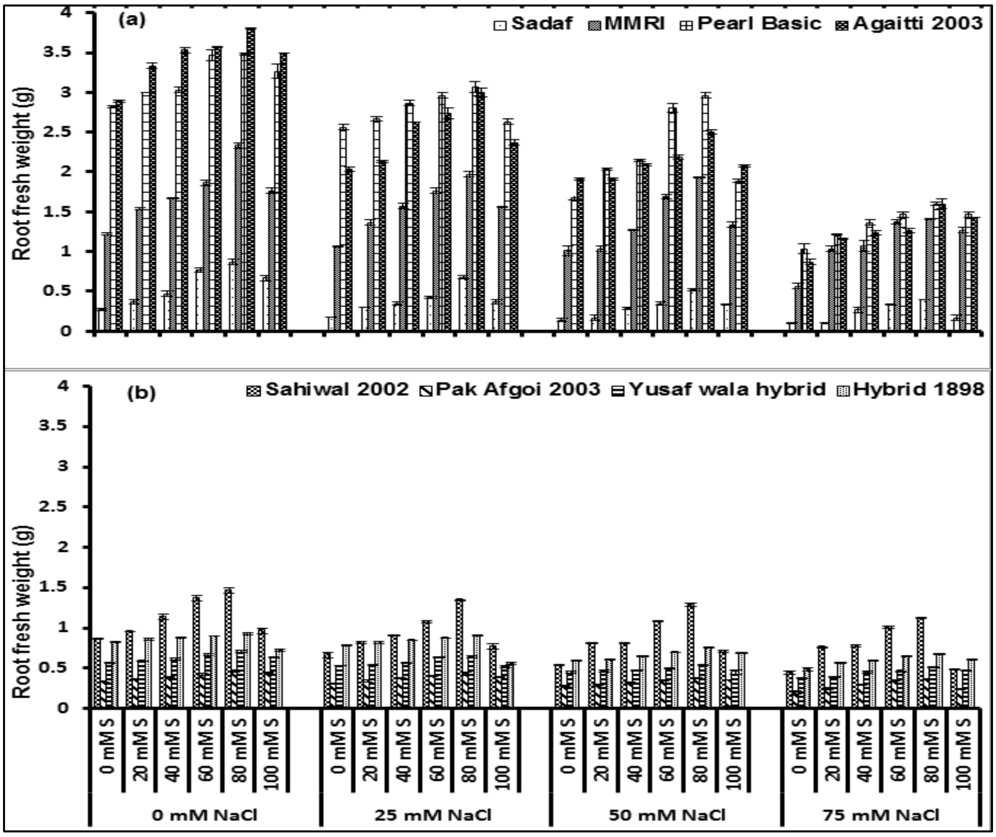

Fig. 4. Effect of different levels of sulfur (S) and NaCl on root fresh weight of different maize cultivars (*Zea mays* L.).

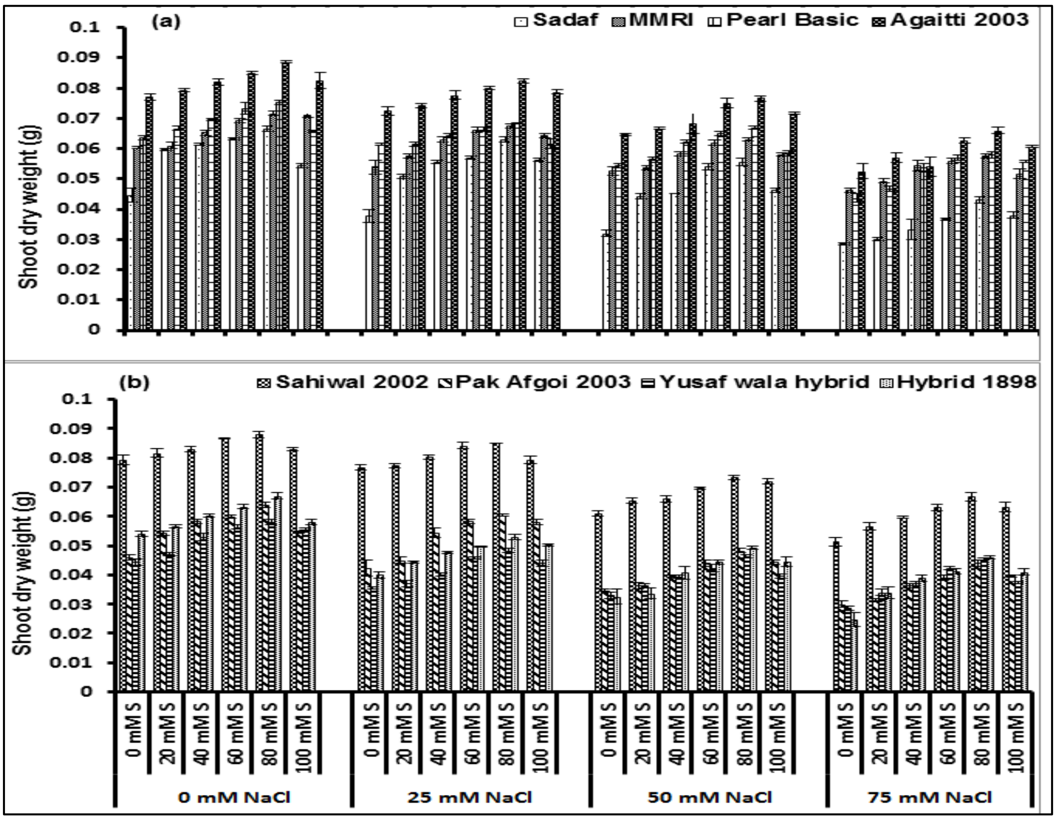

Fig. 5. Effect of different levels of sulfur (S) and NaCl on shoot dry weight of different maize cultivars (*Zea mays* L.).

