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Si INDUCED STRESS TOLERANCE IN WHEAT (*TRITICUM AESTIVUM* L.) HYDROPONICALLY GROWN UNDER WATER DEFICIT CONDITIONS

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Abstract

ALI, A., M. TAHIR M. AMIN, S. M. A. BASRA, M. MAQBOOL and DONG JIN LEE, 2013. Si induced stress tolerance in wheat (*Triticum aestivum* L.) hydroponically grown under water deficit conditions. *Bulg. J. Agric. Sci.,* 19: 951-957

Present water scarcity is an emerging issue and cause of deterioration in quality and productivity of crops to reduce crop yield all over the world. Silicon is known to be better against the deleterious effects of drought on plant growth and development. The current study was conducted to test the ameliorative effect of silicon (Si) on a wheat; under water stress in rain protected net house conditions. Initially the nursery was raised in prewashed sand and then transplanted in pots each containing Johnson's nutrient solution as a medium of plant growth. There were four treatments: Well watered (WW), Drought at -0.6 Mpa (DD), Well watered + Si @ 150 mgL^{-1} (WW+Si) and Drought at -0.6 Mpa + Si (DD+Si). The experiment was laid out in CRD with 5 replications. Silicon applied wheat plants depicted marked enhancement in root shoot fresh and dry weights in comparison to plants grown without Si. More over, they maintained higher water status with increased leaf water potential and relative water contents and higher chlorophyll contents. It was concluded that Si is beneficial to improve the growth of wheat via change in the physiological and biochemical traits.

Key words: salt tolerance, Na-silicate, wheat

Introduction

Wheat is an important major food crop, but its production per unit area is lower is due to imbalance mineral nutrition, sowing time, water shortage and poor seed quality etc. Balance nutrition plays a pivotal role to improve thee crop growth and yield. Along with essential nutrients, the application of some beneficial elements enhances the yield of crop. Silicon also eliminates certain mineral imbalances and certain diseases caused by abiotic stresses (Epstein, 1994).

Silicon is the second most abundant element in earth crust (Marschner, 1995). Silicon creates a positive effect on plants especially under various stresses either biotic or abiotic (Liang et al., 2005; Marschner, 1995, Hattori et al., 2005). Silicon becomes very important in stresses due to its beneficiary effects as compared to the normal condition (Ma, 2004). Silicon is deposited in the xylem vessels and prevents collapse of the vessels under high transpiration due to drought or heat stress and results in increase water use efficiency of plant (Zou et al., 2005). Silicon increases the number and mass grain production of wheat by stimulating shoot and root biomasses (Filho et al., 2005) ultimately enhances the wheat growth and yield especially when applied in water-stressed conditions (Gong et al., 2003). Silicon application enhance dry matter yield of salt sensitive and salt tolerant genotypes of barley under stressful conditions created by salt application (Takashi, 1995). Silicon also improves chlorophyll contents and yield in salt stressed conditions (Amador et al., 2007, Ali et al., 2011). The possible mechanisms responsible to increase drought tolerance in plants are, by maintaining plant water balance, photosynthetic efficiency, and erectness of leaves and structure of xylem vessels under high transpiration rates due to higher temperature and moisture stress (Hattori et al.,

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2005). Silicon enhances water economy and crop growth in moisture stress (Kaya et al., 2006; Gong et al., 2005).

Although, silicon found to be an agronomically important fertilizer element but little work has been done on silicon applications in spring wheat germplasm of Pakistan. Therefore, the present work was aimed to test its efficacy on wheat in the presence and absence of water stress.

Materials and Methods

The experiment was carried out in the 1st week of November 2006. Response of wheat to silicon application under moisture stress was studied by inducing drought condition in solution culture through poly ethylene glycol (PEG 8000); the study was conducted in rain protected net house, while analytical work was carried out in the laboratories of departments of crop physiology, Faculty of Agriculture, University of Agriculture Faisalabad, during the year 2007. Average temperatures in the net house were 20±8°C during the day and 12±6°C at nighttime during the experimental period. The relative humidity remained between 45% (midday) to 87% (midnight). Light intensity ranged between 340 and 1300 µmol photon m^{-2} s^{-1} depending upon the day and cloud conditions. The experimental design was completely randomized design with five repeats of each treatment. Seeds of wheat were collected from Ayub Agricultural Research Institute, Faisalabad (AARI). Seeds of the wheat genotype were germinated in polyethylene lined iron trays containing pre-washed sand. Distilled water was applied to maintain moisture content optimum for seed germination and seedling establishment. Two-week-old seedlings of uniform size were transplanted into foam-plugged holes of thermopal sheet floating on distilled water containing Hoagland's Nutrient Solution. The detail of treatments used were T1 = Well watered (No stress), T2 = Well watered + Si @ 150ppm, T3 = Drought at -0.6 Mpa PEG and T4 = Drought at -0.6 Mpa PEG + Si @ 150ppm.

