



Full Length Article

Foliage Applied Silicon Alleviates the Combined Effects of Salinity and Drought Stress on Wheat Seedlings

Abdul Sattar¹, Mumtaz A. Cheema^{2*}, Ahmad Sher^{1*}, Tahira Abbas¹, Muhammad Irfan³, Muhammad Ijaz¹, Shabir Hussain³ and Qasim Ali¹

¹College of Agriculture, Bahauddin Zakariya University, Bahadur Sub-Campus Layyah, Pakistan

²Grenfell Campus - Boreal Ecosystems Research Initiative, Memorial University of Newfoundland, Corner Brook, NL, Canada

³Department of Agronomy, Faculty of Agriculture Sciences and Technology Bahauddin Zakariya University, Multan-Pakistan

*For correspondence: cheemamumtaz@gmail.com; ahmad.sher@bzu.edu.pk

Abstract

Salinity and drought are the most important abiotic stresses limiting the agricultural productivity worldwide. A greenhouse experiment was conducted to evaluate the potential of silicon (Si) in alleviating the adversities of salinity and drought stressed in wheat seedlings. Experimental treatments included control (no salinity and drought nor Si sprayed), only salinity (50 mM NaCl), only drought (40% water holding capacity), only Si (50 mM), salinity + drought (50 mM NaCl + 40% water holding capacity), salinity + Si (50 mM NaCl + 50 mM Si), drought + Si (40% water holding capacity + 50 mM Si), salinity + drought + Si (50 mM NaCl + 40% water holding capacity + 50 mM Si). Wheat seedlings exposed to salinity and drought stresses produced negative effect on morphological, physiological and biochemical attributes. Morphological attributes (shoot and root dry weight, length and biomass of seedlings); water relations, photosynthetic pigments and gas exchange parameters of wheat seedlings were adversely affected upon exposure to salinity and drought separately or in combination; however the combined effect was more negative. However, foliage applied Si significantly improved the morphology, water relations, photosynthetic pigments and gas exchange parameters of wheat seedlings under salinity and drought stress individually or in combined. Moreover, Si spray improved the activities of enzymatic antioxidants i.e. catalase, superoxide dismutase and peroxidase under salinity, drought and salinity + drought stresses. In conclusion, foliage applied Si may be a potential strategy to improve the salt and drought tolerance in wheat owing to significant rise in enzymatic antioxidants, photosynthetic pigments, photosynthesis rate, stomatal conductance and leaf turgor pressure. © 2018 Friends Science Publishers

Keywords: Antioxidants; Morphological attributes; Salt tolerance; Silicon; Water relations

Introduction

Salinity and drought are major threat to crop production and food security throughout the world (Farooq *et al.*, 2017). These stresses cause adverse effects on morphological, physiological and biochemical processes in plants (Ouzounidou *et al.*, 2014; Noman *et al.*, 2015). There is great similarity among both stresses regarding biochemical, physiological and molecular adversities (Sairam and Tyagi, 2004). Physiological drought occurs when the levels of soluble salts rises in the soil solution and limit water uptake due to low water potential (Hussain *et al.*, 2018). It is found that as the salinity increases, water potential and osmotic potential of plants become more negative (Hussain *et al.*, 2018). Drought and salinity stress together lower down the water potential and relative water contents in barley (*Hordeum vulgare* L.) crop, but it remained unchanged in Tibetan wild barley comparative to control (Ahmed *et al.*, 2013a). In another study, Ahmed *et al.* (2013b) found that chlorophyll content was greatly reduced in barley plants

when it was cultivated under both drought and salinity stresses which led to a severe decline in photosynthesis. The stomatal factors and chlorophyll synthesis reticence caused the photosynthetic inhibition.

The production of reactive oxygen species (ROS) is one of initial biochemical reactions in plant subjected to grow under salinity and drought stress (Apel and Hirt, 2004). To face subsequent defense responses in plants, the production of ROS as a secondary messenger. The ROS like hydrogen peroxide (H₂O₂), superoxide, the hydroxyl radical, and singlet oxygen are produced naturally as by-products during oxygen metabolism under normal conditions. The oxidative stress and cell damage is caused due to over-production of ROS. A composite system of enzymatic antioxidants such as catalase (CAT), ascorbate peroxidase (APX), superoxide dismutase (SOD) and peroxidase (POD). Similarly some important solutes like proline and soluble protein that minimize the effects of drought and salinity stresses in higher plants (Ozkur *et al.*, 2009). Under drought stress the enhanced activities of SOD, APX, and POD known as

antioxidant enzymes, reduced the oxidative damage in the seedlings of caper bush (Ozkur *et al.*, 2009). Yang *et al.* (2009) investigated that the activity of enzymatic antioxidant was increased under 25% field capacity in comparison of 100% field capacity. Seckin *et al.* (2010) opposed arrays in the deeds of enzymatic antioxidants due to NaCl stress in *H. maritimum* and *H. vulgare*.

Now-a-days, silicon (Si) is considered a potent researched nutrient in the biological chemistry of plants. Being a beneficial plant nutrient, Si performs an important part in maintaining and reducing many biotic and abiotic stresses like salt and drought stresses (Ouzounidou *et al.*, 2016). The addition of Si maintained the water status of plant leaves (Romero-Aranda *et al.*, 2006), and increased the photosynthetic activity of maize (Sattar *et al.*, 2016). Silica-cuticle binary layer on the leaf epidermal tissue is formed by exogenous use of Si and it also improved the tissue water status in wheat (Liang *et al.*, 1999). It was observed that barley and wheat seedlings treated with Si to salt considerably enhanced the enzymatic activity and alleviated the lipid peroxidation (LPO) in leaves and roots (Liang *et al.*, 2003; Sattar *et al.*, 2017).

