

Salinization/sodification of soil and physiological dynamics of sunflower irrigated with saline–sodic water amending by potassium and farm yard manure

M. Ashraf, S. Muhammad Shahzad, N. Akhtar, M. Imtiaz and A. Ali

ABSTRACT

Sunflower (*Helianthus annuus* L.) plants were grown with saline–sodic water (SSW) by treating with potassium (K @ 100 and 200 mg K₂O kg⁻¹ soil) and farm yard manure (FYM @ 5 and 10% of soil, w/w). Irrigation with untreated SSW caused soil salinization/sodification, leading to an increase in electrical conductivity (EC) of 165% and sodium adsorption ratio (SAR) 100% with the subsequent increase of 736% in shoot Na⁺, a decrease of 52% in shoot K⁺ and 94% in shoot K⁺:Na⁺ ratio compared to canal water. SSW also decreased physiological activities: 31% relative water content (RWC), 34% membrane stability index (MSI), 51% protein, 33% chlorophyll and 58% photosynthetic rate compared to canal water. Integrated application of K and FYM, at higher level, decreased soil EC by 54% and SAR 43%, and shoot Na⁺ 57% with a corresponding improvement in soil organic matter 166%, shoot K⁺ 360%, shoot K⁺:Na⁺ ratio 987%, RWC 34%, MSI 37%, protein 60%, photosynthetic rate 102%, superoxide dismutase 92%, peroxidase 78% and catalase 52% compared to SSW without K and/or FYM. In conclusion, exogenous application of K and FYM could be a promising approach to use brackish water in agriculture on a sustainable basis.

Key words | amendment, brackish water, physiological activities, salinity, sodicity, sunflower

M. Ashraf (corresponding author)

S. Muhammad Shahzad
Department of Soil and Environmental Sciences,
University College of Agriculture,
University of Sargodha,
Sargodha, Punjab 40100,
Pakistan
E-mail: mashraf_1972@yahoo.com

N. Akhtar

Department of Plant Breeding and Genetics,
University College of Agriculture,
University of Sargodha,
Sargodha, Punjab 40100,
Pakistan

M. Imtiaz

College of Resources and Environment,
Huazhong Agricultural University,
Wuhan,
China

A. Ali

Department of Agronomy, University College of
Agriculture,
University of Sargodha,
Sargodha, Punjab 40100,
Pakistan

INTRODUCTION

World population growth is reaching the point where good quality water is becoming insufficient for the basic needs of mankind, including irrigation of agricultural land as well as industrial and urban uses. Furthermore, the gap between water supply and demand is ever widening due to uneven distribution of water resources and rapid socio-economic development, particularly in arid and semiarid regions of the world (Ashraf *et al.* 2015a). This situation demands efficient utilization of existing canal water resources or exploration of the possibility of utilizing low quality water to supplement the canal water (Hussain *et al.* 2011). In this regard, ground water can contribute to bridge the gap

between existing crop irrigation requirements. However, most of the ground water pumped out is of a brackish nature and injudicious use of such brackish water for crop production results in deterioration of not only crop yield and quality but also soil health and productivity (Izhar-ul-Haq *et al.* 2007). It has been estimated that use of brackish water without proper management measures resulted in the development of soil salinity/sodicity on 0.8 billion hectares worldwide (FAO 2008) and 3 × 10⁶ hectares in Pakistan (Iqbal *et al.* 2014). Na⁺ in brackish water caused the dispersion of clay particles which resulted in clogging of soil pores causing a significant reduction in porosity, permeability, hydraulic conductivity and soil aeration (Oster 2004). Similarly, Hussain *et al.* (2008) reported that when excess Na⁺ is applied to soil through the use of brackish water, the Na⁺ content builds up in soil over time, displacing

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Ca^{2+} ions from in-between the clay particles, causing deflocculation and soil dispersion. Cucci & Lacolla (2013) reported that the higher level of salts in the irrigation water resulted in a progressive salinization and sodification of soil, with decreasing values from the top to the deep soil layers. Long-term use of brackish water without any amendment causes the accumulation of toxic ions, particularly Na^+ and Cl^- in the rhizosphere which initially causes osmotic stress and reduction in water absorption by plants, and their gradual accumulation in plant parts damages the cell membrane integrity, synthesis of carbohydrates, chlorophyll, proteins, nucleic acid, photosynthetic efficiency and, ultimately, growth and yield of crops (Qadir & Oster 2004).

Sunflower (*Helianthus annuus* L.) is moderately sensitive to salinity with a threshold level of 4.8 dS m^{-1} (Francois 1996; Sadak *et al.* 2010) and exhibits a yield reduction of 5% with each unit increase in salinity above the threshold level (Flaggella *et al.* 2004). Brackish water can limit the plant growth by affecting various physiological and biochemical processes including water absorption, nutrient uptake and distribution, photosynthesis, transpiration, protein synthesis and the activities of various enzymes (Jamil *et al.* 2007). Noreen & Ashraf (2008) reported that salt stress caused a significant reduction in shoot fresh and dry weights, net CO_2 assimilation rate, transpiration rate, stomatal conductance and substomatal CO_2 in sunflower. In addition, plants irrigated with brackish water are at constant risk from reactive oxygen species (ROS) including superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (OH^\bullet) and singlet oxygen ($^1\text{O}_2$) which are inevitably produced via numerous cell metabolic pathways (Kanazawa *et al.* 2000). ROS at higher levels interacted with various physiological and metabolic functions within the plant body and subsequently reduced plant growth and yield.

