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IMPROVING DROUGHT TOLERANCE POTENTIAL IN WHEAT (*TRITICUM AESTIVUM* L.) THROUGH EXOGENOUS SILICON SUPPLY

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Abstract

Silicon (Si) an essential plant constituent or metabolite is involved in enhancing metabolic, physiological and structural stability in plants. However, its role under biotic and abiotic stress conditions is still unclear and need to be explored. A study was designed to identify the effective methods of Si application for improving the drought tolerance potential of wheat. Various methods (seed priming, fertigation and foliar spray) of applying Si were evaluated in two local spring wheat genotypes grown in plastic pots under normal and water stress conditions. Water stress caused a marked decrease in water relations and uptake of phosphorous, potassium, magnesium and zinc in plants. The Si application significantly enhanced the plants ability to withstand water deficit conditions through increased Si uptake and improved activity of ascorbate peroxidase (APX), peroxidase (POD) and catalase (CAT). Among Si supply methods, foliar spray was more effective in alleviating the adverse effects of drought. Further to this, results suggested that fertilizer Si should be foliarly applied at tillering than anthesis stage for maintenance of turgor and better accumulation of nutrients in both normal and water stressed wheat plants.

Key words: Silicon, Antioxidant system, Nutrients, Water stress, Wheat.

Introduction

Drought stress is one of the most important limiting factors to agricultural productivity primarily in arid and semi-arid regions of the world (Waraich *et al*., 2011). Water stress negatively influences crop growth and development through changes in various physiological and biochemical processes that ultimately decrease crop yields (Nawaz *et al.,* 2012; Sikuku *et al*., 2012). It results in oxidative stress leading to changed carbon and nitrogen metabolic activities, disturbed water relations and photosynthetic activity in plants (Tawfik, 2008).

Stage specific water conditions are of prime importance for a good crop performance. Drought stress at anthesis stage lessens the pollination leading to fewer grains per spike and ultimately decreases the grain yield (Nawaz *et al*., 2012). Adequate water supply at or after anthesis stage not only increases photosynthetic rate but also increases grain filling period (Zhang *et al*., 1998), which consequently increases final grain yield (Inoue *et al*., 2004). Literature indicated significant effect of water stress on the grain weight of triticale and wheat (Royo *et al*., 2000; Nayyar & Walia, 2004; Nawaz *et al*., 2012) due to early senescence.

Numerous nutrients have been identified to act as stress ameliorants such as salicylic acid (Waseem *et al*., 2006), selenium (Nawaz *et al.,* 2013), nitrogen (Gevrek & Atasoy, 2012, Saifullah *et al*., 2014), phosphorous (Kaya *et al*., 2001), potassium (Raza *et al*., 2012) and zinc (Weisany *et al*., 2012). Silicon (Si) has been reported to be effective in alleviating the adverse effects of various edaphic and epiedaphic stresses such as drought (Hattori *et al*., 2005; Sacala, 2009), water logging (Sacala, 2009), heavy metal toxicities (Gunes *et al*., 2008; Sarwar *et al.,* 2010), soil salinity (Romero-Aranda *et al*., 2006), chilling or frost and heating (Savant *et al*., 1999; Ma *et al*., 2004). It accumulates in leaves that increase vigor and stiffness of cell wall resulting in drop of transpiration from cuticle and uptake and translocation of lethal salts and metal ions from roots to shoots (Gao *et al.*, 2006). Moreover, it reduces lethal effects of reactive oxygen species (ROS) through activation of antioxidant system in plants (Liang *et al*., 2007; Gunes *et al*., 2008).

The fertilization of crop plants with Si to increase crop productivity under unfavorable conditions has been well reported (Scala, 2009; Ashraf *et al*., 2010; Sarwar *et al.*, 2010). However, most of these studies involved either treatment of seeds with Si or its application in soil or as a foliar spray and did not evaluate the comparative efficiency of these methods under water deficit conditions. The present study was therefore carried out with the hypothesis that exogenous Si supply attenuates the damaging effects of water stress in wheat aiming to identify the most effective time and method of Si application for improving the drought tolerance potential of wheat.