It has been detected that in the potato (Levy *et al.*, 2013) and barley (Yousfi *et al.*, 2010), the effects of combined stress on plant is more damaging than effects of single stress. On the other hand, many studies showed that single stress effects the plant (Wu *et al.*, 2013), but very limited knowledge has been established for physiological and biochemical processes underlying the tolerance of plants for integrated salinity and drought stresses. Therefore, current study was aimed to assess the effect of both stresses applied as single and in pair on wheat seedlings through morphological, physiological and biochemical attributes and also evaluate the potential of Si in relieving the adverse consequences of salinity and drought.

Materials and Methods

Growth Conditions and Treatments

A pot experiment was performed in greenhouse to evaluate the influence of foliar spray of Si for enhancing the salt and drought tolerance in wheat plants. Wheat variety Faisalabad-2008 was used as an experimental material that was collected from Ayub Agriculture Research Institute Faisalabad. Ten seeds of wheat were sown in each pot containing 12 kg of well ground and sieved soil. Recommended doses of NPK were applied to sustain the growth of wheat seedlings. Basal dose of nitrogen fertilizer as urea at the rate of 100 mg kg⁻¹, phosphorus (P₂O₅) as diammonium phosphate at the rate of 90 mg kg⁻¹, while potassium (K₂O) as potassium sulphate at the rate of 60 mg kg⁻¹ was added and mixed thoroughly.

Each pot was maintained with five plants. Fifteen days old seedlings were planted in each pot under normal conditions. Artificial soil salinities were developed by adding NaCl solution. Salinity and drought stresses were applied

after 15 days of seedlings development. Treatments included the control (no salinity and drought nor Si applied), only salinity (50 mM NaCl), only drought (40% water holding capacity), only Si (50 mM), salinity + drought (50 mM NaCl + 40% water holding capacity), salinity + Si (50 mM NaCl + 50 mM Si), drought + Si (40% water holding capacity + 50 mM Si), salinity + drought + Si (50 mM NaCl + 40% water holding capacity + 50 mM Si) as salt concentration. Foliar application of Si was done in the evening with 10 mL volume per pot each time. The pots were arranged following the completely randomized design with four replicates.

Growth Parameters

Salinity treatment was continued for 15 days and then data were collected for root and shoot lengths and fresh biomass. For sample collection, wheat plants were cut just above the soil surface and roots were removed from the soil. For dry weights of root and shoot, the samples were kept in drying oven at 75°C till a persistent weight. After that, data were calculated for root shoot ratio and total dry biomass.

Water Relations

To measure the relative water content of plant leaves, fresh and healthy leaves (W_f) (0.5 g) of wheat plants were water rinsed till constant weight and weighed (W_s). The water saturated leaves were then oven dried at 80°C for 24 h to find dry weight (W_d). The formula suggested by (Barrs and Weatherley, 1962) was used to determine RWC as:

$$RWC (\%) = (W_f - W_d) / (W_s - W_d) \times 100$$

Pressure bomb Model 3115 (Soil Moisture Equipment Corp. Santa Barbara, CA, USA) was used to measure the water potential (Ψ_w) of fresh leaves. For osmotic potential (Ψ_s), leaf already used for RWC was frozen and thawed. Sap was squeezed and centrifuged (5000 × g) using an osmometer (Digital Osmometer, Wescor, Logan, UT, USA). The difference of Ψ_w and Ψ_s determined the leaf pressure potential (Ψ_p).

Chlorophyll Contents

Chlorophyll a and b were determined following the method of Arnon (1949). Spectrophotometer (Hitachi-U2001, Tokyo, Japan) was used to record the absorbance of supernatant at 645 and 663 nm wavelengths.

Physiological Attributes

Photosynthetic gas exchange variables such as transpiration rate (E), photosynthetic rate (P_n), and stomatal conductance (g) was measured using Infra-Red Gas Analyzer (IRGA) (Analytical Development Company, Hoddesdon, England). For this purpose, mature fresh leaves from each plant were plucked and placed inside the IRGA. The values of all physiological characteristics were recorded during day time between 10.00 and 12.00 a.m. (Zekri, 1991).

Biochemical Analysis

Five mL of 50 mM phosphate buffer (7.8 pH) was used for extraction of enzymatic antioxidants from material of fresh leaf sample centrifuged at $15000 \times g$ for 20 min. The SOD activity was estimated using the procedure given by Giannopolitis and Ries (1977) at 560 nm and CAT activity was analysed using the procedure given by Chance and Maehly (1955) at 240 nm.