Plants, however, can adopt various protective mechanisms against salt toxicity induced by brackish water irrigation, such as accumulation of inorganic ions including K^+ and Ca^{2+} (Munns & Tester 2008) and organic osmolytes like proline (Taiz & Zeiger 2010). Proline accumulation in stress environment protects protein structures against denaturation, stabilizes cell membranes by interacting with phospholipids and functions as a hydroxyl radical scavenger (Okuma *et al.* 2004; Claussen 2005; Misra & Saxena 2009). Relative water content (RWC) and membrane stability index (MSI),

photosynthetic rate, transpiration rate and protein synthesis are also considered typical physiological indexes for the evaluation of salt effects on plants, reflecting metabolic activities in plant tissues (Hossain *et al.* 2006). According to Ashraf *et al.* (2015b), a promising approach to mitigate deleterious effects of brackish water is to increase the level of endogenous K either by applying K salts or farm yard manure (FYM) because high Na^+ content in brackish water readily reduces the absorption and accumulation of K under brackish water irrigation. Cakmak (2005) also reported that maintenance of an adequate supply of K in rooting medium under salt stress is an important factor for controlling the adverse effects of Na^+ . Some other studies also reported the alleviative effects of K under salt stress, for example, Tabatabaei & Tabatabaei (2008) in perennial ryegrass (*Lolium perenne* var Boulevard), Kaya *et al.* (2007) in melon (*Cucumis melo* L.), Akram & Ashraf (2011) in sunflower (*Helianthus annuus* L.), Ashraf *et al.* (2015b) in sugarcane (*Saccharum officinarum* L.). However, Jafari *et al.* (2009) found that increasing K (20 mM) generally stimulated the negative effects of salinity on plant growth and yield under salt stress in sorghum (*Sorghum bicolor* L.). Addition of organic matter through manuring, composting or biofertilizer application improves physical, chemical and biological properties of soil and hence facilitates the management and reclamation of salt affected soils (Qadir *et al.* 2001). FYM is a form of organic matter which plays a vital role in improving soil health, in addition to supplying different plant nutrients when applied under brackish water irrigation. Robbins (1986) reported that use of waste matter and manures improved water infiltration, water holding capacity and aggregate stability of soil which were otherwise deteriorated under salt stress. The present study is planned to evaluate the effects of saline-sodic water (SSW) either alone or amended with K and/or FYM on salinity/sodicity development in soil and its subsequent effects on Na^+ and K^+ accumulation in plants, physiological relations and antioxidant activities of sunflower (*Helianthus annuus* L.).

MATERIALS AND METHODS

Soil and water characterization

The research was focused on the effect of SSW either alone or amended with K and/or FYM on salinity/sodicity

development in soil and its subsequent effects on sunflower (*Helianthus annuus* L.) physiology. The characteristics of SSW used for irrigation were electrical conductivity (EC) 5.56 dS m^{-1} , sodium adsorption ratio (SAR) 17.3 (mmol L^{-1})^{1/2}, residual sodium carbonate (RSC) 5.24 mmol L^{-1} and of canal water were EC 0.12 dS m^{-1} , SAR 0.1 (mmol L^{-1})^{1/2}, RSC nil (US Salinity Lab. Staff 1954). Experimental soil was non-saline, non-sodic and having clay loam texture as determined by hydrometer method (Gee & Bauder 1986), pH 8.1, EC 2.5 dS m^{-1} and SAR 7.3 (mmol L^{-1})^{1/2} (Bigham 1996), cation exchange capacity $8.5 \text{ C mol (+) kg}^{-1}$ (Thomas 1982), organic matter 0.66% (Nelson & Sommers 1996), N 22.6 mg kg^{-1} , P 3.5 mg kg^{-1} (Tandon 2001) and K 127 mg kg^{-1} (Soltanpour & Worker 1979) and CaCO_3 7.1% (Bouyoucos 1962).

Experimental treatments

Ten treatments comprising T₁: canal water (control); T₂: SSW; T₃: SSW + K100 ($100 \text{ mg K}_2\text{O kg}^{-1}$ soil); T₄: SSW + FYM-1 (5% FYM of soil, w/w); T₅: SSW + FYM-2 (10% FYM of soil w/w); T₆: SSW + K100 + FYM-1; T₇: SSW + K100 + FYM-2; T₈: SSW + K200 ($200 \text{ mg K}_2\text{O kg}^{-1}$ soil); T₉: SSW + K200 + FYM-1; and T₁₀: SSW + K200 + FYM-2 were employed according to a completely randomized design with five replications. The characteristics of FYM used were EC 0.62 dS m^{-1} , N 1.76%, P 0.87%, K 0.74% and organic C 21.9%. The recommended dose of nitrogen and phosphorus fertilizers (0.80 g N as urea and $0.50 \text{ g P}_2\text{O}_5$ as single super phosphate in each pot) was applied at the time of sowing. The required amount of K as K_2SO_4 and FYM was applied and incorporated into the respective pots according to the treatment plan, before sowing.

Plant growth

Achenes of sunflower (cultivar Hysun-33) were obtained from Ayub Agricultural Research Institute, Faisalabad, Pakistan. Achenes were surface sterilized in 5% sodium hypochlorite solution for 10 minutes, then vigorously rinsed with distilled water. Healthy and uniform achenes were sown in earthen glazed pots (28 cm diameter and 45 cm height having a basal hole) filled with 12 kg soil,

collected from the top 20 cm depth of a cultivated field, air-dried and passed through a 2 mm sieve. Initially, three achenes were sown in each pot but 2 weeks after germination, two healthy plants were maintained in each pot. Plants were irrigated with canal water for 2 weeks after germination and then irrigated with SSW according to the treatment plan which continued until maturity. Plants were irrigated twice a week with 0.5 L water for the first 2 weeks and then 1 L until maturity. The plant protection measures were adopted uniformly for all treatments. During the experimental period, maximum temperature ranged from 17.7 to 39.2°C and minimum 8.1 to 29.4°C while relative humidity was between 54 and 83%. A polythene sheet cover was used during periods of rainfall to avoid rain water entering the SSW treatments pots.

Physiological determinations

Chlorophyll content

Leaf samples (second fully expanded leaf from the shoot tip) were collected from sunflower plants during the sixth week of SSW irrigation between 8 a.m. and 9 a.m. Each leaf sample of 0.5 g was cut into small pieces and homogenized in a pre-cooled mortar and pestle using 80% (v/v) acetone. The extract was centrifuged at 3,000 rpm for 15 minutes and made up to 25 mL with 80% (v/v) acetone. The clear solution was transferred and the optical density was measured at 645 nm and 663 nm against blank in Shimadzu double beam spectrophotometer (Shimadzu UV-1600, UK). The chlorophyll content was estimated according to the method of Arnon (1949).