Materials and Methods

Experiment layout and plant material: The research activities were performed at the experimental area, Department of Crop Physiology, University of Agriculture, Faisalabad (Pakistan). The seeds of two local wheat genotypes, viz. Chakwal-50 and Sehar-06, identified as drought tolerant and sensitive respectively, in our earlier experiments (Bukhari, 2014) were sown in plastic pots (25 cm diameter × 22 cm length) filled with 7 kg soil which was air dried, ground, sieved and well mixed before the start of the experiment to avoid any plant residues. Recommended doses of N, P and K in the form of urea (110 kg N ha^{-1}), diammonium phosphate (70 kg $P_2 0_5 ha^{-1}$) and potassium sulphate (50 kg SOP ha^{-1}) were mixed in the soil at the time of soil preparation. Seeds of genotypes were sterilized in 5% sodiumhypochlorite solution for 5 minutes and rinsed thrice with distilled water. Sodium meta-silicate ($Na_2SiO_3.5H_2O$) obtained from Sigma-Aldrich (USA) was used as a source of Si for designated treatments. For seed treatment, healthy, vigorous seeds were soaked in 3 mM Si aerated solution of $Na_2SiO_3.5H_2O$ for 12 hours at room temperature (25°C) and were later shade dried before sowing. The solution for foliar application was prepared by dissolving 0.85 g (4 mM) Si in one litre of distilled water. On the basis of soil weight fertigation treatment was applied by adding 1mM $Na_2SiO_3.5H_2O$ solution per pot. Si treatments viz., fertigation and foliarly supply were repeated once at both the growth stages (tillering and anthesis). All the pots were kept at their respective field capacity levels (100% and 60% FC) from the beginnings and maintained on daily weight basis as described by Ahmad *et al*. (2007). All the pots were protected from rain by manually operated shelter equipped with movable sheet of transparent flexible plastic sheet. After one week of emergence, the plants were thinned out and five healthy plants were kept in each pot. The plants were grown up to maturity and the data on various physiological, biochemical and plants nutrients analyses were recorded as follow:

Leaf water relations: The second leaf from top (fully expanded youngest leaf) of plants from each pot was used to determine leaf water potential ($\Psi_{w)}$. The measurements were made between 8.00 to 10.00 a.m. with Scholander type pressure chamber (ARIMAD-2, ELE- International).

For leaf osmotic potential (Ψ_s) measurements, the same leavesused for water potential measurements, were frozen at -20°C. The frozen leaf material was thawed and cell sap was extracted while crushing the leaves with a glass rod. The sap was directly used for the determination of Ψ_s using an Osmometer (Wescor-5520).

Turgor potential (Ψ_p) was calculated as the difference between Ψ_w and $\Psi_s$values.

$$(\Psi_p) = (\Psi_w) - (\Psi_s)$$

Leaf relative water contents (RWC) were measured following the method of Cornic (1994). Three flag leaves from each treatment were used to record fresh weight (FW) using a digital electrical balance (Chyo, MK-500C) and were later dipped in test tubes containing distilled water for 24 hours. Afterwards leaves were taken out, wiped with the tissue paper and the turgid weight (TW) was recorded. The samples were dried at 65°C for 72 h and dry weight (DW) of each sample was recorded. The following formula was used to calculate RWC:

$$RWC = [(FW-DW) / (TW-DW)] \times 100$$

Determination of antioxidants activity: The activities of peroxidase (POX), catalase (CAT), and ascorbate peroxidase (APX) were determined spectrophotometrically (Hitachi-2800). Leaves were homogenized in a medium composed of 50 m*M* phosphate buffer with 7.0 pH and 1 m*M* dithiothreitol (DTT) as described by Dixit *et al*. (2001).

Catalase activity (CAT) was assayed by measuring the conversion rate of hydrogen peroxide to water and oxygen molecules, following the method described by Chance and Maehly (1955). The activity was assayed in 3 mL reaction solution comprising of 50 m*M* phosphate buffer with 7.0 pH, containing 5.9 m*M* of H_2O_2 and 0.1 mL enzyme extract. The catalase activity was determined by decline in absorbance at 240 nm after every 20 s due to consumption of H_2O_2. Absorbance change of 0.01 unit min^{-1} was defined as one unit catalase activity.

