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Optimizing sulfur for improving salt tolerance of sunflower (*Helianthus annuus* L.)

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Abstract

Soil salinization limit crop yield and deteriorate product quality in arid and semi-arid agroecological regions. Under such conditions regulation of mineral nutrients may help to sustain crop productivity. Therefore, a pot experiment was carried out to identify optimal sulfur level and source for enhancing salt adaptability of sunflower (Helianthus annuus L.). A uniform salinity level of 100 mM NaCl was developed in each pot and then six S treatments viz. S_0, control; S_1, 20 mg sulfur kg^{-1} soil; S_2, 40 mg sulfur kg^{-1} soil; S_3, 60 mg sulfur kg^{-1} soil; S_4, 80 mg sulfur kg^{-1} soil and S_5, 100 mg sulfur kg^{-1} soil were applied by using elemental S and K_2SO_4 as sulfur source. Supplementation of sulfur caused reduction in tissue Na^+ accumulation and electrolyte leakage while increase in tissue K^+ and Ca^{2+} with a subsequent increase in relative water content and photosynthetic rate of sunflower. Increasing sulfur levels in the form of K_2SO_4 depicted linear increase in plant growth and yield attributes up to 80 mg sulfur kg^{-1} soil beyond which there was decline in these growth characteristics suggesting that sulfur as K_2SO_4 at 80 mg sulfur kg^{-1} soil might be optimum in mitigating NaCl toxicity in sunflower.

Keywords: NaCl toxicity, sunflower, morpho-physiological, electrolyte, sulfur

Introduction

Soil salinity is considered a major environmental stress that limits growth and yield of many crop species (Nejat and Mantri, 2017; Ashraf *et al.,* 2017). Globally, more than 800 million hectares of land which accounts for 20% of the irrigated land is affected by salts to varying degree (Shrivastava and Kumar, 2015) that hinders sustainable food production. In Pakistan, more than 6.28 million hectares are affected by salinity and sodicity which restricts the development of agriculture and food security (Rehman *et al.,* 2015). The excessive concentration of sodium (Na^+) and chloride (Cl^-) ions in soil creates osmotic imbalance in plant cells which results in osmotic stress, nutrient deficiency, ion injury and oxidative impairment, subsequently causing complete or partial failure of plant growth and development (Alvarez *et al.,* 2012; Ashraf *et al*., 2015). Tester and Devenport (2003) reported that high soil salinity has negative impact on plant respiration, carbohydrate metabolism and photosynthetic efficiency.

Sunflower (*Helianthus annuus* L.) is a promising oil seed crop and sulfur (S) has significant role in its production. Sulfur interacts with nitrogen (N) and forms significant partnerships during the growth of this crop. This interaction between S and N controls leaf area, which produces photosynthates for developing seeds and florets determining sunflower seed size and seed yield. Sulfur deficiency reduces number of seeds per plant, seed weight and yield. Serafin and Belfield (2008) observed that 1 ton seeds of sunflower remove 5 kg of sulfur from the soil. Cultivation of this crop in regular rotation may remove significant amounts of sulfur over time. The uptake of S is highest (45%) between budding and anthesis, medium (35%) at post-anthesis and lowest (20%) during emergence to budding. Sunflower crop shows moderate sensitivity to saline soils having a threshold level of 1.7 dS m^{-1} (Munns, 2005). Hussain *et al.* (2011) found that salinity stress caused decline in K^+ and increase in Na^+ concentration of sunflower which impaired cell membrane integrity, water uptake, nutrient absorption, ultimately reduced its yield and yield contributing parameters. Effects of salinity appear in the form of reduced seed germination and stunted growth, patchy growth pattern, physiological drought, plant wilting, desiccation, leaf area reduction, root shoot length reduction, decrease in flowering and lesser seed production in sunflower (Mane *et al.,* 2011). Plant metabolisms and cell

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structure like membrane integrity, cytoplasm, chlorophyll and other metabolic reactions of plant are adversely affected by salinity. Salinity affects the protein synthesis, enzyme activities, lipid and energy metabolism which alters the metabolites transportation to seeds and reduce yield (Parvaiz and Satyawati, 2008).

The adverse effects of salinity on crop plants are mitigated by employing various physical, biological and chemical treatments to soil, seeds and seedlings. All these approaches activate osmotic, molecular and physiological mechanisms that enable crop plants to tolerate saline conditions. In addition to this, exogenous provision of essential nutrients as foliar spray or soil application may alleviate salinity stress by manipulating some physiological processes in plants (Rossi *et al.,* 2016). For example, the application of zinc upregulated the activities of Ascorbate peroxidase (APX), Catalase (CAT), Peroxidases (POD) and polyphenol oxidase (PPO) enzymes and lowered the concentration of Malonic dialdehyde (MDA) and Hydrogen peroxide (H_2O_2) in the test plant tissues under salt stress (Weisany *et al.,* 2012). More recently Fathi *et al*. (2017) and Soliman *et al*. (2015) have also reported a positive impact of Fe and Zn application on crop plants under salinity stress environment. Another mechanism behind improving salt tolerance in plants in response to application of essential mineral elements is antagonistic interactions between ions. Foliar or soil application of Ca^{2+}, NO_3, P, K, NO_3, silicon (Si) and salicylic acid to the saline soil has improved salinity tolerance in many crops such as pepper, eggplant, tomato, strawberry, melon, and bean (Maas and Grattan, 1999; Grattan, 2002; Jamil *et al.,* 2018). Flores *et al*. (2001) reported a marked reduction in the harmful effects of salinity on tomato biomass production due to the use of higher NH_4^+ concentration in the nutrient solutions. A significant decrease in the tomato root-to-shoot ratio of Na^+, Cl^- and boron was observed due to the addition of Si in a sodic-B toxic soil (Gunes, *et al*., 2007). Hence, it is an effective strategy to use essential nutrients for improving salt tolerance in crops.