Protein determination

For protein determination, 0.5 g of fresh leaf samples (second fully expanded leaf from the shoot tip) of the same physiological age as for chlorophyll estimation were collected and homogenized in 1 mL phosphate buffer (pH 7.0). The crude homogenate was then centrifuged at 5,000 g for 10 minutes. After that, 0.5 mL 5% of freshly prepared trichloroacetic acid was added and centrifuged at 8,000 g for 15 minutes. The debris was dissolved in 1 mL of 0.1 N NaOH and 5 mL Bradford reagent was added.

Absorbance was measured with a spectrophotometer (Shimadzu UV-1600, UK) at 595 nm using bovine serum albumin (BSA) as a standard. The final concentration of soluble protein was calculated by using BSA standard curve as described by Bradford (1976).

Proline determination

For proline determination, leaf samples (second fully expanded leaf from the shoot tip) were collected during the sixth week of SSW irrigation between 8 a.m. and 9 a.m. Proline was determined according to Bates *et al.* (1973). Absorbance was measured at 520 nm with a UV spectrophotometer (Shimadzu UV-1600, UK).

MSI

Each leaf sample of 0.5 g (second fully expanded leaf from the shoot tip) was cut into discs of 1.0 cm in diameter and placed into plastic tubes containing 50 mL of distilled water. After 24 h, the EC of water containing the leaf sample was measured (C_1) using an electrical conductivity meter (Jenway 4510, Bibby Scientific Ltd, UK). Plastic tubes were then autoclaved at 120 °C in an Autoclave (Tomy SX-500E) for 20 min and their EC was measured (C_2). MSI was calculated according to the method described by Sairam *et al.* (1997):

$$\text{MSI (\%)} = \left[1 - \left(\frac{C_1}{C_2} \right) \right] \times 100$$

RWC

During the sixth week of SSW irrigation, the third fully expanded leaves from the shoot tip were detached and weighed immediately to record fresh weight (FW). The leaves were dipped in distilled water inside a closed glass petridish for 12 h and reweighed after gently wiping water from the leaf surface with tissue paper to get turgid weight (TW). The leaves were then dried at 70 °C for 48 h to get the oven-dried weight (ODW). RWC was determined using

the equation as described by Kaya & Higgs (2003):

$$\text{RWC (\%)} = \left[\frac{(\text{FW} - \text{ODW})}{(\text{TW} - \text{ODW})} \right] \times 100$$

Photosynthesis and transpiration rate

During the sixth week of SSW irrigation, measurements for net photosynthetic rate and transpiration rate were made on the fully expanded youngest leaf by using photosynthesis meter (CI-340 hand-held photosynthesis meter). These measurements were made from 10 a.m. to 12 p.m.

Enzyme assay

Fresh leaf samples (the fourth fully expanded leaf from the shoot tip) were collected during the sixth week of SSW irrigation for enzyme assays. Each sample of 200 mg FW was homogenized in a mortar and pestle with 2 mL of 50 mM sodium phosphate buffer of pH 7.8 including 1 mM ethylene diamine tetra-acetic acid (EDTA) and 0.5 mM polyvinylpyrrolidone. The homogenate was centrifuged at 10,000 rpm for 20 minutes at 4 °C. The supernatant was stored at 4 °C and used for superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) assay. SOD activity was measured spectrophotometrically according to Zhang & Qu (2003) by measuring the inhibition in photochemical reduction of nitroblue tetrazolium (NBT) at 560 nm. The reaction mixture consisted of 50 mM sodium phosphate buffer of pH 7.8, 0.1 mM EDTA, enzyme extract, 13 mM methionine, 2 μM riboflavin and 75 μM NBT. The reaction mixture was kept under white fluorescent light for 20 minutes and then stirred, and absorbance was recorded at 560 nm on a spectrophotometer (Shimadzu UV-1600, UK). One SOD unit was taken to be the amount of enzyme causing 50% inhibition of NBT reduction and expressed in unit mg^{-1} FW. POD activity was determined using guaiacol oxidation method as described by Li (2000). The samples were prepared using 1 mL H_2O_2 (0.2%), 0.95 mL guaiacol (0.2%), 1 mL sodium phosphate buffer of pH 7.0 and 0.05 mL enzyme extract. The increase in absorbance was recorded at 470 nm at 30 second intervals up to 3 minutes with a

UV-1600 spectrophotometer. CAT activity was assayed using the method described by Aebi (1984). The 3 mL reaction mixture contained 50 mM K phosphate buffer (pH 7.0), 45 mM H₂O₂ and 100 µL of enzyme extract. The activity was assayed by monitoring the decrease in absorbance at 240 nm as a consequence of H₂O₂ consumption.

K⁺ and Na⁺ determinations

One plant from each pot was harvested at 45 days after SSW irrigation and used to determine shoot K⁺ and shoot Na⁺ concentrations. The shoot samples were washed and first air dried and then oven dried at 70 °C for 48 h in an oven (EYELA WFO-600ND; Tokyo Rikaikai Co., Ltd, Tokyo, Japan). Oven-dried shoot samples were finely ground in a grinder fitted with stainless steel blades and a chamber (MF 10 IKA-WERKE, GMBH & Co., KG, Germany). A 0.5 g portion of oven-dried shoot samples was digested in a mixture of concentrated nitric acid and perchloric acid (2:1, v/v) at 250 °C. The shoot K⁺ and Na⁺ concentrations were estimated with a flame photometer (Jenway PFP 7, ELE Instrument Co. Ltd, UK) according to Yoshida *et al.* (1976).

Statistical analysis

Data collected were subjected to statistical analysis using Mstat-C (Department of Crop and Soil Sciences, Michigan State University, East Lansing, Michigan, USA) and analysis of variance was used to compare the effects of treatments. Differences between means were compared using the least significant difference test (LSD, $P \leq 0.05$).

RESULTS

Soil characteristics

Results revealed that SSW irrigation without any amendment led to the build-up of salts in soil and caused an increase of 165% in soil EC and 100% in SAR while there was a decrease of 6.4% in soil organic matter content compared to control (canal water), although soil organic matter was slightly increased compared to original value (Table 1).