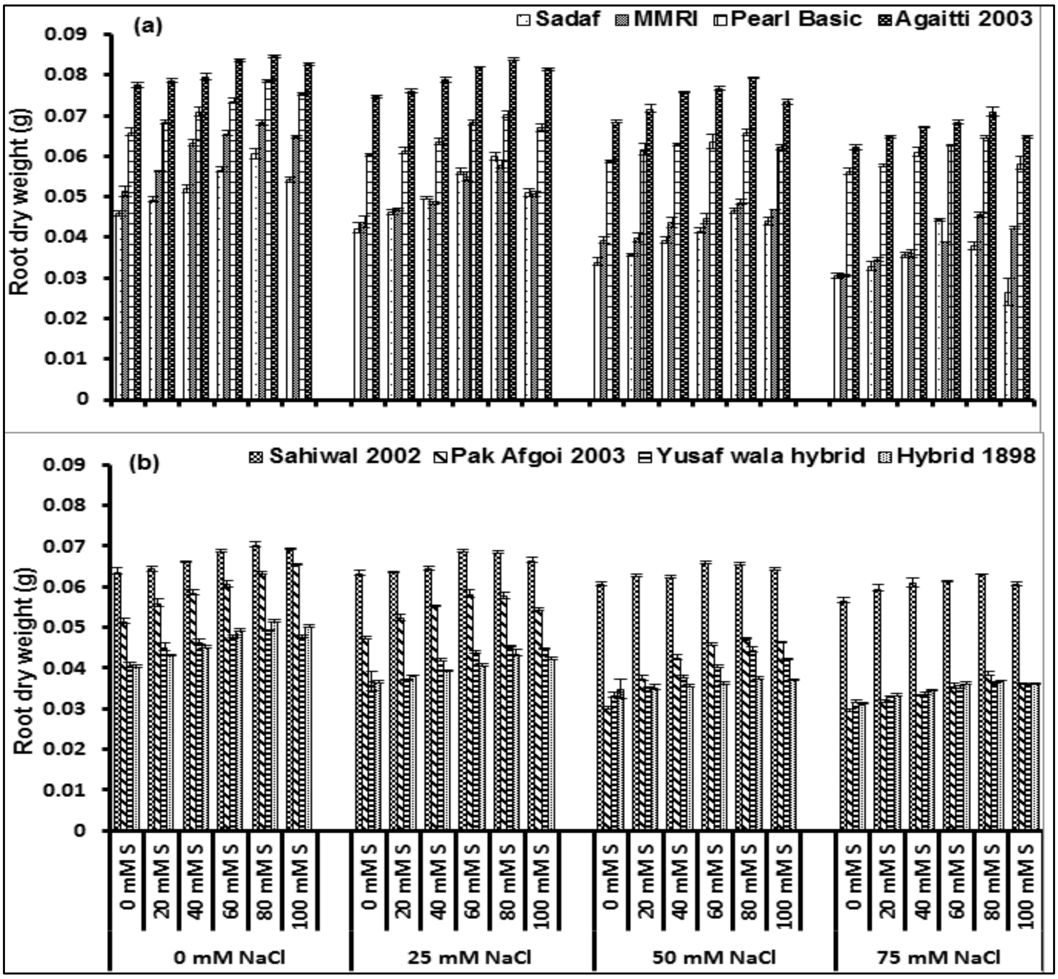

Fig. 6. Effect of different levels of sulfur (S) and NaCl on root dry weight of different maize cultivars (*Zea mays* L.).

Table 1. Mean squares from analysis of variance (ANOVA) of the data for morphological parameters and nutrien contents of maize subjected to salt stress and sulfur application.

SOV	df	SL	RL	SFW	RFW	SDW	RDW	Leaf Na^+ content
Variety (V)	7	1194.04 ***	832.37 ***	1.44 ***	49.45 ***	0.011 ***	0.012 ***	1214.17 ***
Salinity (Sa)	3	479.50 ***	765.15 ***	2.33 ***	14.38 ***	0.011 ***	0.0066 ***	1069.85 ***
Sulfur (S)	5	526.47 ***	344.46 ***	0.71 ***	3.07 ***	0.0024 ***	0.0012 ***	612.96 ***
V × Sa	21	6.56 ***	18.10 ***	0.11 ***	2.14 ***	1.50e-4 ***	1.95e-4 ***	22.58 ***
V × S	35	19.60 ***	7.61 ***	0.024 ***	0.28 ***	2.44e-5 ***	2.15e-5 ***	3 5.11 ***
Sa × S	15	2.36 ***	1.58 ***	0.0085 ***	0.16 ***	1.11e-5 ***	1.68e-5 ***	1.11 ***
V × Sa × S	105	2.18 ***	3.23 ***	0.018 ***	0.11 *	1.16e-5 ***	8.87e-6 ***	0.93 ***
Error	384	0.40	0.34	0.44	0.091	3.55e-6	1.47e-6	0.38
SOV	df	Leaf K^+ content	Leaf Ca^{2+} content	Leaf Ca^{2+}/Na^- content	Leaf K^+/Na^+ content	Leaf NO_3^- content	Leaf PO_4^{3-} content	Leaf SO_4^{2-} content
Variety (V)	7	2412.71 ***	54.76 ***	0.19***	12.3***	2.57***	20.14***	26123.38***
Salinity (Sa)	3	1239.09 ***	72.70 ***	0.25***	8.89***	3.34***	4.06***	15963.83***
Sulfur (S)	5	122.62 ***	1.89 ***	0.03***	2.84***	17.26***	3.54***	5924.52***
V × Sa	21	2.67 ***	1.78 ***	0.006***	0.21***	0.32***	0.62***	577.36***
V × S	35	0.15 ns	0.21 ns	0.001***	0.07***	0.23***	0.35***	170.61***
Sa x S	15	0.13 ns	0.12 ns	0.002***	0.06***	0.24***	0.15***	73.79ns
V × Sa × S	105	0.18 ns	0.05 ns	1.57e-4ns	0.011***	0.04***	0.08***	50.62ns
Error	384	0.21	0.41	5.512e-4	0.002	0.014	0.021	47.62

*, **, *** = significant at 0.05, 0.01 and 0.001 levels, respectively. ns = non-significant, e=exponent.

Abbreviations: Shoot length (SL), Root length (RL), Shoot fresh weight (SFW), Root Fresh Weight (RFW), Shoot dry weight (SDW), Root dry weight (RDW), Sodium(Na^+), Potassium (K^+), Calcium (Ca^{2+}), Nitrate (NO_3^-), Phosphate (PO_4^{3-}), Sulfate (SO_4^{2-}).