Among the major essential nutrients, sulfur (S) is involved in different plant metabolic reactions under salinity and other environmental stresses. Sulfur is primary constituent of some essential amino acids like cystine, cysteine. Sulfur has the ability to suppress absorption of toxic ions such as Na^+ and Cl^- due to antagonistic relationship (Zhang *et al*., 1999), thus its availability in growth medium is helpful not only for enhancing crop yield and quality but also brings improvement in soil environments (Lopez-Berenguera *et al.,* 2007). Sulfur also improves plant selectivity for K^+/Na^+ and increases the ability of Ca^{2+} to mitigate harmful effects of Na^+ on plants (Ali *et al*., 2012). Relative water content, electrolyte leakage and photosynthetic rate are considered as important physiological parameters for assessing salt tolerance in crops (Asfaw, 2011). It is suspected that these physiological parameters might be improved by S supplementation. A thorough review of literature has suggested that S supplementation under salt stress may influence plant tolerance to salinity depending upon many plant and soil factors (Khan *et al*., 2013). Hence, the present study was undertaken to identify best S level and source for enhancing salt adaptation capacity in terms of growth, yield, ionic and physiological relations of sunflower grown under saline environment.

Materials and Methods

Experimental site description

Experiment was carried out in pots at College of Agriculture (32.08° N latitude, 72.67° E longitude), University of Sargodha, Pakistan during 2017. Each Pot was filled with 10 kg soil mixture having 9 kg soil and 1 kg sand taken from top 20 cm layer of cultivated field after drying and passing through 2 mm sieve. Soil physico-chemical characteristics are given in Table 1.

Table 1. Physicochemical properties of experimental soil

Soil properties	Value
Sand (mg kg^{-1})	592
Silt (mg kg^{-1})	189
Clay (mg kg^{-1})	219
Textural class	Sandy loam
EC_e (dS m^{-1})	1.48
pH	7.5
Extractable potassium (mg kg^{-1})	174
Available phosphorus (mg kg^{-1})	2.3
Organic matter (%)	0.55

Salinity level of 100 mM NaCl (10 dS m^{-1} EC) was developed by adding required quantity of NaCl to each pot and then six treatments viz. S_0-control; S_1- 20 mg sulfur kg^{-1} soil; S_2- 40 mg sulfur kg^{-1} soil; S_3-60 mg sulfur kg^{-1} soil; S_4-80 mg sulfur kg^{-1} soil and S_5-100 mg sulfur kg^{-1} soil were applied by using elemental S and K_2SO_4 as sulfur sources. Each treatment was replicated five times.

Experimental details

Sunflower hybrid Hysun-33 was used in the experiment. Three seedlings in each pot were maintained after the germination. The experimental pots were organized randomly at ambient temperature and light conditions in net house. In order to maintain uniform K^+ level in each treatment, KNO_3 was applied in the pots treated with elemental S as a source of sulfur. Uniform level of N (70 mg kg^{-1} soil) in each treatment was maintained by adjusting

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urea dose considering N gain from KNO_3. Recommended dose of P_2O_5 (40 mg kg^{-1} soil) was added in every pot in the form of TSP. During growth of plants all agronomic management practices were performed uniformly. After 60 days of planting, 2 plants from each pot were harvested for measuring relative water content and electrolyte leakage. While, third plant was kept growing up to maturity. At maturity, plant growth and yield characteristics including plant height, stem girth, head size, head weight, number of seeds $head^{-1}$, plant fresh and dry weight, achene yield pot^{-1} and 1000-achene weight were recorded using standard protocol.

Plant analysis

Harvested plants were washed, divided into shoots, roots and weighed to measure fresh biomass. The plant samples were oven dried at 65°C for 48 hours to record dry weight. The dried plant samples were ground to 40 mesh by using a grinder (MF 10 IKA-WERKE, GMBH & Co., KG, Germany). The ions such as Na^+, K^+ and Ca^{2+} were recovered from leaves, stems and roots by adopting wet digestion procedure (Jones and Case, 1990). For ionic analysis, 0.5 g plant sample was taken in conical flask, kept it overnight after adding 10 mL di-acid digestion mixture (HNO_3: $HClO_4$ in the ratio of 2:1). A hot plate at 250ºC was used to digest samples until material became transparent. Digested materials were cooled and diluted up to 50 mL by adding distilled water. Digested samples were filtered with Whatman filter paper # 42 and stored in airtight plastic bottles. Digested leaves, stems and root samples were analyzed for Na^+, K^+ and Ca^{2+} by Atomic Absorption Spectrophotometer calibrated with series of respective standards. Physiological parameters including net photosynthesis and transpiration rate were determined with the help of Infra-red Gas Analyzer (IRGA; Analytical Development Company, Herts, U.K.). Relative water contents were determined by taking 1g of fresh leaves, soaked in the 20 mL distilled water for 4 hours. Leaf samples were taken out from the water, cleaned with the tissue paper and weighed again to get their turgid weight. After that, the leaf samples were placed in the oven at 65°C for 48 hours to get their oven dry weight. Relative water contents were measured by using the given formula:

$$\text{RWC} = \frac{(\text{Fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}} \times 100$$

To measure electrolyte leakage one gram of each leaf sample was placed in 20 mL distilled water for 24 hours and then EC of solution was taken by EC meter and denoted as EC_1. Then these solutions were autoclaved for 15 minutes and again EC were measured as EC_2. Electrolyte leakage were determined with the help of following formula:

$$\text{Electrolyte leakage} = \frac{\text{EC1}}{\text{EC2}} \times 100$$

Statistical analysis

Statistical software Statistica 8.1 was used for data analysis. The effects of treatments were compared by calculating analysis of variance (ANOVA) for the collected data. The differences between the treatments means were compared using the least significant difference test ($p \leq 0.05$) (Steel *et al.,* 1997).

Results

Different sulfur levels and sources significantly affected plant height of sunflower grown at 100 mM NaCl (Table 2). Minimum sunflower plant height of 47.62 cm was recorded when plants were grown at 100 mM NaCl without S. However, plant height was improved by 21.60 and 13.56% with the application of 20 mg S kg^{-1} soil, 29.73 and 28.93% with 40 mg sulfur kg^{-1} soil, 32.38 and 31.54% with 60 mg S kg^{-1} soil, 34.35 and 36.60% with 80 mg sulfur kg^{-1} soil and 14.65 and 10.03% with 100 mg S kg^{-1} soil as elemental S and K_2SO_4, respectively, compared to NaCl treated plants without supplemental S.