Table 1 | Characteristics of experimental soil after the harvest of sunflower (*Helianthus annuus* L.) grown with SSW amended by K and FYM

Treatment	EC (dS m ⁻¹)	SAR (mmol L ⁻¹) ^{1/2}	pH	Organic matter content (%)
CW (control)	2.6e	7.2f	8.1	0.78f
SSW	6.9a	14.8a	8.2	0.73fg
SSW + K100	6.1ab	10.5cd	8.1	0.86f
SSW + FYM-1	5.8ab	9.6de	7.8	0.90f
SSW + FYM-2	4.9b	12.5bc	7.7	1.52bc
SSW + K100 + FYM-1	4.2bc	11.3c	7.7	1.86a
SSW + K100 + FYM-2	3.9cd	10.1cd	7.6	1.67b
SSW + K200	3.6cd	9.8d	8.0	1.88a
SSW + K200 + FYM-1	3.1d	8.8e	7.6	1.74b
SSW + K200 + FYM-2	3.2d	8.4e	7.7	1.94a

Values in a column followed by the same letter are not significantly different at $P \leq 0.05$. EC, electrical conductivity; SAR, sodium adsorption ratio; CW, canal water (control); SSW, saline-sodic water; SSW + K100, saline-sodic water + 100 mg K₂O kg⁻¹ soil; SSW + FYM-1, saline-sodic water + 5% FYM (w/w); SSW + FYM-2, saline-sodic water + 10% FYM (w/w); SSW + K100 + FYM-1, saline-sodic water + 100 mg K₂O kg⁻¹ soil + 5% FYM (w/w); SSW + K100 + FYM-2, saline-sodic water + 100 mg K₂O kg⁻¹ soil + 10% FYM (w/w); SSW + K200, saline-sodic water + 200 mg K₂O kg⁻¹ soil; SSW + K200 + FYM-1, saline-sodic water + 200 mg K₂O kg⁻¹ soil + 5% FYM (w/w); SSW + K200 + FYM-2, saline-sodic water + 200 mg K₂O kg⁻¹ soil + 10% FYM (w/w).

Application of different levels of K and FYM displaced Na⁺ from soil and improved K status of soil with the consequent improvement in soil health as indicated by a decrease in soil EC and SAR and improvement in soil organic matter. The soil EC was decreased by 11.5% with K100, 30% with FYM-1, 39% with FYM-2, 43% with K100 + FYM-1, 48% with K100 + FYM-2, 16% with K200, 55% with K200 + FYM-1 and 54% with K200 + FYM-2 compared with the SSW irrigation without any amendment. SAR was decreased by 29% with K100, 15.5% with FYM-1, 23.6% with FYM-2, 31.7% with K100 + FYM-1, 33.8% with K100 + FYM-2, 35.1% with K200, 40.5% with K200 + FYM-1 and 43% with K200 + FYM-2 compared with the SSW irrigation without any amendment. Organic matter content of soil was increased by 17.8% with K100, 108% with FYM-1, 155% with FYM-2, 129% with K100 + FYM-1, 157% with K100 + FYM-2, 23.3% with K200, 138% with K200 + FYM-1 and 166% with K200 + FYM-2 compared with the SSW irrigation without any amendment. Soil pH was decreased non-significantly by the application of different levels of K and FYM to SSW treatment.

Ionic concentrations

Irrigation of sunflower with SSW caused a significant ($P \leq 0.05$) increase in shoot Na^+ , decrease in shoot K^+ with the concomitant decrease in shoot $\text{K}^+:\text{Na}^+$ ratio compared with the control (canal water) (Table 2). Amendment of SSW by different levels of K and FYM decreased shoot Na^+ but increased K^+ and $\text{K}^+:\text{Na}^+$ ratio and consequently alleviated the deleterious effects of SSW on plant physiological and antioxidant activities. The shoot Na^+ concentration was increased by 736% with SSW irrigation compared with the control. However, shoot Na^+ concentration was decreased by 40.3% with K100, 23.8% with FYM-1, 30.5% with FYM-2, 47.4% with K100 + FYM-1, 51% with K100 + FYM-2, 54.9% with K200, 59% with K200 + FYM-1 and 57% with K200 + FYM-2 compared with the SSW irrigation without any amendment. In contrast, shoot K^+ concentration was decreased by 52% with SSW irrigation compared with the control. The shoot K^+ concentration was increased by 170% with K100, 29.8% with FYM-1, 49% with FYM-2, 212% with K100 + FYM-1, 257% with K100 + FYM-2, 237% with K200, 319% with K200 + FYM-1 and 360% with K200 + FYM-2 compared with the SSW irrigation without

any amendment. The shoot $\text{K}^+:\text{Na}^+$ ratio was decreased by 94% with SSW irrigation compared with the control. When SSW was amended with K and FYM, shoot $\text{K}^+:\text{Na}^+$ ratio was increased by 356% with K100, 71.8% with FYM-1, 115% with FYM-2, 500% with K100 + FYM-1, 635% with K100 + FYM-2, 653% with K200, 933% with K200 + FYM-1 and 987% with K200 + FYM-2 compared with the SSW irrigation without any amendment.

Physiological relations

Physiological relations in terms of RWC, MSI, proline, protein and chlorophyll content, net photosynthetic rate and transpiration rate were significantly ($P \leq 0.05$) influenced by SSW irrigation as well as K and FYM levels (Table 3). The maximum RWC of 86.9% was found in the control which was decreased by 31% with SSW irrigation without any amendment. When SSW was amended with K and FYM, RWC was improved by 22.1% with K100, 13.2% with FYM-1, 17.6% with FYM-2, 25.1% with K100 + FYM-1, 28.7% with K100 + FYM-2, 26.5% with K200, 31.9% with K200 + FYM-1 and 34% with K200 + FYM-2 compared with the SSW irrigation without any amendment. Maximum MSI of 85.8% was found in the control which was decreased by 34% with SSW irrigation without any amendment. Amendment of SSW with K and FYM improved the MSI by 14.7% with K100, 12.2% with FYM-1, 13.6% with FYM-2, 24.9% with K100 + FYM-1, 28.3% with K100 + FYM-2, 23.3% with K200, 32% with K200 + FYM-1 and 37% with K200 + FYM-2 compared with the SSW irrigation without any amendment. In contrast, minimum proline content of $4.4 \mu\text{mol g}^{-1}$ FW was found in the control which was increased by 86.3% with SSW irrigation without any amendment. When SSW was amended with K and FYM, proline accumulation was decreased by 29.2% with K100, 20.7% with FYM-1, 25.6% with FYM-2, 34.1% with K100 + FYM-1, 36.5% with K100 + FYM-2, 36.5% with K200, 40.2% with K200 + FYM-1 and 37.8% with K200 + FYM-2 compared with the SSW irrigation without any amendment. Maximum protein content of 45.96 mg g^{-1} was found in the control, which was decreased by 51% with SSW irrigation without any amendment. However, application of K and FYM to SSW treatment improved protein content by 28.6% with K100, 18.4% with FYM-1, 32.2% with FYM-2, 39.9% with