The activity of peroxidase (POX) was determined by measuring peroxidation of H_2O_2 with guaiacol as an electron donor (Chance and Maehly, 1955). The reaction solution for POD consists of 50 m*M* phosphate buffer with pH 5, 20 m*M* of guaiacol, 40 m*M* of H_2O_2 and 0.1 mL enzyme extract. The increase in the absorbance due to the formation of tetraguaiacol at 470 nm was assayed after every 20 sec. One unit of the enzyme was considered as the amount of the enzyme that was responsible for the increase in OD value of 0.01 in 1 min. The enzyme activity was determined and expressed as unit min^{-1} g^{-1}FW basis.

Ascorbate peroxidase (APX) activity was measured by monitoring the decrease in absorbance of ascorbic acid at 290 nm (extinction coefficient 2.8 *mM* cm^{-1}) in a 1 mL reaction mixture containing 50 m*M*phosphate buffer (pH 7.6), 0.1 m*M* Na-EDTA, 12 m*M* H_2O_2, 0.25 *mM* ascorbic acid and the sample extract as described by Cakmak (1994).

Nutrients analyses: Dried ground material (0.5 g) was taken in digestion tubes and 5 mL of concentrated H_2SO_4 were added to each tube (Wolf, 1982). The tubes were kept overnight at room temperature. Then 0.5 mL of H_2O_2 (35%) poured down the sides of the digestion tube, ported the tubes in a digestion block and heated at 350°C until fumes were produced. They were further heated for another 30 minutes. The digestion tubes were removed from the block, cooled and 0.5 mL of H_2O_2 was slowly added and the tubes were placed back into the digestion block. The above step was repeated till the cooled digested material became colorless. The volume of the extract was maintained up to 50 mL in volumetric flasks. The extract was filtered and used for determination of silicon (Si), phosphorous (P), potassium (K), zinc (Zn)and magnesium (Mg) contents.

Estimation of P and K: Phosphorus (P) and potassium (K) were determined by a spectrophotometer (Hitachi-200, Japan) and flame photometer (Jenway PFP 7, UK), respectively (Jackson, 1962).

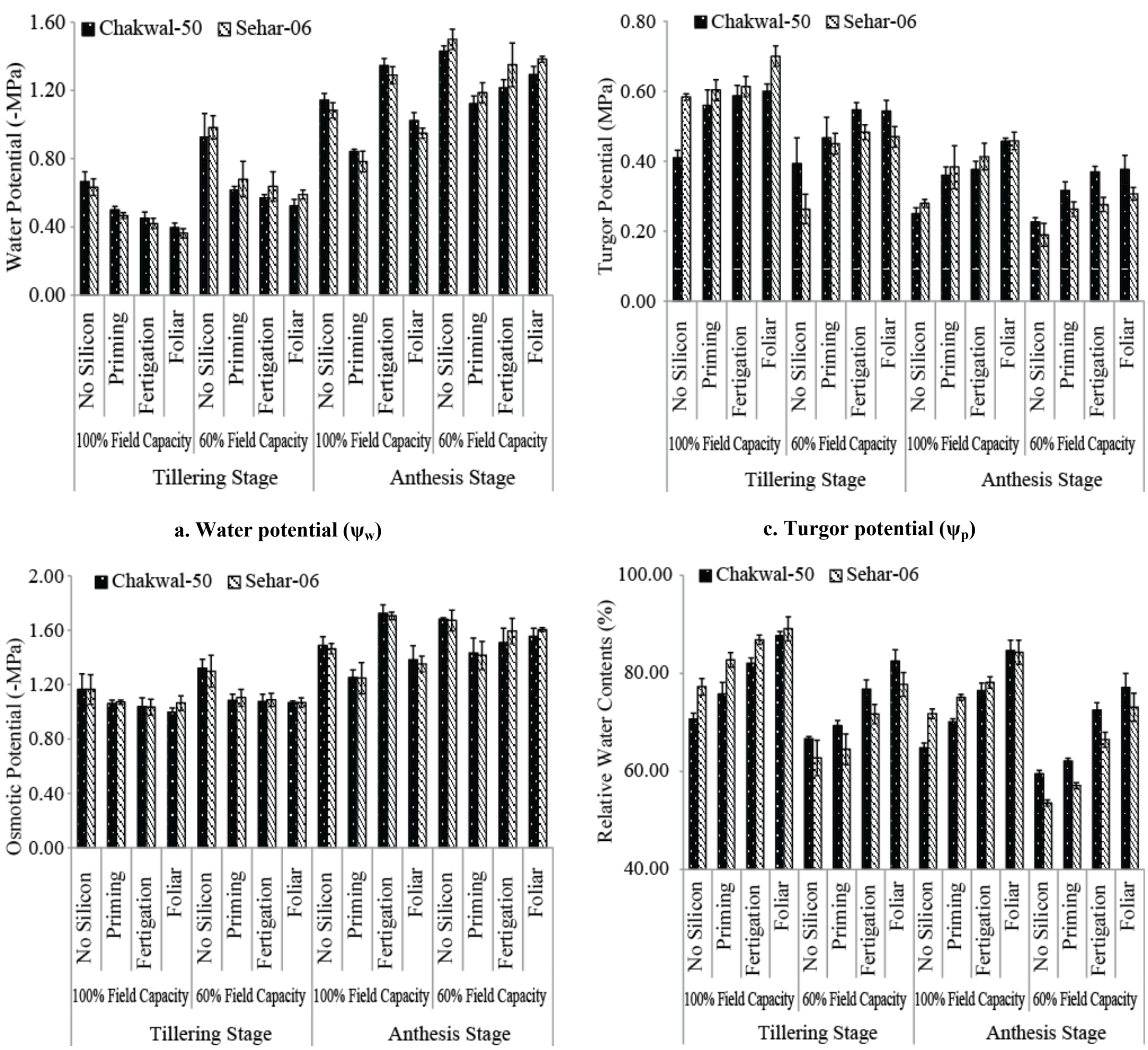
Estimation of Si, Zn and Mg: Digested samples were tested for Si, Mg, and Zn by ICP-OES (Optima-2100 DV Perkin-Elmer).