Effect of sulfur on nutrient contents under salinity

In the present study, application of sulfur improved the calcium, potassium, nitrate, phosphate and sulphate contents and reduced sodium contents as compared to plants with no sulfur application. Results revealed that salinity increased sodium (Na) contents in maize plants. It was evident from statistically significant V × Sa, V × S, Sa × S, and V × Sa × S interactions (Table 1). Sulfur augmentation improved the salt tolerance in maize plants; it was due to the reason that sulfur lowered the sodium uptake (Fig. 7) and significantly improved the uptake of potassium (K^+) and calcium (Ca^{2+}), nitrate (NO_3^-), phosphate (PO_4^{3-}), sulphate (SO_4^{2-}), Ca^{2+}/Na^+ and K^+/Na^+ in maize plants (Table 1). Sulfur application at 60 mM and 80 mM levels showed much effective in reducing the effects of salt stress (Fig. 8-14). Overall salt tolerant maize cultivar Agaitti 2003 accumulated less sodium (Na) contents as compared to Pak Afgoi 2003 (Fig. 7). Also the application of sulfur improved the salt tolerance in Pak Afgoi 2003 by improving the growth parameters and ionic contents.

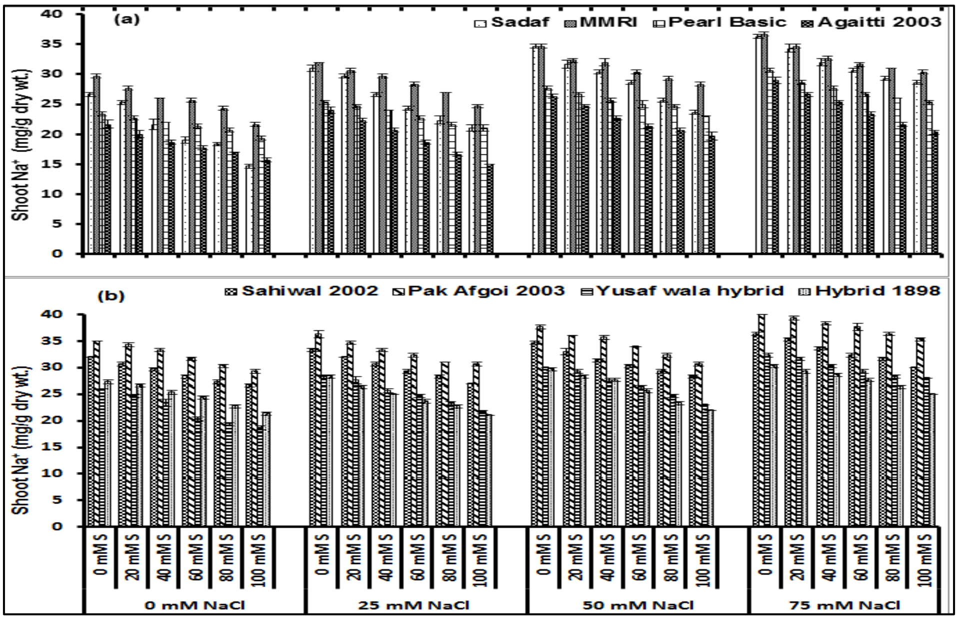

Fig. 7. Effect of different levels of sulfur (S) and NaCl on shoot Na^+ contents of different maize cultivars (*Zea mays* L.).

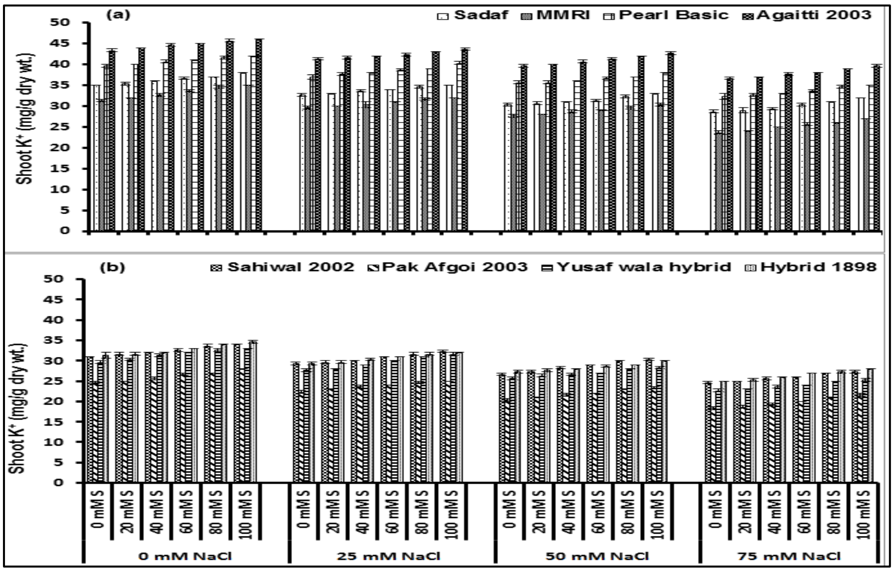

Fig. 8. Effect of different levels of sulfur (S) and NaCl on shoot K^+ contents of different maize cultivars (*Zea mays* L.).