Table 2 | Shoot Na^+ , K^+ and $\text{K}^+:\text{Na}^+$ ratio of sunflower (*Helianthus annuus* L.) grown with SSW amended by K and FYM

Treatments	Shoot Na^+ (mg g^{-1} DW)	Shoot K^+ (mg g^{-1} DW)	Shoot $\text{K}^+:\text{Na}^+$ ratio
CW (control)	5.52f	38.24e	6.92a
SSW	46.20a	18.18g	0.39f
SSW + K100	27.56c	49.12d	1.78e
SSW + FYM-1	35.18b	23.60fg	0.67ef
SSW + FYM-2	32.10bc	27.10f	0.84ef
SSW + K100 + FYM-1	24.26c	56.80cd	2.34d
SSW + K100 + FYM-2	22.60cd	64.92bc	2.87d
SSW + K200	20.80cd	61.26c	2.94d
SSW + K200 + FYM-1	18.90d	76.22ab	4.03c
SSW + K200 + FYM-2	19.70d	83.64a	4.24c

Values in a column followed by the same letter are not significantly different at $P \leq 0.05$. CW, canal water (control); SSW, saline-sodic water; SSW + K100, saline-sodic water + 100 $\text{mg K}_2\text{O kg}^{-1}$ soil; SSW + FYM-1, saline-sodic water + 5% FYM (w/w); SSW + FYM-2, saline-sodic water + 10% FYM (w/w); SSW + K100 + FYM-1, saline-sodic water + 100 $\text{mg K}_2\text{O kg}^{-1}$ soil + 5% FYM (w/w); SSW + K100 + FYM-2, saline-sodic water + 100 $\text{mg K}_2\text{O kg}^{-1}$ soil + 10% FYM (w/w); SSW + K200, saline-sodic water + 200 $\text{mg K}_2\text{O kg}^{-1}$ soil; SSW + K200 + FYM-1, saline-sodic water + 200 $\text{mg K}_2\text{O kg}^{-1}$ soil + 5% FYM (w/w); SSW + K200 + FYM-2, saline-sodic water + 200 $\text{mg K}_2\text{O kg}^{-1}$ soil + 10% FYM (w/w).

Table 3 | Physiological attributes of sunflower (*Helianthus annuus* L.) grown with SSW amended by K and FYM

Treatments	RWC (%)	MSI (%)	Proline ($\mu\text{mol g}^{-1}$ FW)	Protein (mg g^{-1})	Chlorophyll (mg g^{-1} FW)	Net photosynthetic rate ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Transpiration rate ($\text{mmol m}^{-2} \text{s}^{-1}$)
CW (control)	86.90a	85.8a	4.4d	45.96a	1.73a	35.35a	64.10a
SSW	60.25e	56.5e	8.2a	22.63f	1.15f	14.70e	26.74e
SSW + K100	73.60c	64.8d	5.8c	29.10d	1.36cd	23.42cd	41.90c
SSW + FYM-1	68.20d	63.4d	6.5bc	26.80de	1.28d	21.46d	28.50e
SSW + FYM-2	70.90cd	64.2d	6.1c	29.92d	1.29d	22.92cd	33.64d
SSW + K100 + FYM-1	75.40bc	70.6c	5.4cd	31.66cd	1.46c	28.86bc	47.76bc
SSW + K100 + FYM-2	77.55bc	72.5bc	5.2cd	34.60c	1.52c	29.94b	48.96bc
SSW + K200	76.22bc	69.7c	5.2cd	34.50c	1.45c	27.62cd	48.52bc
SSW + K200 + FYM-1	79.50b	74.6bc	4.9cd	37.70bc	1.56bc	30.94b	53.65b
SSW + K200 + FYM-2	80.82b	77.6b	5.1cd	36.25bc	1.58bc	29.63b	53.94b

Values in a column followed by the same letter are not significantly different at $P \leq 0.05$.

RWC, relative water content; MSI, membrane stability index; CW, canal water (control); SSW, saline-sodic water; SSW + K100, saline-sodic water + 100 mg $\text{K}_2\text{O kg}^{-1}$ soil; SSW + FYM-1, saline-sodic water + 5% FYM (w/w); SSW + FYM-2, saline-sodic water + 10% FYM (w/w); SSW + K100 + FYM-1, saline-sodic water + 100 mg $\text{K}_2\text{O kg}^{-1}$ soil + 5% FYM (w/w); SSW + K100 + FYM-2, saline-sodic water + 100 mg $\text{K}_2\text{O kg}^{-1}$ soil + 10% FYM (w/w); SSW + K200, saline-sodic water + 200 mg $\text{K}_2\text{O kg}^{-1}$ soil; SSW + K200 + FYM-1, saline-sodic water + 200 mg $\text{K}_2\text{O kg}^{-1}$ soil + 5% FYM (w/w); SSW + K200 + FYM-2, saline-sodic water + 200 mg $\text{K}_2\text{O kg}^{-1}$ soil + 10% FYM (w/w).