Statistical analyses: The data recorded were analyzed statistically using analysis of variance technique using MSTAT-C. Least Significant Difference (LSD) test at 5% probability level was used to compare the significant means.

Results

Water relations: Drought stress significantly reduced the water relations in wheat as compared to normal conditions, however, exogenous Si supply was found to be effective in maintenance of turgidity. Wheat plants showed maximum values of water relations at tillering than anthesis stage. The foliar application of Si at tillering gave the highest values for Ψ_w (-0.38 MPa), Ψ_s (-1.03 MPa) and, Ψ_p (0.65 MPa) potentials and RWC (88.40%) under normal conditions. Similarly, Si foliarly sprayed stressed plants exhibited higher values for these variables (-0.55 MPa), (-1.06MPa), (0.50 MPa) and (80.13%), respectively compared to other soil and seed based Si application. Among the wheat genotypes, Chakwal-50 showed maximum values for water relations as compared to Sehar-06 under water stress conditions (Fig. 1).

Antioxidant enzymes: The activity of enzymes was increased under drought stress as compared to normal wheat plants however, the application of Si found to be effective in enhancing the enzymes activities. Plants of both wheat genotypes exhibited highest enzymes activity at anthesis than tillering stage. Drought stressed plants of genotype Chakwal-50 showed the maximum activity of ascorbate peroxidase (3.96 ABA digested g^{-1} FW h^{-1}), peroxidase (835.34 Units min^{-1} g^{-1} FW) and catalase (472.92 Units min^{-1} g^{-1} FW) with foliarly applied Si at anthesis stage whereas, genotype Sehar-06plants exhibited the minimum values for ascorbate peroxidase (0.50 ABA digested g^{-1} FW h^{-1}), peroxidase (364.28 Units min^{-1} g^{-1} FW) and catalase (336.64 Units min^{-1} g^{-1} FW) enzymes with no Si supply at tillering stage under drought stress conditions(Fig. 2).