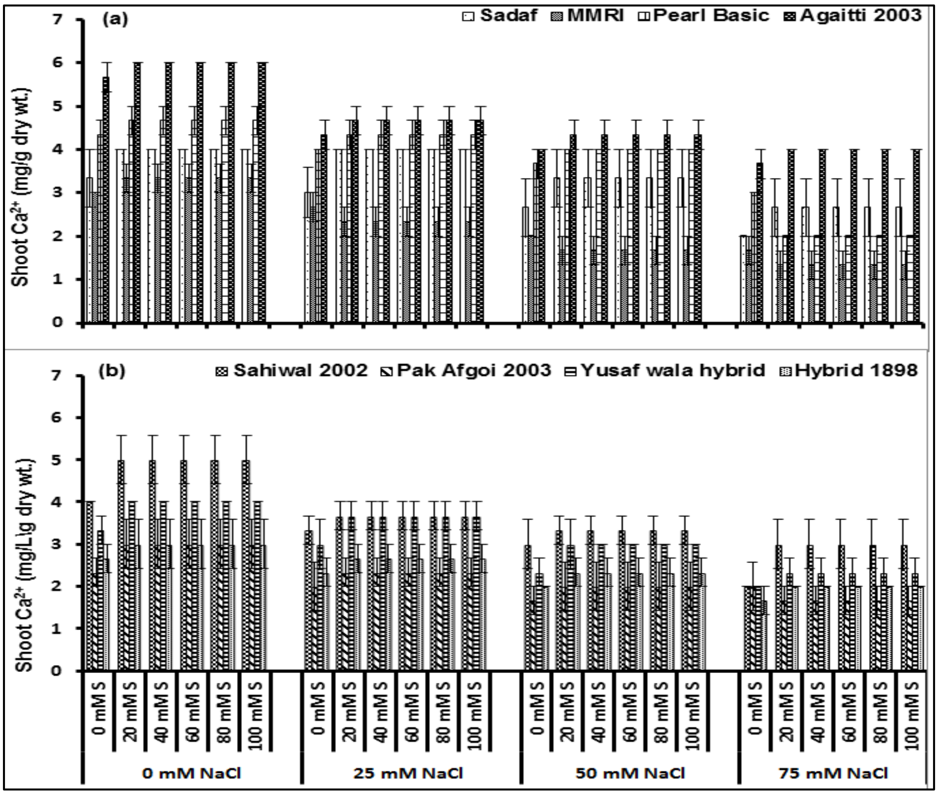

Fig. 9. Effect of different levels of sulfur (S) and NaCl on shoot Ca^{2+} contents of different maize cultivar (*Zea mays* L.).

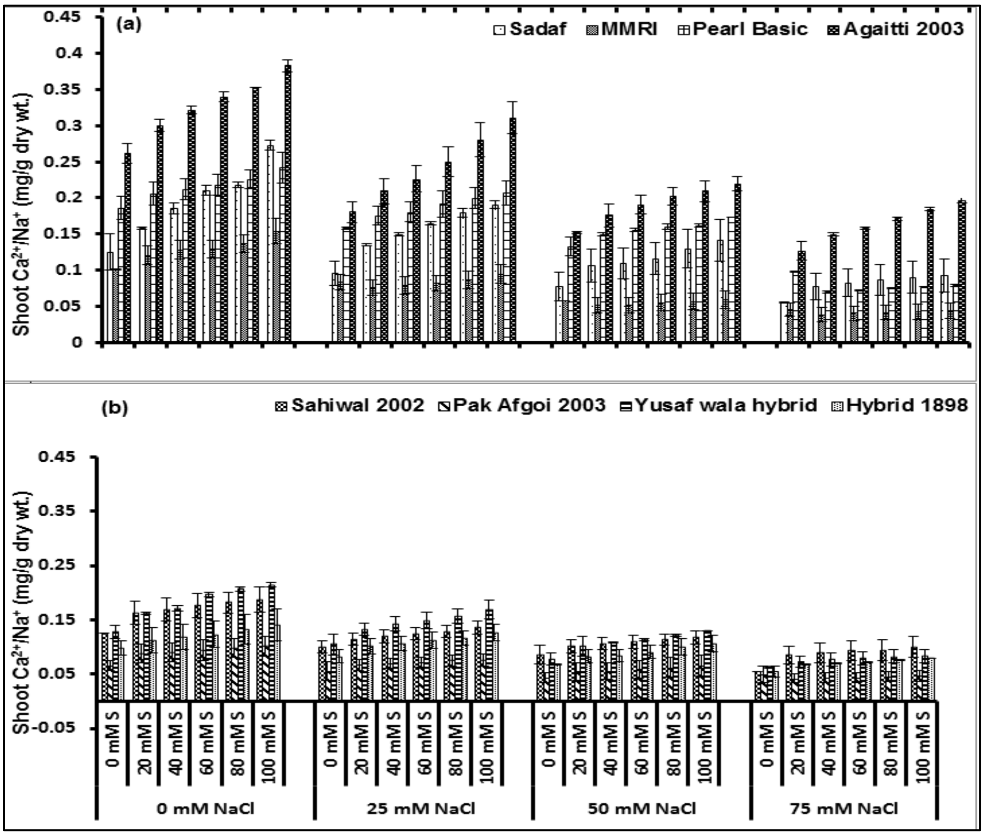

Fig. 10. Effect of different levels of sulfur (S) and NaCl on shoot Ca^{2+}/Na^{+} of different maize cultivar (*Zea mays* L.).