K100 + FYM-1, 52.9% with K100 + FYM-2, 52.4% with K200, 66.6% with K200 + FYM-1 and 60% with K200 + FYM-2 compared with the SSW irrigation without any amendment. Likewise, maximum chlorophyll content of 1.73 mg g^{-1} FW was found in the control which was decreased by 33% with SSW irrigation without any amendment. Amendment of SSW with K and FYM improved the chlorophyll content by 18.2% with K100, 11.3% with FYM-1, 12.1% with FYM-2, 26.9% with K100 + FYM-1, 32.1% with K100 + FYM-2, 26% with K200, 35.6% with K200 + FYM-1 and 37% with K200 + FYM-2 compared with the SSW irrigation without any amendment. Maximum net photosynthetic rate of $35.35 \mu\text{mol m}^{-2} \text{S}^{-1}$ was found in the control which was decreased by 58% with SSW irrigation without any amendment. Amendment of SSW with K and FYM improved net photosynthetic rate by 59.31% with K100, 45.9% with FYM-1, 55.9% with FYM-2, 96.3% with K100 + FYM-1, 103.6% with K100 + FYM-2, 87.9% with K200, 110.4% with K200 + FYM-1 and 102% with K200 + FYM-2 compared with the SSW irrigation without any amendment. Transpiration rate was also found to be maximum ($64.10 \text{ mmol m}^{-2} \text{S}^{-1}$) in control treatment which was decreased by 58.2% with SSW irrigation without any amendment. When SSW was treated with K and FYM, transpiration rate improved by 56.6% with K100, 6.5 with FYM-1, 25.8%

with FYM-2, 78.6% with K100 + FYM-1, 83% with K100 + FYM-2, 81.9% with K200, 100% with K200 + FYM-1 and 101.7% with K200 + FYM-2 compared with the SSW irrigation without any amendment. There was a significant negative correlation of shoot Na^+ concentration with protein content ($R^2 = 0.95$, Figure 1), MSI ($R^2 = 0.92$, Figure 2) and net photosynthetic rate ($R^2 = 0.94$, Figure 3) of sunflower grown with SSW amended by K and FYM.

Antioxidant enzyme activities

The activities of antioxidant enzymes including SOD, POD and CAT in sunflower were also significantly ($P \leq 0.05$)

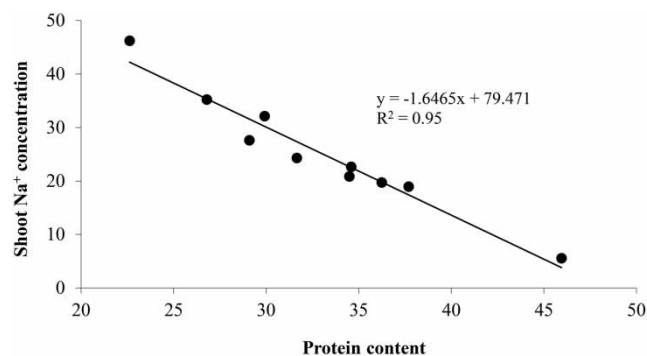


Figure 1 | Correlation between shoot Na^+ concentration and protein content of sunflower (*Helianthus annuus* L.) grown with SSW amended by K and FYM.

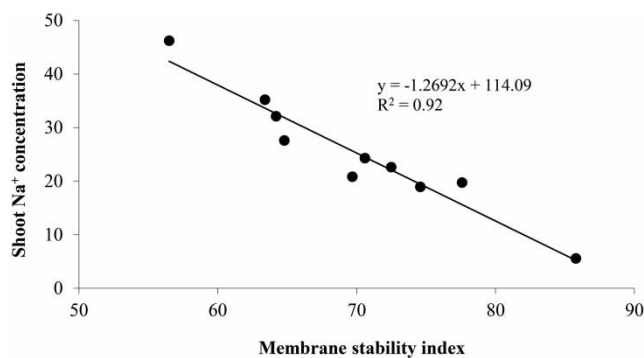


Figure 2 | Correlation between shoot Na^+ concentration and MSI of sunflower (*Helianthus annuus* L.) grown with SSW amended by K and FYM.

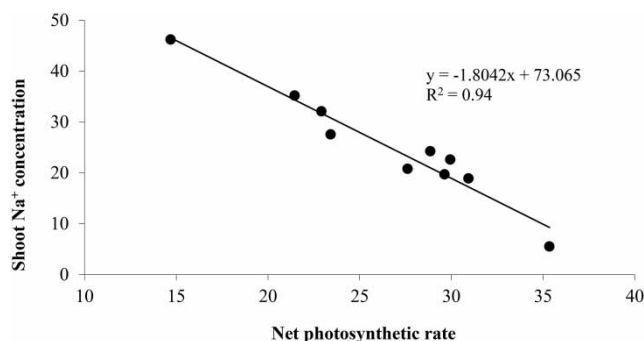


Figure 3 | Correlation between shoot Na^+ concentration and net photosynthetic rate of sunflower (*Helianthus annuus* L.) grown with SSW amended by K and FYM.

influenced by SSW, K and FYM (Table 4). SOD activity was found to be minimum ($32.60 \text{ U mg}^{-1} \text{ FW}$) in control treatment which was increased by 26.5% with SSW irrigation without any amendment. When SSW was amended with K and FYM, SOD activity was increased by 36% with K100, 13.1% with FYM-1, 15.6% with FYM-2, 67.8% with K100 + FYM-1, 87.5% with K100 + FYM-2, 58% with K200, 100% with K200 + FYM-1 and 92% with K200 + FYM-2 compared with the SSW irrigation without any amendment. Minimum POD activity of $53 \text{ U mg}^{-1} \text{ FW}$ was recorded in control treatment which was increased by 205% with SSW irrigation without any amendment. However, when SSW was treated with different levels of K and FYM, POD activity was increased by 38.2% with K100, 18.5% with FYM-1, 32.7% with FYM-2, 51.2% with K100 + FYM-1, 88% with K100 + FYM-2, 56.8% with K200, 76.5% with K200 + FYM-1 and 78% with K200 + FYM-2 compared with the SSW irrigation without any

Table 4 | Antioxidant enzyme activities of sunflower (*Helianthus annuus* L.) grown with SSW amended by K and FYM