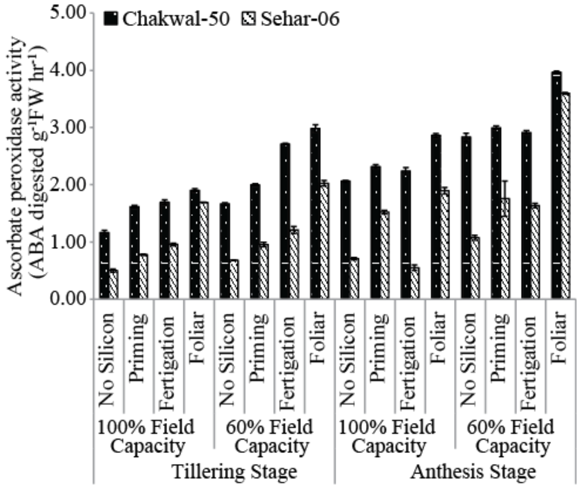
a. Water potential (ψ_w)

c. Turgor potential (ψ_p)

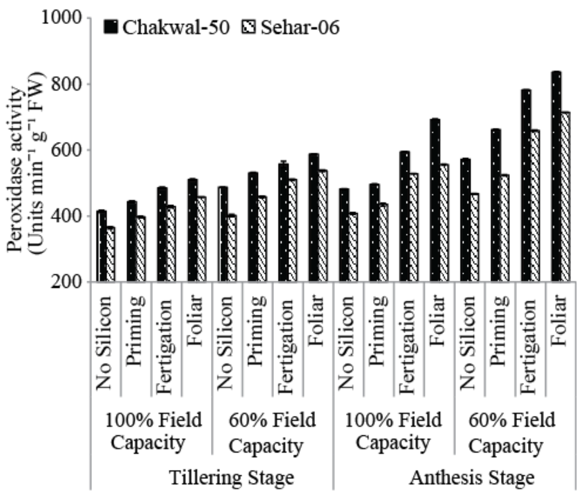
b. Osmotic potential (ψ_s)

d. Relative water contents (RWC)

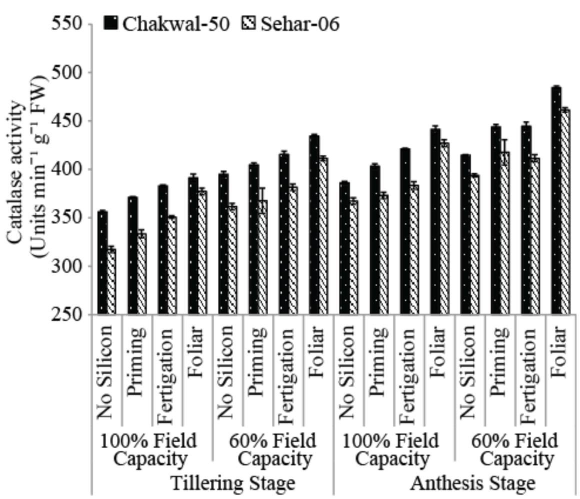
Fig. 1. Effect of silicon applied methods on leaf water relations of two wheat genotypes at tillering and anthesis stages under normal (100% field capacity) and water deficit (60% field capacity) conditions (The values are means of three replicates ± standard error (SE) compared at $p \leq 0.05$)



a. Ascorbate peroxidase (APX)



b. Peroxidase activity (POD)



c. Catalase activity (CAT)

Fig. 2. Effect of silicon applied methods on antioxidant enzymes of two wheat genotypes at tillering and anthesis stages under normal (100% field capacity) and water deficit (60% field capacity) conditions (The values are means of three replicates ± standard error (SE) compared at $p \leq 0.05$).