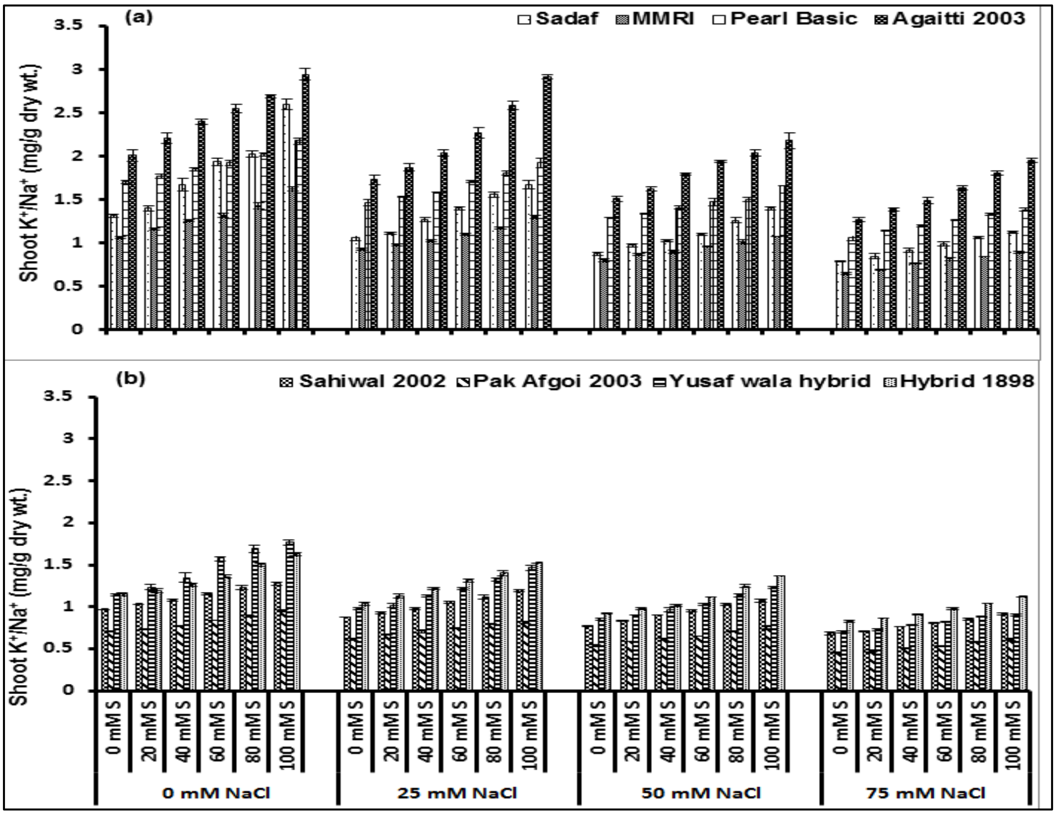

Fig. 11. Effect of different levels of sulfur (S) and NaCl on shoot K^+/Na^+ contents of different maize cultivars (*Zea mays* L.)

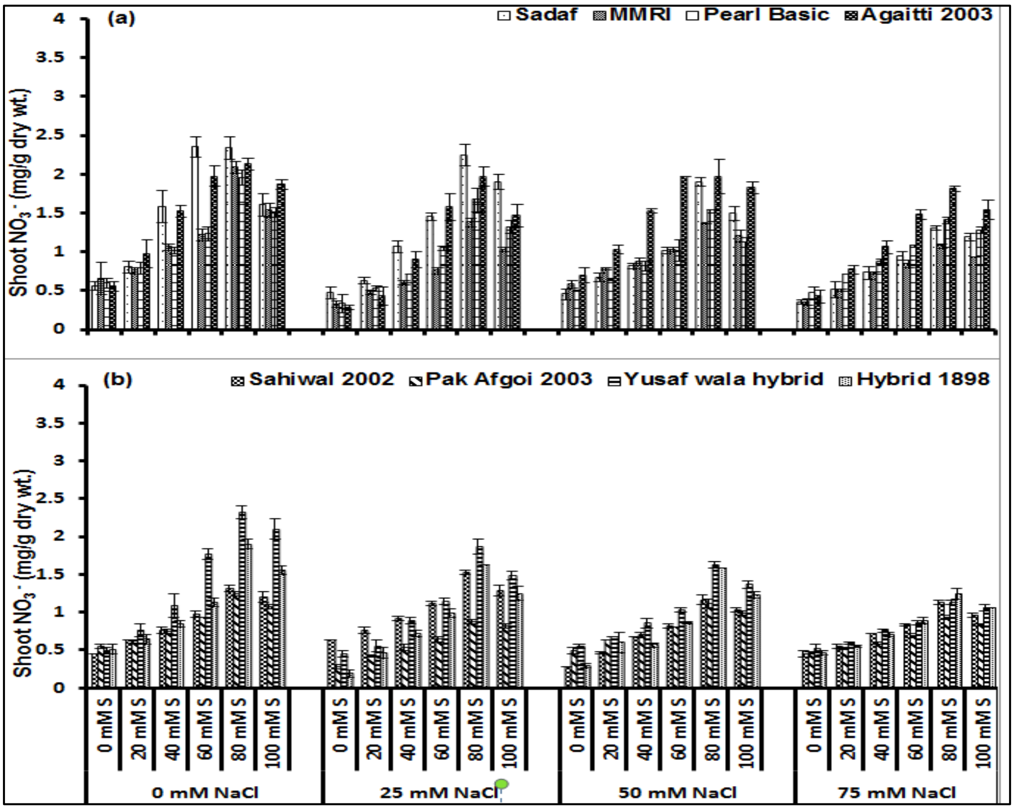

Fig. 12. Effect of different levels of sulfur (S) and NaCl on shoot nitrate (NO_3^-) contents of different maize cultivars (*Zea mays* L.).

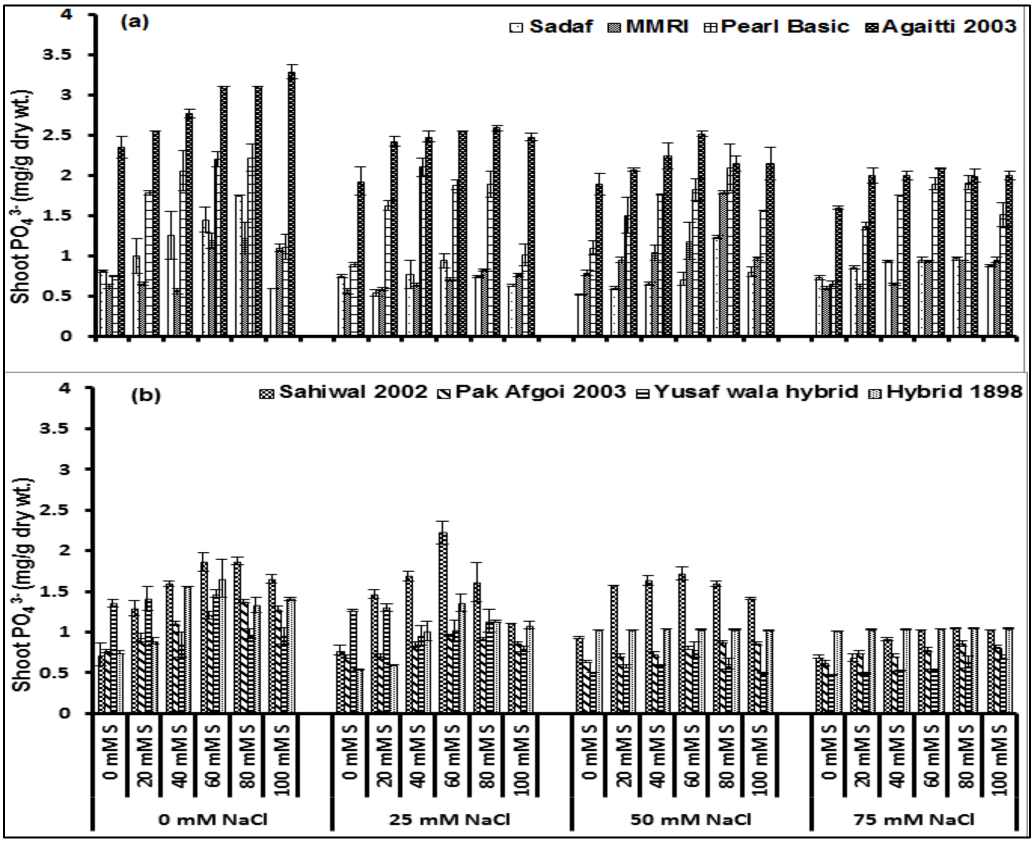

Fig. 13. Effect of different levels of sulfur (S) and NaCl on shoot phosphate (PO_4^{3-}) contents of different maize cultivars (*Zea mays* L.).