Treatments	SOD activity ($\text{U mg}^{-1} \text{ FW}$)	POD activity ($\text{U mg}^{-1} \text{ FW}$)	CAT activity ($\text{U mg}^{-1} \text{ FW}$)
CW (control)	32.60f	53e	18.60fg
SSW	41.25e	162de	39.26d
SSW + K100	56.10cd	224bc	46.73c
SSW + FYM-1	46.65de	192cd	42.55cd
SSW + FYM-2	47.69d	215bc	41.64cd
SSW + K100 + FYM-1	69.22b	245b	49.24bc
SSW + K100 + FYM-2	77.35ab	256ab	54.63b
SSW + K200	65.20bc	254ab	54.82b
SSW + K200 + FYM-1	82.69a	286a	62.96a
SSW + K200 + FYM-2	79.14a	288a	59.74a

Values in a column followed by the same letter are not significantly different at $P \leq 0.05$. SOD, superoxide dismutase; POD, peroxidase; CAT, catalase; CW, canal water (control); SSW, saline-sodic water; SSW + K100, saline-sodic water + $100 \text{ mg K}_2\text{O kg}^{-1}$ soil; SSW + FYM-1, saline-sodic water + 5% FYM (w/w); SSW + FYM-2, saline-sodic water + 10% FYM (w/w); SSW + K100 + FYM-1, saline-sodic water + $100 \text{ mg K}_2\text{O kg}^{-1}$ soil + 5% FYM (w/w); SSW + K100 + FYM-2, saline-sodic water + $100 \text{ mg K}_2\text{O kg}^{-1}$ soil + 10% FYM (w/w); SSW + K200, saline-sodic water + $200 \text{ mg K}_2\text{O kg}^{-1}$ soil; SSW + K200 + FYM-1, saline-sodic water + $200 \text{ mg K}_2\text{O kg}^{-1}$ soil + 5% FYM (w/w); SSW + K200 + FYM-2, saline-sodic water + $200 \text{ mg K}_2\text{O kg}^{-1}$ soil + 10% FYM (w/w).

amendment. Likewise, minimum CAT activity of $18.60 \text{ U mg}^{-1} \text{ FW}$ was found in control treatment which was increased by 111% with SSW irrigation without any amendment. Application of K and FYM to SSW improved CAT activity by 19% with K100, 8.3% with FYM-1, 6% with FYM-2, 25.4% with K100 + FYM-1, 39.1% with K100 + FYM-2, 39.6% with K200, 60.3% with K200 + FYM-1 and 52% with K200 + FYM-2 compared with the SSW irrigation without any amendment. Antioxidant enzyme activities of sunflower grown with SSW amended by K and FYM were significantly positively correlated with shoot K^+ concentration ($R^2 = 0.936$ for SOD, Figure 4), ($R^2 = 0.937$ for POD, Figure 5), ($R^2 = 0.927$ for CAT, Figure 6).

DISCUSSION

In the area of water supply, present-day agriculture is facing the challenge of acute shortage of good quality irrigation water, probably due to uneven distribution of rainfall and water resources both in time and space and strong water competition among various sectors (Ashraf et al. 2015a).

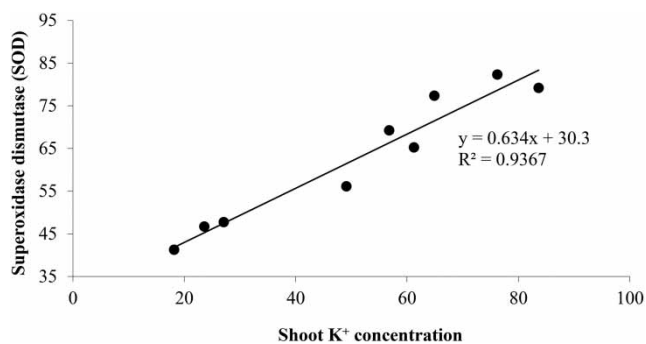


Figure 4 | Correlation between shoot K⁺ concentration and SOD activity of sunflower (*Helianthus annuus* L.) grown with SSW amended by K and FYM.

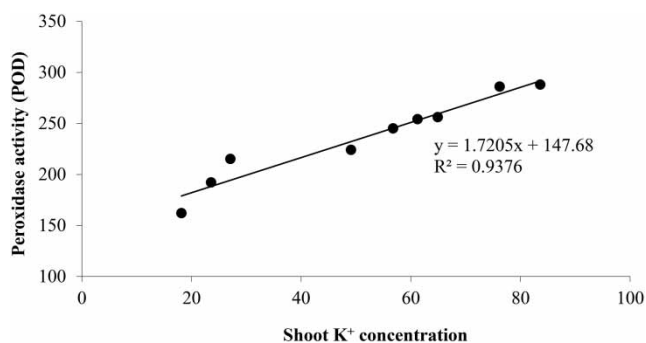


Figure 5 | Correlation between shoot K⁺ concentration and POD activity of sunflower (*Helianthus annuus* L.) grown with SSW amended by K and FYM.

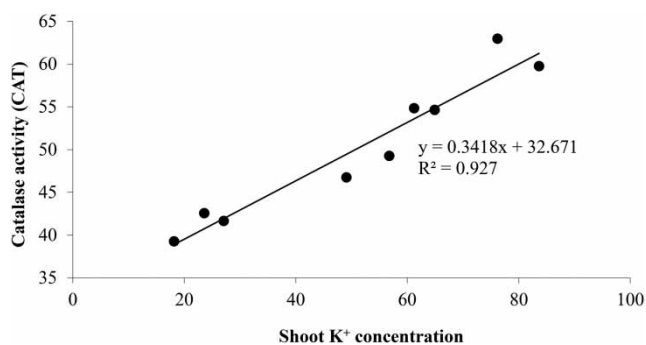


Figure 6 | Correlation between shoot K⁺ concentration and CAT activity of sunflower (*Helianthus annuus* L.) grown with SSW amended by K and FYM.