Stem girth of sunflower plants was significantly affected by the addition of different S levels and sources when planted at 100 mM NaCl (Table 2). Minimum stem girth of 5.12 cm was recorded from sunflower plants grown at 100 mM NaCl without supplemental S. However, stem girth was increased by 15.23 and 21.48% with S_1, 23.82 and 30.86% with S_2, 33.59 and 30.07% with S_3, 34.37 and 58.98% with S_4 and 25.39 and 26.75% with S_5 treatment using elemental S and K_2SO_4, respectively, compared to NaCl treated plants without S.

Data regarding effect of different S levels and sources on root fresh weight of sunflower planted at 100 mM NaCl (Table 2) showed significant differences due to different levels and sources of S. Minimum root fresh weight of 1.38 g was produced by sunflower planted at 100 mM NaCl without supplemental S. Root fresh weight was significantly improved by different S levels and sources showing an increase of 52.89 and 88.40% with 20 mg S kg^{-1} soil, 57.97 and 127.53% with 40 mg S kg^{-1} soil, 66.66 and 131.88% with 60 mg S kg^{-1} soil, 157.24 and 247.10% with 80 mg S kg^{-1} soil and 55.07 and 126.08% with 100 mg S kg^{-1} soil applied as elemental S and K_2SO_4, respectively, compared to NaCl treated plants without S.

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Table 2: Growth and yield characteristics of sunflower (*Helianthus annuus* L.) grown at 100 mM NaCl salinity level as influenced by different levels and sources of S at maturity

Plant parameters	100 NaCl	100 NaCl + 20 S		100 NaCl + 40 S		100 NaCl + 60 S		100 NaCl + 80 S		100 NaCl + 100 S	
	Control	S°	K_2SO_4	S°	K_2SO_4	S°	K_2SO_4	S°	K_2SO_4	S°	K_2SO_4
PH (cm)	47.62f	57.91c	54.80d	61.78 b	61.4b	63.40ab	62.64b	63.98ab	65.50a	54.60d	52.40e
SG (cm)	5.12f	5.90e	6.22d	6.34d	6.70b	6.84b	6.66bc	6.88b	8.14a	6.42cd	6.49cd
RFW (g plant^{-1})	1.38h	2.11fg	2.60c	2.18fg	3.14b	2.30ef	3.20b	3.55cd	4.79a	2.14fg	3.12b
SFW (g plant^{-1})	3.20h	6.90e	10.47d	7.85e	14.79b	6.85e	11.74c	11.44c	17.86a	5.37f	13.62b
TFW (g plant^{-1})	4.40h	9.10f	13.07d	10.03ef	17.93b	9.15f	14.94c	14.99c	22.65a	7.51g	16.74b
RDW (g plant^{-1})	0.64f	0.93d	0.98d	0.97d	1.42bc	1.30 cd	1.48bc	1.72 b	1.89 a	0.94 d	1.09cd
SDW (g plant^{-1})	1.32g	2.75e	3.24cd	2.98d	3.86b	2.79e	3.37c	3.33c	4.34a	2.90f	3.58bc
TDW (g plant^{-1})	1.96g	3.68e	4.22d	3.95de	5.28bc	3.82de	4.854cd	5.50c	6.23a	3.30f	4.67cd
HD (cm)	5.12g	5.72e	6.88c	6.24d	7.05b	6.72c	7.46a	7.20b	7.68a	6.10d	5.44f
HW (g)	4.88g	8.70e	12.24d	10.85d	14.45c	10.70e	17.06b	14.22c	21.26a	8.72e	6.83f
NAH	158g	203f	270cd	227e	273cd	230e	289b	242d	312a	197f	178h
1000-AW (g)	29.82g	40.89f	52.68e	52.68e	64.72b	54.28de	56.45cd	58.40c	69.58a	54.73de	42.02f
AY (g plant^{-1})	4.71g	6.30e	10.51cd	9.96cd	12.76bc	10.48cd	13.86b	12.13bc	15.64a	8.10d	5.53f

Means sharing the same letter in a row do not differ significantly at $p \leq 0.05$; 100 NaCl: 100 mM NaCl; 100 NaCl+20 S: 100 mM NaCl + 20 mg S kg^{-1} soil; 100 NaCl+40 S: 100 mM NaCl + 40 mg S kg^{-1} soil; 100 NaCl+60 S: 100 mM NaCl + 60 mg S kg^{-1} soil; 100 NaCl+80 S: 100 mM NaCl + 80 mg S kg^{-1} soil; 100 NaCl+100 S: 100 mM NaCl + 100 mg S kg^{-1} soil, S°: Elemental S, PH: Plant height, SG: Stem girth, RFW: Root fresh weight, SFW: Shoot fresh weight, TFW: Shoot fresh weight, RDW: Root dry weight, SDW: Shoot dry weight, TDW: Shoot dry weight, HD: Head diameter, HW: Head weight, NAH: Number of achene head^{-1}, 1000-AW: 1000-achene weight, AY: achene yield

Similarly, sunflower planted at 100 mM NaCl without application of S produced minimum (3.02 g) shoot fresh weight (Table 2). Different levels and sources of S influenced shoot fresh weight and caused an increase of 128.47 and 246.68% with S_1, 153.97 and 389.73% with S_2, 119.68 and 288.74% with S_3, 278.80 and 491.39% with S_4, and 77.81 and 350.99% with S_5 treatment applied as elemental S and K_2SO_4, respectively, over NaCl treated plants without S.

Total fresh weight of sunflower plants was significantly influenced by different S levels and sources grown at 100 mM NaCl (Table 2). Minimum total fresh weight of 4.40 g was produced by sunflower plants when cultivated at 100 mM NaCl without S. An increase of 70.68 – 240% and 197-414% in total fresh weight of sunflower was recorded with the application of different levels of elemental S and K_2SO_4, respectively, over no S control.