The SOD activity was determined by the prevention of nitroblue tetrazolium (NBT) at 560 nm as result of photochemical reduction. This was further expressed as SOD IU per minute per mg of the protein. The reaction mixture was consisted of 1 mL NBT (50 μ M), 50 μ L enzyme extract, 1 mL riboflavin (1.3 μ M), 500 μ L methionine (13 mM), 500 μ L EDTA (75 mM) and 950 μ L (50 mM) phosphate buffer. The reaction was initiated by holding reaction mixture under illuminations of 30 W fluorescent lamp. After 5 min of lamp turned off, the reaction was stopped. Blue formazane formed by the NBT photo reduction that was further employed to take the absorbance at 560 nm. The same reaction mixture having no enzyme extract in dark was taken as blank. The catalase activity was recorded at 240 nm using a UV-visible spectrophotometer by measuring the change in the absorbance due to H_2O_2 produced as a result of enzyme reaction. Reaction was initiated by adding 100 μ L enzyme extract into the reaction mixture that contained 900 μ L H_2O_2 (5.9 mM) and 2 mL phosphate buffer (50 mM). The catalase activity was described as μ mol of H_2O_2 per minute per mg of protein (Chance and Maehly, 1955). The peroxidase activity was estimated following the procedure of Kara and Mishra (1976). The reaction mixture was composed of 100 μ L enzyme extract, 5 mL pyrogallol (10 mM), 5 mL of H_2O_2 (5 mM) and 5 mL of Tris-HCL buffer (0.1 M). The enzyme activity was measured by recording the decrease in the absorbance at 425 nm that was due to the H_2O_2 dependent oxidation of pyrogallol.

Statistical Analysis

Design of the experiment was completely randomized with three replications. Experimental data was analyzed using Fisher's Analysis of Variance technique and treatment means were compared with Least Significant Difference (LSD) test (Steel *et al.*, 1997) by using STATISTIX 8.1 computer software.

Results

Both salinity and drought stresses caused reduction in growth characteristics of wheat seedlings individually or in combination which was counteracted to some extent by the foliar application of Si. The Si application improved shoot dry weight (9.42%) without any stress. The Si enhanced the plant dry weight by 30.51% against salinity, 13.67% against drought and 34.91% against collectively induced stresses

(salinity and drought) in wheat seedlings as compared to their respective controls (Table 1). Maximum percentage increase of root dry weight of seedlings were 72.19%, followed by 28.45% and 24.5%, when Si was applied to plants subjected to stresses (salinity + drought), salinity and drought, respectively when compared to stress treatment without Si application. Plant shoot length was improved by 21.30%, under salinity stress, 15.35% under drought stress and 50.91% under salinity + drought conditions, as a result of Si supplementation. With the foliar application of Si under salinity, drought and both salinity + drought conditions, root length was also noted to be increased by 56.95, 40.16 and 74.03%, respectively (Table 1). Root: shoot ratio decreased by 1.69% with Si application under saline conditions but increased by 9.67% and 27.41% under drought and both salinity + drought conditions with Si application. Total seedling biomass was noted to increase by 29.78%, 17.94% and 47.42%, with foliar application of Si under salinity, drought and salinity + drought conditions (Table 1).

Abiotic stresses resulted in reduction in water contents, water potential, osmotic potential and turgor potential of wheat seedlings. Foliar application of silicon supported water relations of wheat seedlings under abiotic stresses. Maximum percentage increase of 23.21% was recorded in relative water contents of wheat seedlings subjected to combine stress of salinity + drought, followed by 13.06% under salinity and 12.33% under drought conditions, with foliar applied Si. The Si stabilized water potential of wheat plants as recorded with percent increase of 37.83% in salinity + drought, 32.96% in drought and 16.27% under salinity treatments (Table 2). The percentage increase in osmotic potential under the influence of Si applied was 1.73% in salinity, 10% in drought and 6.89% in salinity + drought. Turgor potential was influenced by Si application to increase by 12.64% under saline conditions, 59.49% under drought and 23.15% under salinity + drought conditions (Table 2).

The Si applied under saline conditions improved Chl *a* by 23.22%, Chl *b* by 51.11%, Chl *a+b* by 30% and Chl *a/b* by 17.94%. The percentage increase was 20.98% in Chl *a*, 43.75% in Chl *b*, 26.19% in Chl *a+b* and 16.23% in Chl *a/b* under drought conditions, supplied with Si. Under saline + drought conditions, Si application improved Chl *a* by 72.58%, Chl *b* by 162.06%, Chl *a + b* by 88.31% and Chl *a/b* by 34.19% (Table 3).

Salt and drought stress caused reduction in *Pn*, *E*, *gs*. The Si ameliorated this adverse effect by improving *Pn* under salt stress (47.58%), drought conditions (50.24%) and salinity + drought stresses (34.18%). The percentage increase in *E* was 60.80% under saline conditions, 67.93% under drought conditions and 20.53% under salinity + drought conditions (Table 4). The *gs* was supported with an increase of 50.17, 61.15 and 96.51% under salinity, drought and salinity+ drought conditions, respectively.

Stress conditions caused an increment in antioxidant enzyme activity, which was further augmented by Si application under stressed conditions.

Table 1: Effect of foliage applied Si on growth characteristics of wheat seedlings experience to combined salinity and drought stresses