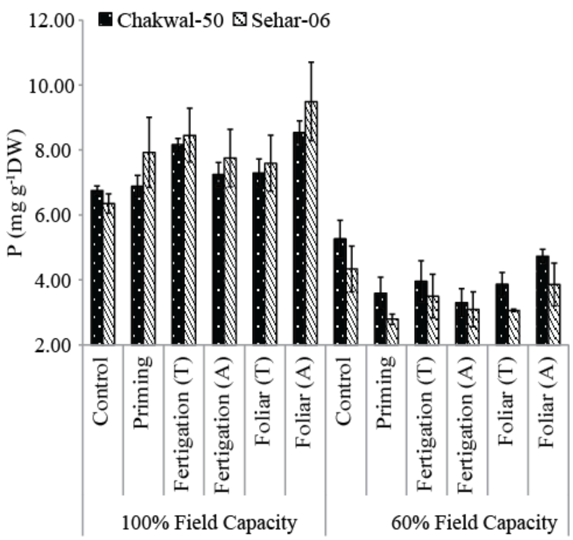
Nutrients: Drought stress significantly lessens the plant nutrients uptake than normal conditions. However, the exogenous Si supply at anthesis stage gave the highest values in accumulating plant nutrients under normal conditions. Control plants of drought stress treatment exhibited the maximum values for P (4.80 mg g^{-1} DW), K (13.28 mg g^{-1} DW), Mg (2.0 mg g^{-1} DW) and Zn (0.03 mg g^{-1} DW) contents than those treated with Si(3.20 mg g^{-1} DW) and (8.94 mg g^{-1} DW) obtained from seed priming,(1.72 mg g^{-1} DW) and (0.01mg g^{-1} DW)gained from foliar spray at tillering stage, respectively (Figs. 3a-d). Plants of both wheat genotypes improved their Si contents under both normal and drought stress conditions with the exogenous Si supply at both the stages. The highest leaf Si (19.96 mg g^{-1} DW) contents were observed in genotype Chakwal-50 with foliarly sprayed Si at anthesis stage under normal conditions, while, the lowest (5.74 mg g^{-1} DW) were seen in drought stressed plants of genotype Sehar-06, which received no Si (Fig. 3).

Discussion

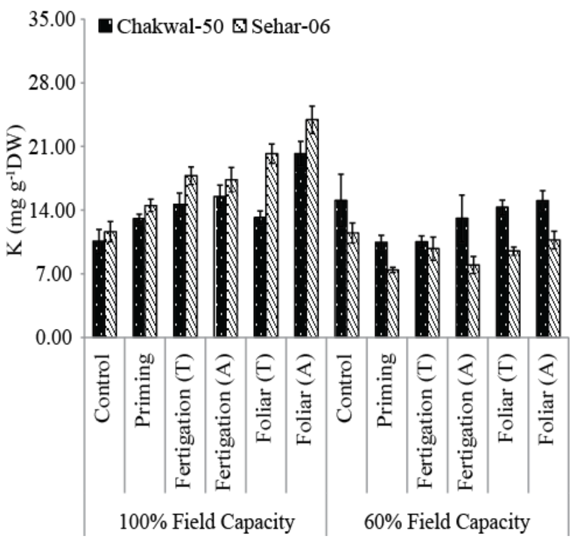
The results of our study indicate that drought markedly declined water relation parameters and nutrients uptake whereas, production of antioxidants enzymes increased. The drought induced reduction in water relations is due to onset of osmotic stress as secondary stress (Ashraf & Khan, 1993; Akram 2011; Nawaz *et al*., 2010, 2012). Decrease in nutrient uptake could be due to drop in transpiration rate, active transport and membrane permeability observed by Alam (1999). However, drought induced increase in antioxidant enzymes might be due to the signaling of ROS or oxidative homeostasis (Agarie *et al*., 1998; Apel & Hirt, 2004; Suzuki & Mittler, 2006).

In present study under stress conditions from vegetative to reproductive stages water relations attributes decreased while nutrients accumulation and antioxidants enzymes were increased. This decline in water relations from tillering to anthesis stage could be due to the accumulation of more osmolytesat anthesis stages (Madan *et al*., 1995; Sairam & Srivastava, 2002). Wheat plants showed the increasing behavior in antioxidant enzymesat later stage that might be due to more production of ROS which enhance the capability of cell antioxidant defense mechanisms (Almeselmani *et al*., 2006) and nutrients uptake can be due to their chemical, biochemical and structural functions in plant metabolism (Taiz & Zeiger, 2006).