The sunflower plants grown at 100 mM NaCl salinity produced minimum root dry weight of 0.64 g (Table 2) when no S was added. Different S levels and sources significantly influenced root dry weight which was increased by 45.31 and 53.12% with 20 mg S kg^{-1} soil, 51.56 and 121.87% with 40 mg S kg^{-1} soil, 60.93 and 131.25% with 60 mg S kg^{-1} soil, 168.75 and 195.31% with 80 mg S kg^{-1} soil, and 46.87 and 70.31% with 100 mg S kg^{-1} soil applied as elemental S and K_2SO_4, respectively as compared to NaCl treated plants without S.

Sunflower shoot dry weight was significantly affected by the addition of different doses and sources of S under 100 mM NaCl saline conditions (Table 2). Sunflower cultivated at 100 mM NaCl salinity produced minimum shoot dry weight (1.32 g plant^{-1}) when no S was added. All doses of S showed a substantial increase in shoot dry weight over control indicating its potential to mitigate salinity stress. The incorporation of S at 80 mg S kg^{-1} soil using K_2SO_4 as a source recorded maximum (228.78%) increase in sunflower shoot dry weight over control. However, minimum (58.33%) improvement in shoot dry biomass was recorded by the addition of 100 mg S kg^{-1} soil as elemental S in comparison with NaCl treated plants receiving no S.

Total dry weight of sunflower plants was significantly affected by different S levels and sources when planted at 100 mM NaCl (Table 2). Minimum total dry weight of 1.96 g was produced by sunflower cultivated at 100 mM NaCl without S. However, total dry weight was enhanced by 87.75 and 115.30% by the addition of 20 mg S kg^{-1} soil, 101.53 and 169.38% with 40 mg S kg^{-1} soil, 94.89 and 147.44% with 60 mg S kg^{-1} soil, 157.65 and 217.85% with 80 mg S kg^{-1} soil and 54.59 and 138.26% with 100 mg S kg^{-1} soil using elemental S and K_2SO_4 source, respectively, in comparison to NaCl treated plants without S application.

Application of different amounts of S using different sources showed a significant influence on head diameter of sunflower when grown at 100 mM NaCl (Table 2). Sunflower plants cultivated at 100 mM NaCl deprived of

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supplemental S produced minimum head diameter. Head diameter was improved by 11.71 and 34.37% with S_1, 21.87 and 37.69% with S_2, 31.25 and 45.70% with S_3, 37.10 and 50% with S_4 and 17.38 and 6.25% with S_5 treatments applied as elemental S and K_2SO_4, respectively, compared to plants produced without S under 100 mM NaCl salinity.

Head weight of sunflower was significantly influenced by different levels and sources of S grown at 100 mM NaCl (Table 2). Minimum head weight of 4.88 g was recorded in sunflower plants cultivated at 100 mM NaCl and receiving no S. Different S levels had positive effect on head weight under NaCl stress. An improvement in sunflower head weight was observed in the range of 78.27 and 150.81% with addition of 20 mg S kg^{-1} soil, 106.35 and 196.10% with 40 mg S kg^{-1} soil, 122.33 and 249.59% with 60 mg S kg^{-1} soil, 191.39 and 335.65% with 80 mg S kg^{-1} soil and 78.68 and 39.95% with 100 mg S kg^{-1} soil using elemental S and K_2SO_4 source, respectively, compared with NaCl treated plants receiving no S.

Data regarding the effects of different S levels and sources on number of achene $head^{-1}$ of sunflower cultivated at 100 mM NaCl is presented in Table 2. Results revealed that minimum number of achene $head^{-1}$ (158) was found in sunflower planted at 100 mM NaCl without application of S. Different S levels had positive effect on number of achene $head^{-1}$ under NaCl stress. An increase of 28.48 and 70.88% with 20 mg S kg^{-1} soil, 43.67 and 72.78% with 40 mg S Kg^{-1} soil, 45.56 and 77.84% with 60 mg S kg^{-1} soil, 53.16 and 97.46% with 80 mg S kg^{-1} soil, and 24.68 and 12.65% with 100 mg S kg^{-1} soil as elemental S and K_2SO_4, respectively, compared to NaCl treated plants without added S.

Similarly, 1000-achene weight of sunflower was significantly influenced by different S levels and sources when cultivated at 100 mM NaCl (Table 2). Minimum 1000-achene weight of 29.82 g was obtained from sunflower plants cultivated at 100 mM NaCl without addition of S. Different S levels had positive effect on 1000-achene weight under NaCl stress and caused an increase of 37.12 and 76.65% with S_1, 76.65 and 117.03% with S_2, 82.02 and 89.30% with S_3, 95.84 and 133.33% with S_4, and 83.53 and 41.51% with S_5 incorporated as elemental S and K_2SO_4, respectively compared to NaCl treated plants receiving no S.

Likewise, sunflower plants grown at 100 mM NaCl supplied with S as elemental S and K_2SO_4 showed a significant improvement in achene yield (Table 2). Results revealed that minimum achene yield pot^{-1} (4.71 g) was recorded from sunflower plants cultivated at 100 mM NaCl and S was not added. Different S levels had shown a positive influence on achene yield pot^{-1} under NaCl stress. An increase of 33.75 and 123.14% with 20 mg S kg^{-1} soil, 111.46 and 170.91% with 40 mg S kg^{-1} soil, 122.50 and 194.26% with 60 mg S kg^{-1} soil, 157.53 and 232.05% with 80 mg S kg^{-1} soil and 71.97 and 17.40% with 100 mg S kg^{-1} soil was recorded by elemental S and K_2SO_4 source, respectively, in comparison with NaCl treated plants without the addition of S.

It was observed that Na^+ concentration in sunflower root grown at 100 mM NaCl was significantly influenced by different levels and sources of S (Table 3). Results depicted that maximum root Na^+ concentration of 36.24 mg g^{-1} was recorded in sunflower plants cultivated at 100 mM NaCl without S addition. Increasing S levels in the growth medium had ameliorative effects on root Na^+ concentration under NaCl stress and caused a decrease of 18.26 and 27.48% with S_1, 26.93 and 34.32% with S_2, 31.78 and 41.55% with S_3, 43.95 and 47.68% with S_4 and 35.84 and 32.28% with S_5 applied as elemental S and K_2SO_4, respectively, than NaCl treated plants without S.