	Shoot dry weight (g)	Root dry weight (g)	Shoot length (cm)	Root length (cm)	Root : shoot	Total seedlings biomass (g)
Control	21.86 ± 0.90 b	13.68 ± 0.43 a	43.54 ± 0.73 a	9.06 ± 0.87 a	0.62 ± 0.011 c	35.54 ± 1.31a
Salinity	12.52 ± 0.59 ef	7.50 ± 0.44 ef	28.56 ± 0.62 e	3.81 ± 0.26 d	0.59 ± 0.006 cd	20.01 ± 1.03 cd
Drought	13.67 ± 0.55 de	8.58 ± 0.68 de	31.00 ± 1.54 d	4.73 ± 0.36 cd	0.62 ± 0.026 cd	22.24 ± 1.23 c
Silicon	23.92 ± 0.78 a	13.71 ± 0.60 a	41.58 ± 0.71 a	9.83 ± 0.71 a	0.57 ± 0.033 d	37.62 ± 0.92 a
Salinity + drought	10.97 ± 0.80 f	6.87 ± 0.53 f	24.71 ± 0.58 f	3.62 ± 0.32 d	0.62 ± 0.007 cd	17.84 ± 1.33 d
Salinity + silicon	16.34 ± 0.56 c	9.63 ± 0.47 cd	34.67 ± 0.58 c	5.98 ± 0.35 bc	0.58 ± 0.009 cd	25.97 ± 1.04 b
Drought + silicon	15.54 ± 0.43 cd	10.69 ± 0.56 bc	35.76 ± 0.58 bc	6.63 ± 0.42 b	0.68 ± 0.016 b	26.23 ± 1.00 b
Salinity+ drought + silicon	14.80 ± 0.53 cd	11.83 ± 0.53 b	37.29 ± 0.27 b	6.30 ± 0.64 bc	0.79 ± 0.007 a	26.30 ± 1.07 b
LSD _{≤0.05}	2.02	1.59	2.31	1.59	0.051	3.32
Coefficient of variance	7.15	8.92	3.86	14.64	4.63	7.25
EMS (df=4)	1.33**	0.84**	1.78**	0.83**	0.008**	3.69**

LSD = Least significant difference, EMS = Error mean square, df = degree of freedom, values represent mean ± SE (n = 3). Different small letters indicated that the means are significantly different ($P \leq 0.05$)

Table 2: Effect of foliage applied Si on water relations of wheat seedlings experience to combined salinity and drought stresses

	Relative water Contents (%)	Water potential (-MPa)	Osmotic potential (-MPa)	Turgor potential (MPa)
Control	89.43 ± 2.74 a	0.44 ± 0.029 d	1.98 ± 0.015 a	1.53 ± 0.035 a
Salinity	68.33 ± 2.06 cd	0.86 ± 0.050 b	1.73 ± 0.034 c	0.87 ± 0.079 cd
Drought	66.35 ± 2.65 de	0.91 ± 0.024 b	1.70 ± 0.030 c	0.79 ± 0.048 d
Silicon	90.23 ± 2.96 a	0.32 ± 0.017 d	1.27 ± 0.033 d	0.95 ± 0.044 c
Salinity + drought	58.67 ± 1.89 e	1.11 ± 0.067 a	1.74 ± 0.043 c	0.63 ± 0.060 e
Salinity + silicon	76.76 ± 0.89 b	0.72 ± 0.029 c	1.70 ± 0.026 c	0.98 ± 0.026 c
Drought + silicon	75.02 ± 4.11 bc	0.61 ± 0.017 c	1.87 ± 0.020 b	1.26 ± 0.021 b
Salinity + drought + silicon	72.29 ± 2.35 bcd	0.69 ± 0.095 c	1.86 ± 0.037 b	1.17 ± 0.066 b
LSD _{≤0.05}	7.67	0.144	0.091	0.150
Coefficient of variance	5.95	11.73	3.04	8.49
EMS (df=4)	19.67**	0.006**	0.002**	0.007**

LSD = Least significant difference, EMS = Error mean square, df = degree of freedom, values represent mean ± SE (n = 3). Different small letters indicated that the means are significantly different ($P \leq 0.05$)

Table 3: Effect of foliage applied Si on photosynthetic pigments of wheat seedlings experience to combined salinity and drought stresses

	Chlorophyll a (mg g ⁻¹)	Chlorophyll b (mg g ⁻¹)	Chlorophyll a + b (mg g ⁻¹)	Chlorophyll a/b (mg g ⁻¹)
Control	2.82 ± 0.035 a	0.96 ± 0.011 a	3.78 ± 0.045 a	2.94 ± 0.022 b
Salinity	1.55 ± 0.058 d	0.45 ± 0.017 c	2.00 ± 0.066 d	3.40 ± 0.148 b
Drought	1.62 ± 0.044 d	0.48 ± 0.042 c	2.10 ± 0.012 d	3.45 ± 0.371 b
Silicon	2.77 ± 0.064 a	0.92 ± 0.026 a	3.69 ± 0.062 a	3.02 ± 0.129 b
Salinity + drought	1.24 ± 0.056 e	0.29 ± 0.017 d	1.54 ± 0.038 e	4.24 ± 0.435 a
Salinity + silicon	1.91 ± 0.032 c	0.68 ± 0.017 b	2.60 ± 0.049 c	2.79 ± 0.036 b
Drought + silicon	1.96 ± 0.014 c	0.69 ± 0.050 b	2.65 ± 0.065 c	2.89 ± 0.186 b
Salinity + drought + silicon	2.14 ± 0.026 b	0.76 ± 0.026 b	2.90 ± 0.053 b	2.79 ± 0.062 b
LSD _{≤0.05}	0.131	0.086	0.153	0.66
Coefficient of variance	3.80	7.61	3.33	12.07
EMS (df=4)	0.058**	0.0025**	0.078**	0.148**

LSD = Least significant difference, EMS = Error mean square, df = degree of freedom, values represent mean ± SE (n = 3). Different small letters indicated that the means are significantly different ($P \leq 0.05$)

Foliar application of Si under saline conditions resulted in increased CAT, SOD and POD activity by 98.29, 78.31 and 35.1%, respectively (Table 5). The increased in CAT, SOD and POD under drought conditions was 83.01, 77.16 and 43.88% respectively by the application of Si. The wheat seedlings which experienced salinity + drought conditions with foliar application of Si depicted an increase of 40.04% in CAT, 38.8% in SOD and 9.89% in POD (Table 5).