Under these existing situations, water scarcity can be overcome by either increasing water use efficiency (Niakan & Ahmadi 2014) or using brackish water for irrigation (Izhar-ul-Haq et al. 2007). Injudicious use of brackish water for irrigation may result in build-up of salts in the soil, causing deterioration of the physical and chemical properties of

the soil with a consequent reduction in plant growth and yield (Ashraf & Gill 2005). In the present study, it was found that SSW irrigation without any amendment increased the accumulation of soluble salts and exchangeable Na⁺ in the rhizosphere as evidenced by a significant ($P \leq 0.05$) increase in soil EC and SAR while pH was increased slightly. Past studies also reported a sharp increase in soil EC and SAR due to the use of brackish water with a resultant deterioration in physical and chemical properties of the soil depending upon the irrigation water quality, water consumption and management strategies employed (Qadir et al. 2009; Ashraf & Gill 2005; Iqbal et al. 2014). When SSW was amended with different levels of K and FYM, EC and SAR were decreased significantly and soil pH non-significantly. The decline in soil EC and SAR with K and FYM was attributed to the interaction of K⁺ with Na⁺ to replace it from in-between the clay particles, and thus along with FYM, improved physical and chemical properties of the soil which were otherwise damaged by SSW irrigation, with more pronounced effects in the case of combined application. Izhar-ul-Haq (2007) found that gypsum and FYM were significantly effective to reduce the deleterious effects of brackish water by lowering the EC, pH, SAR and lime content while increasing K and organic matter content of the experimental soil.

A high concentration of Na⁺ in the root zone induced by SSW irrigation without any amendment competed with K⁺ not only for binding sites in the roots but also for long-distance transport from root to shoot and resulted in high Na⁺, low K⁺ and low K⁺:Na⁺ ratio in shoots of sunflower when irrigated with SSW without any amendment. Past studies, for example, de Lacerda et al. (2003), Mohamedin et al. (2006), Hussain et al. (2008), Tahir et al. (2011), George et al. (2012) and Iqbal et al. (2014) also reported a significant increase in Na⁺ and decrease in K⁺ accumulation of salt-stressed plants, and they added that high levels of Na⁺ inhibited K⁺ uptake and translocation and caused a decrease in K⁺:Na⁺ ratio. Application of K and/or FYM interacted with Na⁺, restricted its uptake and accumulation, enhanced K⁺ concentration and K⁺:Na⁺ ratio in the plant body and subsequently improved plant resistance to high levels of salts induced by SSW irrigation. According to Akram et al. (2009), higher concentration of Na⁺ under the condition of high salts in the rooting medium readily

displaced K^+ from membrane binding sites, and therefore, maintenance of higher levels of endogenous K^+ by applying K salts or organic manure could be a promising approach to sustain crop productivity with brackish water irrigation. Singh *et al.* (1992) evaluated different organic manures to improve plant growth under brackish water irrigation and found that these manures not only improved plant growth and yields by reducing Na^+ accumulation and improving mineral nutrient uptake, particularly of K^+ , under brackish water-induced salt toxicity, but also ameliorated the soil chemically as well as physically.

The marked decrease in physiological attributes of sunflower in terms of RWC, MSI, protein and chlorophyll synthesis, net photosynthetic rate and transpiration rate was attributed to high build-up of salts in soil, their uptake and accumulation within the plant and subsequent interference with plant metabolism under SSW irrigation without any amendment. High salt accumulation in the rooting medium by SSW irrigation initially disturbed water relations in the soil, reduced the plant's ability to take up water and resulted in lower RWC in sunflower. Subsequently, the gradual accumulation of salts in plant parts damaged membrane structure and caused lower MSI, inhibited chlorophyll synthesis, reduced leaf area with a resultant decrease in net photosynthesis and transpiration rate, and disturbed protein synthesis in sunflower plants grown with SSW without any amendment. Arzani (2008) reported that high Na^+ build-up in soil as the result of brackish water irrigation caused osmotic changes in soil, disturbed the uptake of water and mineral nutrients from the soil with a consequent reduction in plant growth and yield. Application of K and FYM under SSW irrigation improved water uptake through osmotic adjustment, and hence improved RWC, and helped to scavenge ROS and improved MSI, chlorophyll and protein content. Ashraf *et al.* (2015b) found that exogenous application of K contributed to osmotic adjustment, water economy, and improved RWC under salt stress. Proline content was significantly increased with SSW irrigation without any amendment but decreased after the application of K and FYM suggesting a protective role of K and FYM in sunflower plants against SSW irrigation. Therefore, the level of proline accumulated in K- and FYM-treated plants was not as high as in SSW irrigated plants without K and FYM supplementation.

In the present study, increased activities of SOD, POD and CAT were found in sunflower plants irrigated with SSW compared to canal water, although the increase was insufficient to alleviate oxidative stress as these plants still exhibited a marked decrease in MSI, chlorophyll and protein synthesis. Al-aghabary *et al.* (2004) also reported an increase in SOD, POD and CAT activities with increasing salt concentration in the rooting medium which is often considered among the first signals of plant adaptation to a stressful environment. Application of K and FYM to SSW treatment further increased the activities of SOD, POD and CAT, and consequently improved MSI, chlorophyll and protein synthesis, indicating a reduction in oxidative stress. K and FYM supplementation generally reduced salt ions uptake, improved mineral nutrient status of plants with a consequent stimulation in antioxidant enzyme activities and alleviation of oxidative damage caused by SSW in sunflower.

CONCLUSION

SSW irrigation of sunflower without any amendment led to salt build-up in the soil and resulted in significant increase of soil EC and SAR with a slight change in soil pH. Advanced uptake of salts, particularly Na^+ , by plants inhibited K^+ uptake and translocation to shoots and increased shoot Na^+ with a corresponding decrease in shoot K^+ and $K^+ : Na^+$ ratio, RWC, MSI, protein and chlorophyll synthesis and net photosynthesis in sunflower grown with SSW. However, when SSW was amended with K and FYM at different levels, K^+ readily displaced Na^+ from soil, and along with FYM ameliorated the soil physically as well as chemically. Subsequently, there was a significant reduction in Na^+ uptake and translocation to aerial plant parts with an increase in K^+ accumulation, $K^+ : Na^+$ ratio as well as physiological and antioxidant activities. Finally, displacement of Na^+ from soil and plant binding sites by K^+ , increase in $K^+ : Na^+$ ratio, RWC, MSI, protein content, net photosynthetic rate and stimulation of antioxidant enzyme activities were the main protective mechanisms induced by K and FYM against SSW irrigation. However, the results need to be confirmed under field conditions and the economic feasibility should be calculated.

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