Data regarding the effects of different S levels and sources on sunflower stem Na^+ concentration planted at 100 mM NaCl (Table 3) revealed that maximum stem Na^+ concentration of 42.84 mg g^{-1} was found in sunflower plants grown at 100 mM NaCl without supplemental S. Increasing S levels in the growth medium had ameliorative effects on stem Na^+ concentration under NaCl stress and caused a decrease of 21.28 and 31.88% with S_1, 31.72 and 35.06% with S_2, 33.17 and 38.74% with S_3, 46.72 and 54.50% with S_4, and 45.96 and 43.09% with S_5 applied as elemental S and K_2SO_4, respectively, compared to NaCl treated plants without supplemental S. Similarly, maximum leaf Na^+ concentration of 45.22 mg g^{-1} was found in sunflower plants cultivated at 100 mM NaCl without supplemental S (Table 3).

Addition of S in the growth medium alleviated toxic effects of salinity and showed a decrease of 27.81 and 37.61% with 20 mg S kg^{-1} soil, 39.27 and 47.72% with 40 mg S kg^{-1} soil, 44.89 and 54.97% with 60 mg S kg^{-1} soil, 51.65 and 61.76% with 80 mg S kg^{-1} soil and 47.92 and 45.57% with 100 mg S kg^{-1} soil supplied as elemental S and K_2SO_4, respectively, over NaCl treated plants without S.

Potassium plays an important role in plant osmotic regulation and resultantly it improves plant ability to survive in saline environment. Root K^+ contents in sunflower cultivated at 100 mM NaCl were significantly ($p \leq 0.05$) altered by different sources and levels of S (Table 3). Results discovered that minimum root K^+ concentration of 18.54 mg g^{-1} was found in sunflower plants cultivated at 100 mM NaCl without supplemental S. Increasing S levels in the growth medium had positive effects on root K^+

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concentration under NaCl stress and caused an increase of 25.94 and 46.38% with the addition of 20 mg S kg^{-1} soil, 37.21 and 69.95% with 40 mg S kg^{-1} soil, 57.92 and 79.34% with 60 mg S kg^{-1} soil, 86.24 and 101.72% with 80 mg S kg^{-1} soil and 61.48 and 51.56% with 100 mg S kg^{-1} soil supplied as elemental S and K_2SO_4, respectively, over plants without S.

A very similar trend of sunflower stem K^+ concentration was observed. Minimum stem K^+ concentration of 20.22 mg g^{-1} was recorded from sunflower cultivated at 100 mM NaCl without S. Addition of S in the growth medium depicted an increase in stem K^+ concentration of sunflower plants grown under NaCl stress. The supply of 80 mg S kg^{-1} soil as elemental S and K_2SO_4, showed a maximum increase of 80.90 and 91.14% in stem K^+ concentration, respectively, over NaCl treated plants without supplemental S.

Leaf K^+ concentration in sunflower plants cultivated at 100 mM NaCl was significantly ($p \leq 0.05$) altered by different levels and sources of S (Table 3). Minimum leaf K^+ concentration of 22.61 mg g^{-1} was found in sunflower cultivated at 100 mM NaCl without supplemental S. Increasing S levels in the growth medium had positive effects on leaf K^+ concentration under NaCl stress and caused an increase of 24.72 and 32.46% with S_1, 47.10 and 60.99% with S_2, 58.86 and 66.52% with S_3, 73.15 and 84.52% with S_4, and 42.68 and 38.87% with S_5 applied as elemental S and K_2SO_4, respectively, in comparison with plants subjected to NaCl without supplemental S.

Root Ca^{2+} concentration in sunflower plants grown at 100 mM NaCl was significantly ($p \leq 0.05$) altered by different levels and sources of S (Table 3). Minimum root Ca^{2+} concentration of 8.65 mg g^{-1} was recorded from sunflower plants cultivated at 100 mM NaCl without addition of S. Increasing S levels in the growth medium depicted positive effects on root Ca^{2+} concentration under NaCl stress and caused an increase of 18.61 and 29.82% with the application of 20 mg S kg^{-1} soil , 43.58 and 71.32% with 40 mg S kg^{-1} soil, 57.68 and 80.92% with 60 mg S kg^{-1} soil, 91.44 and 111.44% with 80 mg S kg^{-1} soil and 41.62 and 29.24% with 100 mg S kg^{-1} soil using elemental S and K_2SO_4, respectively, compared to NaCl treated plants without S.

Stem Ca^{2+} concentration of plants grown in saline environment determine their adaptability to survive in stress environment. Data regarding the effects of different S levels and sources on stem Ca^{2+} concentration in sunflower plants cultivated at 100 mM NaCl (Table 3) indicated that minimum stem Ca^{2+} concentration of 9.14 mg g^{-1} was found in sunflower plants cultivated at 100 mM NaCl when S was not added. Increasing S levels in the growth medium showed positive effects on stem Ca^{2+} concentration under salinity stress and caused an increase of 25.38 and 48.14% with S_1, 41.68 and 55.68% with S_2, 67.17 and 79.43% with S_3, 87.63 and 110.28% with S_4,

Table 3: Ionic contents in different parts of sunflower plant grown at 100 mM NaCl salinity level as influenced by different levels and sources of S 60 days after planting