Discussion

This study indicated that drought and salinity stress severely hampered the root growth of wheat. Drought and salt stresses are known to reduce root growth, shoot growth and biomass of wheat (Hussain *et al.*, 2018). Initially, the root growth is increased due to water stress. However, prolonged drought may reduce the root growth (Lipiec *et al.*, 2013) as was observed in this study.

Table 4: Effect of foliage applied Si on photosynthetic attributes of wheat seedlings experience to combined salinity and drought stresses

	Net photosynthetic rate (<i>Pn</i>) ($\mu\text{mol m}^{-2}\text{s}^{-1}$)	Transpiration rate (<i>E</i>) ($\text{mmol m}^{-2}\text{s}^{-1}$)	Stomatal conductance (<i>gs</i>) ($\text{mmol m}^{-2}\text{s}^{-1}$)
Control	15.38 \pm 0.89 a	7.70 \pm 0.64 a	5.37 \pm 0.41 ab
Salinity	6.20 \pm 0.31 d	3.24 \pm 0.23 d	2.93 \pm 0.29 de
Drought	6.19 \pm 0.36 d	3.93 \pm 0.21 cd	2.78 \pm 0.56 e
Silicon	15.19 \pm 1.44 a	7.43 \pm 0.32 a	5.68 \pm 0.34 a
Salinity + drought	7.84 \pm 0.26 cd	5.21 \pm 0.36 bc	2.01 \pm 0.36 e
Salinity + silicon	9.15 \pm 0.32 bc	5.12 \pm 0.41 bc	4.40 \pm 0.37 bc
Drought + silicon	9.30 \pm 0.19 bc	6.60 \pm 0.79 ab	4.48 \pm 0.20 bc
Salinity +drought + silicon	10.52 \pm 0.42 b	6.28 \pm 0.77 ab	3.95 \pm 0.12 cd
LSD \leq 0.05	1.94	1.52	1.05
Coefficient of variance	11.29	15.50	15.46
EMS (df=4)	1.26**	0.77**	0.37**

LSD = Least significant difference, EMS = Error mean square, df = degree of freedom, values represent mean \pm SE (n = 3). Different small letters indicated that the means are significantly different ($P \leq 0.05$)

Table 5: Effect of foliage applied Si on antioxidants of wheat seedlings experience to combined salinity and drought stresses

	Catalase (unit mg^{-1} of protein)	Superoxide dismutase (unit mg^{-1} of protein)	Peroxidase (unit mg^{-1} of protein)
Control	2.55 \pm 0.28 d	55.20 \pm 6.38 d	1.32 \pm 0.16 c
Salinity	5.88 \pm 0.46 c	148.16 \pm 19.14 c	4.70 \pm 0.31 b
Drought	6.24 \pm 0.41 c	162.64 \pm 13.21 bc	4.99 \pm 0.27 b
Silicon	2.83 \pm 0.62 d	72.28 \pm 3.20 d	1.94 \pm 0.38 c
Salinity + drought	8.94 \pm 0.42 b	193.08 \pm 9.55 b	6.47 \pm 0.35 a
Salinity + silicon	11.66 \pm 0.61 a	264.19 \pm 15.70 a	6.35 \pm 0.34 a
Drought + silicon	11.42 \pm 0.20 a	288.14 \pm 5.86 a	7.18 \pm 0.38 a
Salinity + drought + silicon	12.52 \pm 0.68 a	268.01 \pm 6.16 a	7.11 \pm 0.42 a
LSD \leq 0.05	1.45	32.93	1.00
Coefficient of variance	10.80	10.49	11.56
EMS (df=4)	0.70**	362.1**	0.33**

LSD = Least significant difference, EMS = Error mean square, df = degree of freedom, values represent mean \pm SE (n = 3). Different small letters indicated that the means are significantly different ($P \leq 0.05$)

The root growth response to drought and saline conditions, as an aspect, contributes significantly to drought tolerance (Ali *et al.*, 2016).

However, foliar application of Si significantly enhanced the shoot and root dry weight as well as total seedlings biomass of wheat under both the stresses whether exposed alone or in combination. Our results showed that foliar spray Si may be a schematic approach to enhance the crop growth cultivated under drought or saline soils. Likewise, as Si can enhance drought tolerance potential of plants, its application may alleviate the water requirement that sequentially would relieve the salinization of cultivated soil (Guntzer *et al.*, 2012; Van Bockhaven *et al.*, 2013) by reducing the use of irrigational water. Si utilization enhances the growth and also keeps the plant leaves and stem rigid that reduces the self-shading and ultimately improves the *Pn* (Salman *et al.*, 2012) under the abiotic stresses. Indirect beneficial effects of Si application include low *E* and more shoot and root growth (Kamenidou *et al.*, 2009) as was observed in this study.