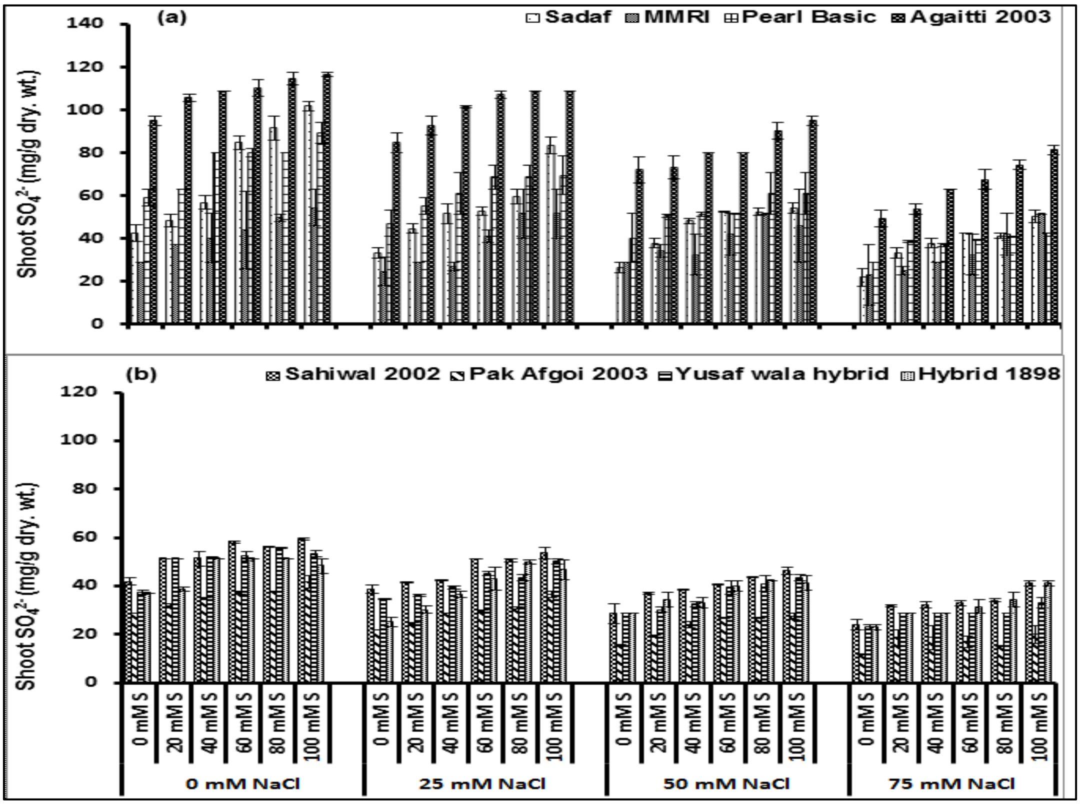

Fig. 14. Effect of different levels of sulfur (S) and NaCl on shoot sulfate (SO_4^{2-}) contents of different maize cultivars (*Zea mays* L.).

Determination of variation in salt tolerance of maize cultivars

The data of all studied growth attributes and ionic contents of maize cultivars was fed to NTSysPC software (v2.10m) for constructing phylogenetic tree created on SAHN method. The tree was formed on the basis of distance coefficient (DC). Less distance between two groups means higher variation in the salt tolerance ability modulated by sulfur. And more distance in group means that sulfur application has induced less variation in salt tolerance ability of maize cultivars.

Determination of variation based on growth parameters

The cluster analysis clearly divided all maize cultivars in two groups (DC=4.03) showing higher variation in maize growth (Table 2). Salt tolerant group (Sadaf, MMRI, Sahiwal-2002, Pearl Basic, Agatti-2003) and salt sensitive group (Pak Afgoi 2003, Hybrid-1898, Yusaf Wala Hybrid) (Fig. 15). In salt tolerant group, Agaitti 2003 and Pearl Basic branched at higher distance (DC=1.5) reflecting their higher salt tolerant ability by sulfur application. MMRI and Sahiwal branched at equal distance (DC=1.09) categorised as moderately salt tolerant and sadaf at lower distance (DC=1.645) ranked as salt sensitive variety. In salt sensitive group, Yusaf wala hybrid was branched at most distance (DC=2.385) categorized as moderately salt sensitive. Other two cultivars i-e Pak Afgoi 2003 and Hybrid 1898 branched at least distance (DC=1.3) showing salt sensitive cultivars (Table 2). Overall, among all cultivars, Agaitti 2003 and Pearl Basic responded to sulfur application by improving growth under salt stress condition while Pak Afgoi 2003 and Hybrid 1898 at least improved by sulfur fertilization.