Ionic contents (mg g^{-1})	100 NaCl	100 NaCl + 20 S		100 NaCl + 40 S		100 NaCl + 60 S		100 NaCl + 80 S		100 NaCl + 100 S	
	Control	S°	K_2SO_4	S°	K_2SO_4	S°	K_2SO_4	S°	K_2SO_4	S°	K_2SO_4
Root Na^+	36.24a	29.62bc	26.28c	26.48c	23.08d	24.72cd	21.18e	20.31e	18.96f	23.25d	24.54d
Stem Na^+	42.84a	33.72b	29.18bc	29.25bc	27.82c	28.63c	26.24cd	22.95de	19.49e	23.15d	24.38d
Leaf Na^+	45.22a	32.64c	28.21cd	27.46d	23.64de	24.92de	20.36e	21.86e	17.29f	23.55de	24.61de
Root K^+	18.54e	23.85de	27.14cd	25.44d	31.51bc	29.28c	33.25b	34.53b	37.04a	29.94c	28.10cd
Stem K^+	20.22e	26.75de	29.15cd	27.86d	34.03b	31.45c	35.52ab	36.58ab	38.65a	29.94cd	28.88cd
Leaf K^+	22.61e	28.02de	29.95de	33.26c	36.04b	35.92bc	37.65b	39.15ab	41.72a	32.26cd	31.40d
Root Ca^{2+}	8.65de	10.26cd	11.23cd	12.42c	14.82b	13.64c	15.65b	16.56ab	18.29a	12.25c	11.18cd
Stem Ca^{2+}	9.14de	11.46cd	13.54c	12.95c	14.23bc	15.28b	16.40b	17.15ab	19.22a	13.67c	12.96c
Leaf Ca^{2+}	10.26d	11.75cd	13.36c	13.94c	15.22bc	16.25b	17.80ab	18.21a	19.75a	13.68c	12.60cd

Mean s sharing the same letter in a row do not differ significantly at $p \leq 0.05$; 100 NaCl: 100 mM NaCl; 100 NaCl+20 S: 100 mM NaCl + 20 mg S kg^{-1} soil; 100 NaCl+40 S: 100 mM NaCl + 40 mg S kg^{-1} soil; 100 NaCl+60 S: 100 mM NaCl + 60 mg S kg^{-1} soil; 100 NaCl+80 S: 100 mM NaCl + 80 mg S kg^{-1} soil; 100 NaCl+100 S: 100 mM NaCl + 100 mg S kg^{-1} soil, S°: Elemental S

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and 49.56 and 41.79% with S_5 treatment applied as elemental S and K_2SO_4, respectively, compared to NaCl treated plants without S. Likewise Ca^{2+} concentration in sunflower leaves treated with different doses of S also varied. Plants grown at 100 mM NaCl salinity without addition of S showed minimum leaf Ca^{2+} concentration of 10.26 mg g^{-1}. Addition of S in growth medium showed an increase in sunflower leaf Ca^{2+} concentration under NaCl stress. An increase in leaf Ca^{2+} concentration of 14.52 and 30.21% with 20 mg S kg^{-1} soil, 35.86 and 48.34% with 40 mg S kg^{-1} soil, 58.38 and 73.48% with 60 mg S kg^{-1} soil, 77.48 and 87.62% with 80 mg S kg^{-1} soil, and 33.33 and 22.80% with 100 mg S kg^{-1} soil applied as elemental S and K_2SO_4 was noted, respectively, compared to NaCl treated plants without supplemental S.

A detectable indication of salinity stress is alteration in plant physiological characteristics such as relative water contents, electrolyte leakage, net photosynthetic rate and transpiration rate. Water levels in plant tissue and preservation of transpiration enables a plant to continue its growth. Effects of different S levels and sources on relative water content of sunflower plants cultivated at 100 mM NaCl (Table 4) showed that minimum relative water content of 66.81% was observed when S was not added in growth media. Addition of S in the growth medium had positive influence on relative water content under NaCl stress. An increase in relative water contents in order of 12.97and 18.41% with 20 mg S kg^{-1} soil, 13.03 and 24.17% with 40 mg S kg^{-1} soil, 17.87 and 22.40% with 60 mg S kg^{-1} soil, 20.77 and 24.78% with 80 mg S kg^{-1} soil, and 22.64 and 32.04% with 100 mg S kg^{-1} soil applied as elemental S and K_2SO_4 was noted, respectively, than NaCl treated plants without S.

Electrolyte leakage is a symbol of plant's response to stress environment. This is a widely used test for determining stress-induced plant injury and tolerance. The electrolyte leakage is universal and can be activated by different stress factors including salinity. The data showed that the electrolyte leakage was significantly reduced by increasing S contents in 100 mM NaCl saline growth medium (Table 4).

Maximum electrolyte leakage of 94.66% was found in sunflower plants cultivated at 100 mM NaCl without addition of S. Different S levels had ameliorative effect on electrolyte leakage under salinity stress. Electrolyte leakage was found to be decreased by 6.10 and 12.75% with S_1, 7.07 and 17.32% with S_2, 10.01 and 18.88% with S_3, 17.13 and 34.49% with S_4, and 11.68 and 20.91% with S_5 treatments applied as elemental S and K_2SO_4, respectively, compared to NaCl treated plants without supplemental S.

A substantial reduction in a plant's stomatal conductance can be observed under salinity stress. The resultant decrease in internal CO_2 slows down the activity of many enzymes including RuBisCo, hence carboxylation and net photosynthetic rate is declined. Net photosynthetic rate of sunflower cultivated at 100 mM NaCl was significantly influenced by different levels and sources of S (Table 4). Minimum net photosynthetic rate was observed in sunflower plants (up to 11.50 µmol m^{-2} s^{-1}) cultivated at 100 mM NaCl without S. Increasing S levels in the growth medium under NaCl stress demonstrated positive effects on net photosynthetic rate and caused an increase of 19.13 and 39.91% with 20 mg S kg^{-1} soil, 72.34 and 81.21% with 40 mg S kg^{-1} soil, 93.65 and 112.78% with 60 mg S kg^{-1} soil, 130.08 and 174.95% with 80 mg S kg^{-1} soil, and 85.04 and 72.73% with 100 mg S kg^{-1} soil applied as elemental S and K_2SO_4, respectively, compared to NaCl treated plants without supplemental S.