In this study, a decline in relative water content and water, osmotic and turgor potentials of wheat seedlings was detected under drought/salinity stresses. Under abiotic stresses like salinity, wheat seedlings absorb less water (Heidari *et al.*, 2011; Naeem *et al.*, 2015). Low plant uptake of water might also be due to the fact that high salt

concentration has loosened the sap flux flow that decreased the root hydraulic conductivity and relative water content (Hussain *et al.*, 2018). Si foliar spray lessened salt and water stress by lowering the *E*. Lobato *et al.* (2009) documented that high concentration of Si promoted the water retention of leaf tissue. These outcomes recommend that Si application may be suitable to increase the drought and salinity tolerance by improving the water relation of the crop plants. The improved water retention ability of Si-treated plants can be attributed to the decreased rate, enhanced tissue water status (Gao *et al.*, 2006), and the development of binary layer of silica-cuticle on leaves that reduce the stomatal opening and improves water potential in leaves under Si application (Hattori *et al.*, 2005). The Si also induces the modifications in lignin and suberin processing and displacement that decrease the loss of water and evapotranspiration rate under stressed conditions (Sonobe *et al.*, 2009; Amin *et al.*, 2016). It has also been documented that Si strengthens the lignification in sorghum that induced the increased xylem resilience to water loss (Sivakumar *et al.*, 2000; Hattori *et al.*, 2005).

The leaf gas exchange parameters were negatively influenced by drought and salt stress alone or in combination. However, Si application decreased the disintegration of photosynthetic pigments. Si application minimized the reduction of leaf chlorophyll contents. In this study, high

concentration of chlorophyll *a* content may have caused significant increase in the net photosynthetic rate as also previously documented for wheat (Ahmad and Haddad, 2011) which improved the stay green of wheat under drought and salinity stress. In another study, Mateos-Naranjo *et al.* (2013) found that detrimental effects of salinity stress on pigment concentrations (Chl*a* and Chl*b*) and PSII productivity were minimized by Si application for a halophytic grass *Spartina densiflora*.

Both salinity and drought stresses considerably *gs* and *Pn* and *E* but foliar application of Si improved these traits. It has been argued that, under stress conditions such as salinity and drought, CO₂ limitation following stomatal closure inhibits photosynthetic biochemical reactions (Gong and Chen, 2012; Hajiboland, 2014). Recent research work has shown that Si influences the photosynthesis by promoting the water uptake and transport. Gong *et al.* (2005) stated that Si could enhance the photosynthesis of wheat plants under drought and this fact can be linked with enhanced functions of photosynthetic enzymes and increased chlorophyll content. Outcomes of *E* and *gs* are in accordance with Gong *et al.* (2005) who documented that application of Si promoted the *E* and *gs* of wheat plants grown under drought stress. Sonobe *et al.* (2009) perceived that Si implementation resulted in amplified *gs* and *E* and also remediated the water uptake reduction in hydroponic sorghum subjected to polyethylene glycol water stress. The increased *gs* and *E* were due to the fact that Si stimulated the improvement in hydraulic conductance in the leaves. Increased level of CO₂ might be due to regulation of stomatal conductance in reaction to Si application.

In the present study, the activity of CAT, SOD and POD in wheat was increased in the leaves under salinity and/or drought, while such intensification was more significant and consistent in Si treatment than in other treatments. Gong *et al.* (2005) observed that under drought stress the foliar spray of Si surged the antioxidant activity in wheat. During metabolic processes plants release H₂O₂ that adversely damages the cell oxidation function, while CAT can remove H₂O₂ and play a crucial role in stress tolerance. It has been suggested that Si can boost the antioxidant defense mechanism and as a result diminish oxidative stress in plants under stressed environments (Gong *et al.*, 2005, 2008).

Conclusion

The Si application decrease damages to wheat seedlings under salt and/or drought stress probably via improving water relations, chlorophyll contents and antioxidant activity, which resulted in better growth attributes. Wheat seedlings were found to have higher activities of SOD, CAT and POD activities, more water relations attributes and chlorophyll contents during the salt and/or drought-stress period when treated with Si. Thus, our findings suggest that seedlings treated with Si are an effective strategy that can be used to enhance salt and drought tolerance of wheat crop.