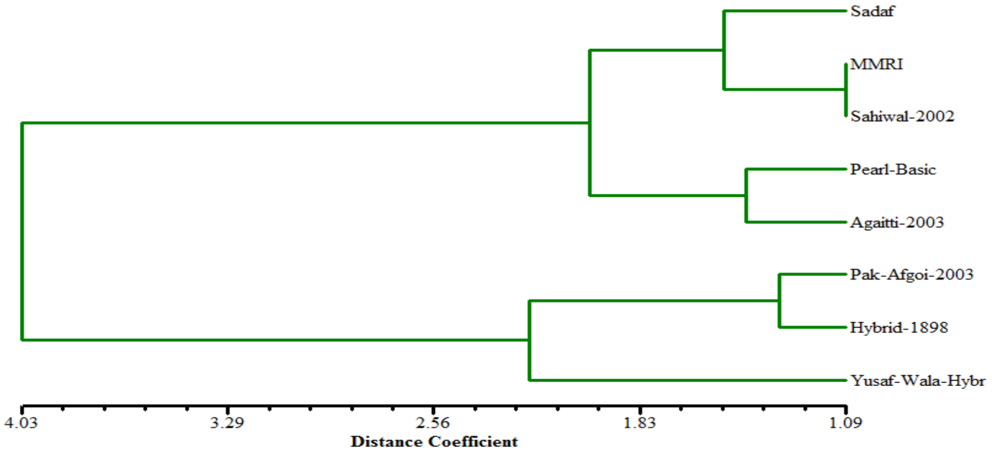

Fig. 15. The clustering analysis based on phylogenetic distance formed by NTS Sys PC for growth of maize (*Zea mays* L.) cultivars ameliorated by sulfur under saline conditions.

Determination of variation based on nutrient contents

Sulfur application improved the beneficial ionic contents (K^+, Ca^{2+}, NO_3^-, PO_4^{3-}, SO_4^{2-}, Ca^{2+}/Na^+, K^+/Na^+) and lower down the sodium contents (Na^+) by developing salt tolerance potential in all maize cultivars. However, the tree representing ionic contents showed variable response to sulfur application (Fig. 16). Two groups were observed in this tree (DC=7.36). Salt tolerant group (Agitti 2003, Pearl Basic) and salt sensitive group (Sadaf, Yusaf wala hybrid, Hybrid 1898, MMRI, Sahiwal 2002, Pak Afgoi 2003). In salt tolerant group Agaitti 2003 and Pearl Basic branched at higher distance (DC=3.29) showing highly salt tolerant. In salt sensitive group, MMRI and Sahiwal 2002 branched at equal distance (DC=1.43) categorized as least salt sensitive.

Yusaf wala hybrid and Hybrid 1898 branched at least but equal distance (DC=1.8) ranked as moderately salt sensitive, Sadaf branched at lower distance (DC=3) showed salt sensitive variety and Pak Afgoi 2003 branched at least distance coefficient (DC=5.6) so ranked as highly salt sensitive variety (Table 2).

Table 2. The clustering based on growth attributes and ionic contents of maize (*Zea mays* L.) cultivars as ameliorated by sulphur application under salinity.

Growth parameters				Ionic contents			
Group	Cultivars	Distance coefficient (DC)	Salt tolerance ability	Group	Cultivars	Distance coefficient (DC)	Salt tolerance ability
Salt tolerant	Agaitti 2003	1.5	Highly salt tolerant	Salt tolerant	Agaitti 2003	3.29	Highly salt tolerant
	Pearl Basic	1.5	Highly salt tolerant		Pearl Basic	3.29	Highly salt tolerant
	MMRI	1.09	Moderately salt tolerant	Salt sensitive	MMRI	1.43	Least salt sensitive
	Sahiwal 2000	1.09	Moderately salt tolerant		Sahiwal 2002	1.43	Least salt sensitive
	Sadaf	1.645	Least salt tolerant		Yusafwala Hybrid	1.8	Moderately salt sensitive
Salt sensitive	Yusafwala Hybrid	2.385	Moderately salt sensitive		Hybrid 1898	1.8	Moderately salt sensitive
	Pak Afgoi 2003	1.3	Highly salt sensitive		Sadaf	3	Highly salt sensitive
	Hybrid 1898	1.3	Highly salt sensitive		Pak Afgoi 2003	5.6	Highly salt sensitive

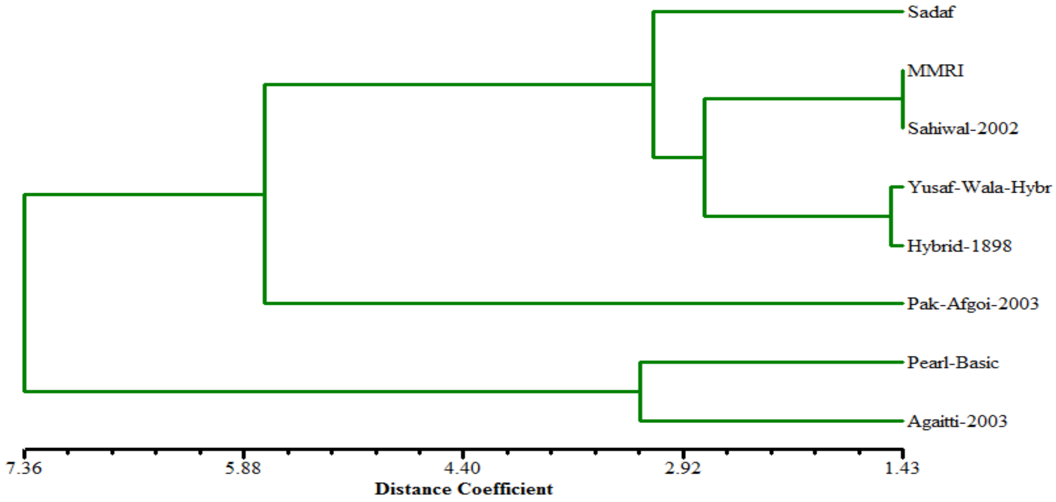

Fig. 16. The clustering analysis based on phylogenetic distance formed by NTS Sys PC for ionic contents of maize (*Zea mays* L.) cultivars ameliorated by sulfur under saline conditions.

Discussion

Results showed that salt stress reduced shoot and root length. It might be due to high concentration of Na^+ and Cl^- in the rooting zone (Zhang *et al.*, 2010) that lower water potential in growing media, resulting in loss of cell turgor. This causes reduction in photosynthetic rate, cell division and cell elongation that ultimately reduces plant length (Zekri, 1991; Ali-Dinar *et al.*, 1999; Ebert *et al.*, 2002). The reduction in root and shoot length by imposition of salinity has been reported in various studies (Werner and Frankelstein, 1995; Okcu *et al.*, 2005; Atak *et al.*, 2008; Asaadi, 2009). However, application of sulfur improved root and shoot length in the current study.