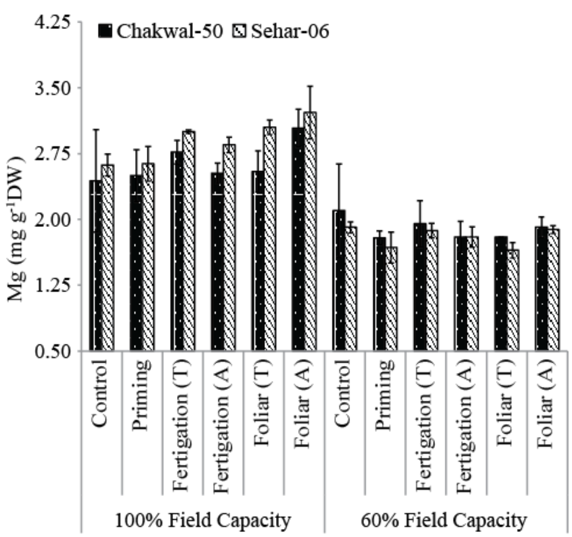
Our results showed that under water deficit stress genotype Chakwal-50 exhibited higher values for water relations, nutrients uptake and antioxidant enzymes as compared to genotype Sehar-06.Chakwal-50 responded Si comparatively better than Sehar-06. This might be due to the variation in the plant genetic sensitivity to drought stress (Ashraf & Khan, 1993; Glombitza *et al*., 2004; Brisson & Casals, 2005).



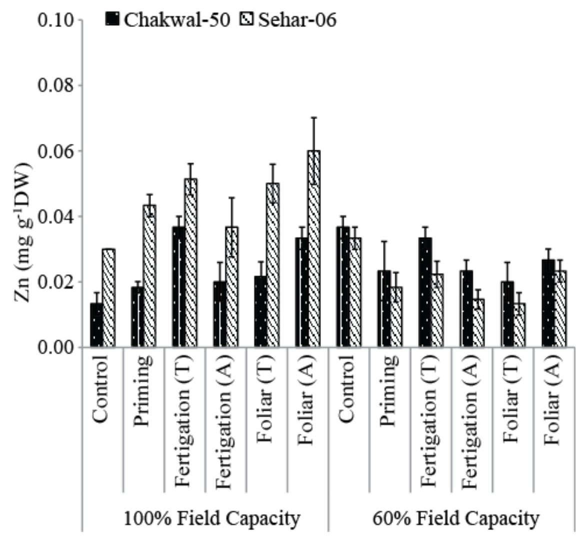
a. Leaf phosphorous (P) contents



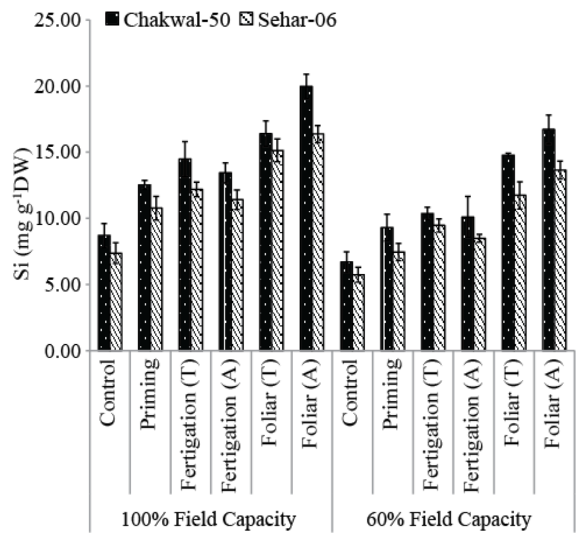
b. Leaf potassium (K) contents



c. Leaf magnesium (Mg) contents



d. Leaf zinc (Zn) contents



e. Leaf silicon (Si) contents

Fig. 3. Effect of silicon applied methods on leaf nutrients uptake of two wheat genotypes at tillering and anthesis stages under normal (100% field capacity) and water deficit (60% field capacity) conditions (The values are means of three replicates ± standard error (SE) compared at $p \leq 0.05$).

Foliar application proved to be the best method that could be attributed to readily availability to the mesophyll tissues where it is actually required. Our findings are in line with Matoh *et al*. (1991) who reported that exogenous Si application in water stressed rice plants improved ψ_w due to deposition of Si as silica cuticle double layer on leaf epidermal tissues. Romero-Aranda *et al*. (2006) reported that application of Si to tomato plants under salinity improved ψ_p by 42% than those without Si applied through the accumulation of osmolytes. Our results exhibited that due to foliar spray of Si Ψ_p of wheat plants increased under stress that may be attributed to accumulation of solutes so the water moves into the cell from surroundings, ultimately plants maintain their turgor and carry on their metabolic activities (Subbarao *et al*., 2000). Similarly, improvement in drought tolerance by Si supply may be associated with enhancement of more water uptake from the soil solution (Hattori *et al*., 2005 and 2007).