References

- Ahmad, S.T. and R. Haddad, 2011. Study of silicon effects on antioxidant enzyme activities and osmotic adjustment of wheat under drought stress. *Czech J. Genet. Plant*, 47: 17–27
- Ahmed, I.M., H.X. Dai, W. Zheng, F.B. Cao, G.P. Zhang, D.F. Sun and F.B. Wu, 2013a. Genotypic differences in physiological characteristics in the tolerance to drought and salinity combined stress between Tibetan wild and cultivated barley. *Plant Physiol. Biochem.*, 63: 49–60
- Ahmed, I.M., F. Cao, M. Zhang, X. Chen, G. Zhang and F. Wu, 2013b. Difference in yield and physiological features in response to drought and salinity combined stress during anthesis in Tibetan wild and cultivated barleys. *PLoS One*, 8: 77–86
- Ali, M.L., J. Luetchens, A. Singh, T.M. Shaver, G.R. Kruger and A.J. Lorenz, 2016. Greenhouse screening of maize genotypes for deep root mass and related root traits and their association with grain yield under water-deficit conditions in the field. *Euphytica*, 207: 79–94
- Ali, Q., Z.A. Zahir, H.N. Asghar and A. Jamil, 2017. Inoculation with rhizobial consortium for improving the growth, yield and quality of maize under salt-stressed conditions. *Pak. J. Agric. Sci.*, 54: 97–105
- Amin, M., R. Ahmad, A. Ali, I. Hussain, R. Mahmood and M. Aslam, 2016. Influence of silicon fertilization on maize performance under limited water supply. *Silicon*, 10: 177–183
- Apel, K. and R. Hirt, 2004. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annu. Rev. Plant Biol.*, 55: 373–99
- Arnon, D.T., 1949. Copper enzyme in isolated chloroplasts polyphenoloxidase in *Beta vulgaris*. *Plant Physiol.*, 24: 1–15
- Barrs, H.D. and P.E. Weatherley, 1962. A re-examination of the relative turgidity technique for estimating water deficits in leaves. *Aust. J. Biol. Sci.*, 15: 413–428
- Chance, M. and A.C. Maehly, 1955. Assay of catalases and peroxidases, *Methods Enzymol.*, 2: 764
- Donaldson, E., 1996. Crop traits for water stress tolerance. *Amer. J. Altern. Agric.*, 11: 83–93
- Farooq, S., M. Hussain, K. Jabran, W. Hassan, M.S. Rizwan and T.A. Yasir, 2017. Osmopriming with CaCl₂ improves wheat (*Triticum aestivum* L.) production under water-limited environments. *Environ. Sci. Pollut. Res.*, 24: 13638–13649
- Gao, X., C. Zou, L. Wang and F. Zhang, 2006. Silicon decreases transpiration rate and conductance from stomata of maize plants. *J. Plant Nutr.*, 29: 1637–1647
- Giannopolitis, C.N. and S.K. Ries, 1977. Superoxide dismutase. I. Occurrence in higher plants, *Plant Physiol.*, 59: 309–314
- Gong, H.J. and K.M. Chen, 2012. The regulatory role of silicon on water relations, photosynthetic gas exchange and carboxylation activities of wheat leaves in field drought conditions. *Acta Physiol. Plant.*, 34: 1589–1594
- Gong, H.J., K.M. Chen, Z.G. Zhao, G.C. Chen and W.J. Zhou, 2008. Effects of silicon on defense of wheat against oxidative stress under drought at different developmental stages. *Biol. Plant*, 52: 592–596
- Gong, H.J., X.Y. Zhu, K.M. Chen, S.M. Wang and C.L. Zhang, 2005. Silicon alleviates oxidative damage of wheat plants in pots under drought. *Plant Sci.*, 169: 313–321
- Guntzer, F., C. Keller and J.D. Meunier, 2012. Benefits of plant silicon for crops: a review. *Agron. Sustain. Dev.*, 32: 201–213
- Hajiboland, R., 2014. Reactive oxygen species and photosynthesis. In: *Oxidative Damage to Plants, Antioxidant Networks and Signaling*, pp: 1–63 Ahmad, P. (ed.). Academic Press, USA
- Hattori, A., S. Inanaga, H. Araki, P. An, S. Mortia and M. Luxova, 2005. Application of silicon enhanced drought tolerance in *Sorghum bicolor*. *J. Plant Physiol.*, 123: 459–466
- Heidari, A., M. Toorchi, A. Bandehagh and M.R. Shakiba, 2011. Effect of NaCl stress on growth, water relations, organic and inorganic osmolytes accumulation in Sunflower (*Helianthus annuus* L.). *Lines Univ. J. Environ. Res. Technol.*, 1: 351–362
- Hsiao, T.C., 1973. Plant responses to water stress. *Ann. Rev. Plant Physiol.*, 24: 519–570