Table 4. Physiological characteristics of sunflower plant grown at 100 mM NaCl salinity level as influenced by different levels and sources of S 60 days after planting

Physiological traits	100 NaCl	100 NaCl + 20 S		100 NaCl + 40 S		100 NaCl + 60 S		100 NaCl + 80 S		100 NaCl + 100 S	
	Control	S°	K_2SO_4	S°	K_2SO_4	S°	K_2SO_4	S°	K_2SO_4	S°	K_2SO_4
RWC (%)	66.81f	75.48e	79.11d	75.52e	82.96bc	78.75d	81.78bc	80.69cd	83.37b	81.94bc	88.22a
EL (%)	94.66a	88.88b	82.59d	87.96b	78.26e	85.18c	76.78ef	78.44e	62.01g	83.60cd	74.86f
NPR (µmol m^{-2} s^{-1})	11.50f	13.70e	16.09de	19.82d	20.84d	22.87cd	24.47c	26.46bc	31.62a	21.28cd	19.83d
TR (mmol m^{-2} s^{-1})	2.59g	4.65d	4.66d	5.76b	5.91ab	5.19c	6.04ab	6.83a	6.98 a	4.97c	4.73cd

Means sharing the same letter in a row do not differ significantly at $p \leq 0.05$. 100 NaCl: 100 mM NaCl; 100 NaCl+20 S: 100 mM NaCl + 20 mg S kg^{-1} soil; 100 NaCl+40 S: 100 mM NaCl + 40 mg S kg^{-1} soil; 100 NaCl+60 S: 100 mM NaCl + 60 mg S kg^{-1} soil; 100 NaCl+80 S: 100 mM NaCl + 80 mg S kg^{-1} soil; 100 NaCl+100 S: 100 mM NaCl + 100 mg S kg^{-1} soil, S°: Elemental S, RWC: Relative water content, ELL: Electrolyte leakage, NPR: Net photosynthetic rate, TR: Transpiration rate

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Literature review suggests that the maintenance of normal transpiration rates by a plant under salinity stress is an important determinant of salt tolerance. The effect of different S levels and sources on transpiration rate of sunflower plant cultivated at 100 mM NaCl (Table 4) revealed that minimum transpiration rate of 2.59 mmol m^{-2} s^{-1} was found in pots where sunflower plants did not receive any S input.

Addition of S in the growth medium under NaCl stress had positive effects on transpiration rate. In comparison with control an increase of 79.53 and 79.92% with 20 mg S kg^{-1} soil, 122.39 and 128.18% with 40 mg S kg^{-1} soil, 100.38 and 133.20% with 60 mg S kg^{-1} soil, 163.70 and 169.49% with 80 mg S kg^{-1} soil, and 91.89 and 82.62% with 100 mg S kg^{-1} soil applied as elemental S and K_2SO_4, respectively, was recorded.

Discussion

Saline soil conditions suppress plant growth by disturbing different biochemical and physiological processes. Hussain *et al.* (2011) observed that saline growth medium caused excessive absorption of Na^+ and reduced K^+ uptake by sunflower, which impaired cell membrane integrity, hindered water and nutrient absorption. This salinity induced variations ultimately reduce yield and yield contributing parameters such as head diameter, number of seeds $head^{-1}$, achene weight $head^{-1}$ and 1000-achene weight. To avoid or tolerate salt toxicity plants have evolved different mechanisms. Regulation of nutrients may serve as possible short-term solution to develop salt tolerance in many crops/plants. Among the major plant nutrients, S is considered the master key element because of its role in S-containing compounds like methionine, cysteine that are essential constituents of protein, coenzyme A (CoA), sulfolipids, peptides thiamine and biotin. These S-comprising compounds show a great role in the plant defense mechanisms against stress conditions, including salinity stress. Pakistani soils have S deficiency, but it is essentially required for the protein and oil synthesis in oil seed crops. Mcgrath and Zhao (1996) reported that for the production of one ton of seed in oil seed crops, 16 kg S is required, and the exogenous application of S increased the crop yield and S-containing compounds by improving stress tolerance. Minimum growth and yield of sunflower plants cultivated at 100 mM NaCl without supplemental S could be due to ion injury, decreased RWC, excessive electrolyte loss and ionic imbalance. Early studies (El Kheir *et al.,* 2000) have also established a similar reduction in sunflower growth and yield due to saline growth medium. A study conducted by Lauchli and Epstein (1990) depicted that plant characteristics like plant height, leaves number, stem width, leaf area and head diameter were significantly reduced due to salinity stress mainly because of specific ion toxicity and osmotic stress. Ashraf *et al.* (2008) also observed that excessive absorption and accumulation of Na^+ in plants suppress uptake of essential nutrients such as K^+ and Ca^{2+} and induce phytotoxicity. Moreover, less water absorption under salt stress environment disturb cell multiplication and/or extension and influence metabolic functions in plants which leads to stunted plant growth and poor yield. Munns (2002) reported that soil salinity decreased the plant's ability of water uptake, leading to reduction in growth along with metabolic changes similarly as occurred due to drought stress. Tabatabaei and Ahmad (2010) reported that essential mineral absorption and water supply through roots is restricted because of abnormal osmotic potential and Cl^- and Na^+ toxicity. However, application of S at all levels via elemental S and K_2SO_4 were significantly effective in improving growth and yield characteristics of sunflower cultivated at 100 mM NaCl. S application at various rates increased the plant growth and yield probably due to its involvement in defense mechanism of sunflower with more synthesis of proteins, vitamins and S-containing compounds, these compounds improve the defense mechanism of plants against the oxidative stress and scavenging of the ROS species. Sing *et al.* (2000) also reported a significant improvement in sunflower growth supplied with different doses of S (0, 30 and 45 kg S ha^{-1}). They reported that S-induced increase in growth and yield of sunflower under salt-stress was attributed to more synthesis of chlorophyll. Khan *et al.* (2003) demonstrated that application of S at 50 kg ha^{-1} was found to be more effective in increasing fresh biomass, dry matter, head diameter, head weight, 1000-achene weight and total seed yield of sunflower than other levels of S treatments. Results revealed that increasing S contents in the growth media were found effective in improving sunflower growth and yield attributes up to 80 mg S kg^{-1} soil above which plant growth and yield were declined. These results are supported by Ahmad *et al.* (2013) who found that a higher dose of 100 kg S ha^{-1} from both sources (elemental S and K_2SO_4) decreased the plant growth and crop yield in terms of plant fresh biomass, head weight, head diameter, 1000-achene weight. Vala *et al.* (2014) described that the higher doses of S via different sources decreased plant growth and achene yield of sunflower. This may be due to SO_4^{-2} toxicity in saline soils by production of Sous acid in leaf mesophyll cells in the stomatal cavities which directly reduced the CO_2 assimilation. This is the reductive step

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in the net photosynthesis of the plant with resultant decrease in plant growth. While comparing the efficiency of both S sources, K_2SO_4 was found to be more effective in improving sunflower growth and yield at 100 mM NaCl soil salinity.