In the present study results showed that with the foliar spray of Si activities of enzymes (APX, POD and CAT) enhanced under drought at anthesis stages. Ahmad and Haddad (2011) reported that application of Si (2*mM* of sodium silicate kg^{-1} soil) under drought stress (applied by gypsum block, -1.0 MPa) significantly increased the activities of SOD, CAT, APX and POD enzymes in wheat. Additionally, Si supply through soil (0.1167 g m^{-2} as sodium silicate) enhanced the activity of POD enzyme in wheat plants at booting stage as compared to grain filling stage under drought (Gong *et al*., 2008). Moreover, Ma *et al*. (2004) reported that under drought exogenous Si supply (120 and 240 mg kg^{-1} of soil as SiO_2) improved the activity of CAT but did not affect the POD activity and suggested that Si improved the drought tolerance, primarily by taking part in the plants metabolism. The increase in the enzyme activity under Si treatment coincided with a variable increase in the individual isoform expression. In the present study, higher activity of APX, POD and CAT by Si application under water limited conditions might be due to the decomposition of POD into H_2O_2 by the oxidation of phenolic compounds.

The results of the present study revealed that leaf P, K, Mg, Zn and Si contents of both wheat genotypes were improved under normal condition plants by the application of Si than stressed plants. Under water deficit conditions, decrease in uptake of these minerals was more pronounced as compared to control plants of water stress. Under water stress, application of Si by different means improved the leaf Si contents than those plants which received no Si (Fig. 3-e). Uptake of Si was decreased considerably as soil water contents gradually decrease (Ahmad *et al*., 2007; Crusciol *et al*., 2009). Ali *et al*. (2009) reported that silicon application at 150 mg/kg of soil significantly increased Si content in wheat flag leaf under both saline and non-saline conditions as compared to Si absence plants. Ma and Takahashi (2002) noted higher phosphorous uptake in leaves of rice plants with the application of Si, which directly associated with the improved yield and growth of normal water plants. Similarly, Singh *et al*. (2002) reported that the application of Si (180 kg ha^{-1}) enhanced phosphorus in to well water rice straws and grains. Soratto *et al*. (2012) reported that foliarly applied Si (0.8% soluble Si) to wheat flag leaves enhanced the K^+, Si and Mg^{2+} (Mg^{2+} from 1.5 to 4.0 g kg^{-1}) contents under normal conditions as compared to droughted plant leaves. Results of our study are in agreement with Chen *et al*. (2011) who reported that application of Si under water deficit stress improved the leaf Si contents in rice but reduced K, Mg, Ca, Na and Fe contents. Similar, findings were observed by Pei *et al*. (2010), they reported that wheat plants treated with Si under PEG-6000 induced osmotic stress were reduced the concentration of Ca, Mg and K ions as compared to control plants of water stress treatment. Chen *et al*. (2011) found that applying 1.5 *mM* silicon to drought-stressed rice reduced leaf tissue concentrations of K, Na, Ca, Mg, and Fe. Hafeez *et al*. (2013) macronutrient cations such as Ca, Mg and K inhibit the absorption of Zn by plants in a solution culture study. Epstein (1994) reported that Si application to the plants remedied a problem of Zn deficiency. The results potentially suggest that these ions did not contribute to osmotic adjustment under the water stress used in this study but it needs further investigation to explore the mechanism of different mineral nutrients and their interaction in the presence of Si (Gao *et al*., 2004; Pei *et al*., 2010; Chen *et al*., 2011).

Conclusion

The results of the present study conclude that drought stress significantly reduced the water relations and nutrient uptake by both wheat genotypes. Under drought stress genotype Chakwal-50 performed better than Sehar-06. Supplemental Si was found to be effective in maintenance of turgor and activity of antioxidant system under water deficit conditions. The uptake of nutrients increased by Si application under normal conditions, however, nutrients uptake decreased under limited water supply. Present study shows that the exogenous Si supply as foliar spray was the effective one at tillering stage.

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