The increase in root length by the application of sulfur might be due to the reason that sulfur enhances the cell division and cell elongation in the meristematic region of the plant. Therefore, application of sulfur keeps root system very healthy (Chandel *et al.,* 2002). Diepenbrock (2000) reported that sulfur maintains health of root which in turn transports the nutrient to the upper parts of the plant that results in gaining in the fresh biomass of the plant. Bejandi *et al.* (2009) reported that sulfur application significantly enhanced the shoot length in soyebean.

Salinity reduced fresh and dry weight of all maize cultivars. The findings of current investigation are in accordance to earlier studies (Netondo *et al.*, 2004; Ashraf *et al.*, 2008; Akhzari *et al.*, 2012; Mantri *et al.*, 2012). In various plants the reduction in fresh and dry biomass by salt stress has been reported e.g. *Zea mays* L., *Raphanus sativus* L., *Kyllinigia peruviana* L., *Bruguiera gymnorrhiza* and *Brassica campestris* L. (Cicek and Cakirlar, 2002; Jamil *et al.*, 2007; Ha *et al.,* 2008; Rui *et al.*, 2009; Memon *et al.*, 2010). Sulfur has very significant role in formation of proteins and a number of metabolites necessary for increase in fresh and dry weights of plants (Ali *et al.*, 1990; Zhao *et al.*, 1993). Previous studies revealed that use of sulfur improved shoot and root fresh and dry weights (Gilbert *et al.,* 1984).

Salinity causes reduction in water potential, imbalance in nutrient composition and transport and ultimately disrupting ionic homeostasis in plants (Chinnusamy *et al.*, 2005; Parida and Das, 2005; Genc *et al.*, 2007; Zhang *et al.*, 2013). While sulfur application enhances the nutrient availability in plants under salt stress conditions which has been supported by previous studies (Aslam *et al.*, 2001; Prasad, 2003; Ali *et al.*, 2008). In this study salt stress increased the Na^+ contents and reduced the K^+ contents in all studied maize cultivars. Under salt stress conditions, Na^+ contents become very high that compete with K^+ ions and reduces its uptake. This reduction in K^+ uptake causes the reduction in K^+/Na^+ ratio (Kaya *et al.*, 2007). In this study, using sulfur, the harmful effects of salt stress were reduced by reducing the Na^+ and improving K^+ contents in maize plants. This property is due to the reason that sulfur has such metabolites that modulate the physiological and molecular process under saline conditions. This modulation creates salt tolerance in crop plants (Khan *et al.*, 2014). Reich *et al.* (2016) found that the use of sulfur improved the potassium contents in plants. It also improves the K^+/Na^+ ratio inside the plant. Hence, developing salt tolerance, as the rise in K^+/Na^+ ratio indicates the salt tolerance in plants (Shirazi *et al.*, 2002; Kaya *et al.*, 2002).

Results showed that salinity reduced the Ca^{2+} ions and Ca^{2+}/Na^+ ratio in plants. It was supported by previous studies. Salt stress reduces the Ca^{2+} ions that reduce the Ca^{2+}/Na^+ ratio in the plant (Khan, 2001), while sulfur application improved the Ca^{2+} and Ca^{2+}/Na^+ ratio in maize cultivars. Aulakh and Dev (1978) found positive correlation between sulfur and calcium. Sulfur improves the availability of Ca^{2+} ions to the plant that lower the toxic effects of salinity on plant health and vigor (Badr *et al.*, 2002). Improvement in Ca^{2+} ions not only exclude Na^+ but also helps the plant to efficiently use nitrogen (Aslam *et al.*, 2001; Mahmood *et al.*, 2009). The adequate amount of sulfur maintains Ca^{2+}/Na^+ ratio in the plants that reduce the toxic effects of salinity and helps in establishment of crop plants (Aslam *et al.*, 2001).

In this study it was found that salinity reduced NO_3^-, PO_4^{3-}, and SO_4^{2-} contents in plants while sulfur application enhanced the salt tolerance in maize plants by balancing the nutrient concentration, uptake and transport of beneficial ions (NO_3^-, PO_4^{3-}, and SO_4^{2-}). These findings have been reported in previous studies. Perez-Alfocea *et al.* (2015) reported that salt stress reduced the nitrate and total nitrogen contents in tomato plants. However, sulfur application improves the nitrate contents in plants by improving the activities of the enzymes involved in nitrate uptake. Prosser *et al.* (2001) found that a reduction in sulfur lowered the nitrate reductase activity in maize and spinach (Prosser *et al.*, 2001). Salinity also reduces the phosphate contents in maize plants. It was supported by findings of Aslam *et al.* (1996), who found that salinity reduces the phosphate contents in rice. Similarly, Turhan and Atilla (2004) evaluated that by increasing the NaCl level from 500 to 2000m g/L the phosphorus contents were decreased. The application of sulfur in low quantity improved the phosphorous uptake in the plant which improved the nutrient deficiency under stress conditions. It was related to earlier researches. Randhawa and Arora (2000) reported that the sulfur and phosphorous has significant positive interaction. The sulfate contents were reduced by high level of salinity in comparison to the plants with no salt application.

It has been reported that the NaCl salinity reduced the SO_4^{2-} contents in *Brassica rapa*. Moreover, sulfur contents were increased by application of sulfate salts (Reich *et al.*, 2017). For the determination of relative improvement in salt tolerance ability by sulfur application, cluster analysis was constructed and distance coefficients (DC) was constructed by SAHN method. This procedure is very helpful in determination of cultivar variation to salt tolerance modulated by sulfur. In previous studies, such methods have been used for various germination and growth experiments (Saboora *et al.,* 2006; Ali *et al.*, 2014).

Conclusion

In conclusion, although applied salinity decreased the growth of maize plants, the use of sulfur was found efficient in alleviation of harmful effect of salt stress. Sulfur improves availability of macronutrients in the plants which are key regulators in alleviation of salt stress. The effectiveness of the use of applied level of S was from 60 to 80 mM concentration. So, sulfur could be applied at about 60 mM to 80 mM for improving nutrient contents and ultimately growth of maize under saline conditions.

Acknowledgement

The authors acknowledge the partial financial assistance provided by University of Agriculture Faisalabad under UAF Ph.D. Research Grant (2907/ORIC) for completion of this study.

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