- Hussain, M., S. Ahmad, S. Hussain, R. Lal, S. Ul-Allah and A. Nawaz, 2018. Rice in saline soils: physiology, biochemistry, genetics, and management. *Adv. Agron.*, 148: 231–287
- Kamenidou, S., T.J. Cavins and S. Marek, 2009. Evaluation of silicon as a nutritional supplement for greenhouse *Zinnia* production. *Sci. Hortic.*, 119: 297–301
- Kara, M. and D. Mishra, 1976. Catalase, peroxidase, polyphenoloxidase activities during since leaf senescence. *Plant Physiol.*, 54: 315–319
- Lee, S.K., E.Y. Sohn, M. Hamayun, J.Y. Yoon and I.J. Lee, 2010. Effect of silicon on growth and salinity stress of soybean plant grown under hydroponic system. *Agrofor. Syst.*, 80: 333–340
- Levy, D., W.K. Coleman and R.E. Veilleux, 2013. Adaptation of potato to water shortage: irrigation management and enhancement of tolerance to drought and salinity. *Amer. J. Potat. Res.*, 90: 1–21
- Liang, Y., Q. Shen, A. Zhang and Z. Shen, 1999. Effect of calcium and silicon on growth of and nutrient uptake by wheat exposed to simulated acid rain. *Yingyong Shengtai Xuebao*, 10: 589–592
- Liang, Y., Q. Chen, Q. Liu, W. Zhang and R. Ding, 2003. Exogenous silicon (Si) increases antioxidant enzyme activity and reduces lipid peroxidation in roots of salt stressed barley (*Hordeum vulgare* L.). *J. Plant Physiol.*, 160: 1157–1164
- Liang, Y., J. Zhu, Z. Li, G. Chu, Y. Ding, J. Zhang and W. Sun, 2008. Role of silicon in enhancing resistance to freezing stress in two contrasting winter wheat cultivars. *Environ. Exp. Bot.*, 64: 286–294
- Lipiec, J., C. Doussan, A. Nosalewicz and K. Kondracka, 2013. Effect of drought and heat stresses on plant growth and yield: a review. *Int. Agrophys.*, 27: 463–477
- Lobato, A.K.S., G.K. Coimbra, M.A.M. Neto, R.C.L. Costa, F.B.G. Santos, C.F. Oliveira, L.M. Luz, A.G.T. Barreto, B.W.F. Pereira, G.A.R. Alves, B.S. Monterio and C.A. Marochio, 2009. Protective action of silicon on water relation and photosynthetic pigments in pepper plants induced to water deficit. *Res. J. Biol. Sci.*, 4: 617–623
- Mateos-Naranjo, E., L. Andrades-Moreno and A.J. Davy, 2013. Silicon alleviates deleterious effects of high salinity on the halophytic grass *Spartina densiflora*. *Plant Physiol. Biochem.*, 63: 115–121
- Naeem, M.K., M. Ahmad, M. Kamran, M.K.N. Shah and M.S. Iqbal, 2015. Physiological responses of wheat (*Triticum aestivum* L.) to drought stress. *Int. J. Plant Soil Sci.*, 6: 1–9
- Noman, A., Q. Ali, F. Naheed, M. Rizwan, S. Ali and K. Irshad, 2015. Foliar application of ascorbate enhances the physiological and biochemical attributes of maize (*Zea mays* L.) cultivars under drought stress. *Arch. Agron. Soil Sci.*, 6: 1659–1672
- Ouzounidou, G., I.F. Ilias, A. Giannakoula and I. Theocharidou, 2014. Effect of water stress and NaCl triggered changes on yield, physiology, biochemistry of broad bean (*Vicia faba* L.) plants and on quality of harvested pods. *Biologia*, 69: 1010–1017
- Ouzounidou, G., A. Giannakoula, I. Ilias and P. Zamanidis, 2016. Alleviation of drought and salinity stresses on growth, physiology, biochemistry and quality of two *Cucumis sativus* L. cultivars by Si application. *Braz. J. Bot.*, 39: 531–539
- Ozkur, O., F. Ozdemir, M. Bor and I. Turkan, 2009. Physiochemical and antioxidant responses of the perennial xerophyte *Capparis ovata* Desf. to drought. *Environ. Exp. Bot.*, 66: 487–492
- Romero-Aranda, M.R., O. Jurado and J. Cuartero, 2006. Silicon alleviates the deleterious salt effect on tomato plant growth by improving plant water status. *J. Plant Physiol.*, 163: 847–855
- Sairam, R.K. and A. Tyagi, 2004. Physiology and molecular biology of salinity stress tolerance in plants. *Curr. Sci.*, 86: 407–421
- Salman, D., S. Morteza, Z. Dariush, G.M. Abbas, Y. Reza, G.D. Ehsan and N.L. Reiza, 2012. Application of nitrogen and silicon rates on morphological and chemical lodging related characteristics in rice (*Oryza sativa* L.) at north of Iran. *J. Agric. Sci.*, 6: 12–18
- Sattar, A., M.A. Cheema, H. Ali, A. Sher, M. Ijaz, M. Hussain, W. Hassan and T. Abbas, 2016. Silicon mediates the changes in water relations, photosynthetic pigments, enzymatic antioxidants activity and nutrient uptake in maize seedling under salt stress. *Grassl. Sci.*, 62: 262–269
- Sattar, A., M.A. Cheema, T. Abbas, A. Sher, M. Ijaz and M. Hussain, 2017. Separate and combined effects of silicon and selenium on salt tolerance of wheat plants. *Russ. J. Plant Physiol.*, 64: 341–348
- Seckin, B., I. Turkan, A.H. Sekmen and C. Ozfidan, 2010. The role of antioxidant defense systems at differential salt tolerance of *Hordeum marinum* Huds. (seabardley grass) and *Hordeum vulgare* L. (cultivated barley). *Environ. Exp. Bot.*, 69: 76–85
- Sivakumar, P., P. Sharmila and P.P. Saradhi, 2000. Proline alleviates salt stress induced enhancement in ribulose-1-5-bisphosphate oxygenase activity. *Biochem. Biophys. Res. Commun.*, 279: 512–515
- Sonobe, K., T. Hattori, P. An, W. Tsuji, E. Eneji and K. Tanaka, 2009. Diurnal variations in photosynthesis, stomatal conductance and leaf water relation in Sorghum grown with or without silicon under water stress. *J. Plant Nutr.*, 32: 433–442
- Steel, R.G.D., J.H. Torrie and D.A. Dickey, 1997. *Principles and Procedures of Statistics: A Biometric Approach*, 3rd edition. McGraw Hill Book Co. Inc., New York, USA
- Taiz, L. and E. Zeiger, 2006. *Plant Physiology*, 4th edition. Sinauer Associates, Sunderland, Van Bockhaven, M.A., J.D. De Vleeschauwer and M. Hofte, 2013. Towardsestablishing broad-spectrum disease resistance in plants: silicon leads the way. *J. Exp. Bot.*, 64: 1281–1293
- Wu, D., S. Cai, M. Chen, L. Ye, Z. Chen, H. Zhang, F. Dai, F.B. Wu and G.P. Zhang, 2013. Tissue metabolic responses to salt stress in wild and cultivated barley. *PLoS One*, 8: e55431
- Yang, F., X. Xu, X. Xiao and C. Li, 2009. *Responses to Drought Stress in Two Poplar Species Originating from Water & Nitrogen Use Efficiency in Plants and Crops*. Marston, Lincolnshire, UK
- Yousfi, S., M.D. Serret, J.L. Araus, X. Draye, J. Foulkes, M. Hawkesford and E. Murchie, 2010. A comparative effect of salinity and drought on growth, ion concentration and $\delta^{13}C$ and $\delta^{15}N$ in barley. *Assoc. Appl. Biol.*, 105: 73–81
- Zekri, M., 1991. Effects of NaCl on growth and physiology of sour orange and Cleopatra mandarin seedlings. *Sci. Hortic.*, 47: 305–315

(Received 19 March 2018; Accept 25 June 2018)