The increase in Na^+ and reduction in K^+ as well as Ca^{2+} in different plant tissues of sunflower planted at 100 mM NaCl without supplemental S were the main opposing effects of NaCl. This was due to antagonistic interactions of Na^+ with K^+ and Ca^{2+} as depicted by excessive absorption of Na^+ by sunflower under salt stress conditions. Cuin *et al.* (2009) observed that 150 mM NaCl induce excessive Na^+ absorption by plant roots which brings reduction in K^+ contents, indicating antipathy between K^+ and Na^+ in durum wheat (*Triticum turgidum* L. spp. durum) and bread wheat (*Triticum aestivum* L.). In present study, use of S in growth medium decreased uptake of Na^+, increased K^+ and Ca^{2+} concentration, which are expressed as important salt tolerance mechanism. According to Badr-uz-Zaman (2002), S application in saline environment improves sunflower tolerance to salinity. As S have synergistic relation with K^+ and Ca^{2+} and both of these elements play an important role in sustaining water content of plant tissues, hence, positive results could be observed at 75 mM of NaCl along with 4 mM of applied S. Plant leaves were fully opened depicting that they had sustained their turgor pressure by improving their ion content through modifying osmotic potential in relation to outside environment. S-induced increased uptake of K^+ and Ca^{2+} might be the due to antagonistic relationship between Na^+ and these elements at the plasmalemma (Epstein, 1969). Devitt *et al.* (1981) reported that the main salt obstacle to nutrient absorption is by K-Na competition. Higher K^+ and Ca^{2+} improve leaf turgor pressure (Devitt *et al.,* 1981). In plants subjected to salinity, the capability to regulate rapid alterations in water potential includes accumulation of K^+ ion. Mass and Poss (1989) also reported that the application of S under salt stress environment might be contributed to the vegetative, reproductive growth parameters along with the Ca^{+2} and K^+ concentration of plant leaves, shoot and root with the amelioration of salinity. Koprivova *et al.* (2000) found that S decreased the toxic effects of Na^+ ion and make a check and balance in the K^+/Na^+ ratio. Rennenberg *et al.* (2007) reported that the concentration of the Na^+ and Cl^- was increased in the leaf, stem and root of sunflower due to the salt stress while the concentration of K^+ and Ca^{+2} decreased. However, the application of S reduced the Na^+ accumulation in the plant body and increased the K^+ concentration. Among different levels of applied S, 80 mg S kg^{-1} soil was found to be superior in reducing Na^+ and increasing K^+ and Ca^{2+} in root, stem and leaves of sunflower grown at 100 mM NaCl compared to all other levels.

High salt contents in soil solution suppress plant's ability to absorb water which leads to abnormal growth. The maintenance of plant water potential by addition of different levels of S in terms of high relative water content under NaCl stress might be attributed to the contribution of S in enhancing water use efficiency, stomatal resistance and lowering transpiration rate. The cell membrane injury due to saline growth medium is indicated by Electrolyte leakage. The higher value of electrolyte leakage in plants cultivated at 100 mM NaCl without adding supplemental S may be attributed to the buildup of reactive oxygen species (ROS) who destroyed the membrane structure and subsequently enhanced electrolyte leakage. Hashemi *et al.* (2010) found that saline soil (150 mmol L^{-1} NaCl) decreased the activities of antioxidant enzymes, enhanced the accumulation of ROS which resulted in oxidative stress in canola (*Brassica napus* L.). The application of different levels of S in salt-stressed medium through elemental S and K_2SO_4 may improve the activities of antioxidant enzymes, decrease the synthesis of ROS and consequently reduce electrolyte leakage. Earlier experiments depicted that S could stabilize membrane structure by influencing peroxidation of membrane lipids. In early growth of plants S plays a vital physiological role. The S-H group of cysteine amino acid is oxidized to produce S-S bond and this disulfide bond is crucial in sustaining three-dimensional structure of many enzymes. The S requirement of sunflower is higher as compared to other oil seed crops (Nabi *et al.,* 1989). Mamatha (2007) also reported that application of S via potassium sulfate at 80 kg ha^{-1} after the 50 days of emergence showed a positive reflection in the plant fresh biomass, membrane stability and water content of the plant. However, the application of elemental S at 20 mg kg^{-1} soil and 80 mg kg^{-1} soil showed a good reflection in the plant height, fresh biomass, relative water content and membrane stability. A S level of 80 mg kg^1 soil again produced maximum ameliorative effects in terms of physiological characteristics. Among both sources of S, K_2SO_4 proved better in improving sunflower plant growth and yield under NaCl stress. This might be due to immediate availability of SO_4^{2-} form of S for plant uptake compared to S^o source which must be converted to SO_4 in soil before plant uptake and it needs a certain amount of time. The extent of conversion time is increased by soil environment and hence the effectiveness of SO_4^{2-} form of S outclass elemental form of S (Roy *et al.,* 2006).

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Conclusion

The addition of S in the soil was effective in overcoming NaCl toxicity in sunflower by reducing Na^+ accumulation and electrolyte leakage while increasing K^+ and Ca^{2+} in plant tissues with the subsequent improvement in plant physiological, growth and yield characteristics. There was a linear increase in plant growth and yield with increasing S levels in growth medium up to 80 mg S kg^{-1} soil above which there was a slight decline suggesting that 80 mg S kg^{-1} soil might be optimum in mitigating NaCl toxicity and enhancing growth and yield of sunflower. The current study also suggests that application of S in the form of K_2SO_4 proved superior over elemental S as its oxidation to sulfate **S** (SO_4^{2-}) is required before absorption which depends on microbial activity, soil temperature, humidity and aeration.

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