Polyethylene glycol (PEG 8000) 0 and 19g were dissolved in 100ml of half strength Hoagland's nutrient solution separately to create control and water stress treatment (-0.6Mpa). Silicon was added as sodium silicate ($Na_2SiO_3.H_2O$).

Various observations were recorded as given below:

Morphology and Biomass production

Plants were harvested to record the biomass data 45 days after transplanting. The harvested fresh samples were washed thoroughly and separated into roots and shoots. Root and shoot lengths and fresh weights were measured immediately, while to record dry weights, the samples were oven-dried at 70°C till a constant. Root shoot ratio was taken as ratio between root and shoot dry weights.

Determination of Si from flag leaf

The leaves of harvested plants were oven dried and grinded in a Wiley mill built-in with stainless steel chamber into fine powder. The grinded samples (0.5g) were digested in 2 mL 50% hydrogen peroxide (H_2O_2) and 4.5 g 50% NaOH in open vessels (Teflon beakers) on a hot plate at 150ºC for 4 hours. Si concentration was measured using calorimetric amino molybdate blue color method (Elliot and Synder, 1991). To 1mL of supernatant filtrate liquid, 10 mL of ammonium moblybdate (54g L^{-1}) solution and 25 mL of 20% acetic acid was added in 50 mL of polypropylene volumetric flask. After five minutes, 5 mL of 20% tartaric acid and 1 mL of reducing solution was added in flask and volume was made with 20% citric acid. After 30 minutes, the absorbance was measured at 650 nm wavelengths with a UV visible spectrophotometer (Shimdzu, Spectronic 100, Japan). The reducing agent was prepared by dissolving 0.5 g 1 amino-2-naphthol-4-sulfonic acid, 1 g Na_2SO_3 and 30 g $NaHSO_3$ in 200 mL water (Elliott and Synder, 1991).

Chlorophyll contents

The chlorophylls *a* and *b* were determined according to the method of Arnon (1949). Fresh leaves (0.2 g) were cut and extracted overnight with 80% acetone at 0-4°C. The extracts were centrifuged at 10 000 x *g* for 5 min. Absorbance of the supernatant was read at 645, 663 and 480 nm using a spectrophotometer (Hitachi-U2001, Tokyo, Japan).

The chlorophylls *a* and *b* were calculated by the following formulae:

Chl a = [12.7 (OD 663) -2.69 (OD 645)] x V/1000 x W

Chl b = [22.9 (OD 645) -4.68 (OD 663)] x V/1000 x W

V = volume of the extract (mL)

W = weight of the fresh leaf tissue (g)

a and b were added to get total chlorophyll content

Relative water content, %:

For relative water content (%), a sample consisting of 5 flag leaves was taken from each pot. Fresh weight of each sample was measured. Leaves were soaked in distilled water for 14-16 hours. After soaking period, the leaves were wiped with tissue paper and soaked weight was measured. Afterwards, samples were oven dried at 80ºC to determine dry weight for each sample. For each pot, relative water content was calculated by using the formula given below proposed by Turner (1986).

$$\text{RWC} = \frac{\text{Fresh weight - Dry weight}}{\text{Soaked weight - Dry weight}}$$

Water Potential (MPa)

A fully expanded flag leaf was excised at booting stage to determine the leaf water potential. Leaf water potential was measured with water potential apparatus (Chas W. Cook & Sons. Birmingham B 42, ITT England)) following the method described by Scholander et al. (1964). A single leaf (flag leaf) was sealed in the pressure chamber with the cut surface protruding out of the hole. Pressure was applied to the leaf from a cylinder of compressed gas until xylem sap appeared at the cut surface. This balancing pressure was regarded as the tension originally existing in the xylem sap and approximately equal to water potential of the cells. Sampling was done between 6.00 and 8.00 A.M. to avoid evaporation losses. The leaves were placed in the pressure chamber as quickly as possible and measurements made on three leaves from control and stressed treatments separately.

Data analysis

Analysis of variance of the data for each attribute was computed using the MSTAT-C computer programme (MSTAT Development Team, 1989), and data collected were analyzed according to standard statistical procedure of ANOVA and Least Significance Difference (LSD) test was applied to treatment means at 5 % probability (Steel et al., 1997) to observe the response of wheat to silicon application under moister stress.

Results

Data (Table 1) revealed that shoot fresh weight was increased greatly where silicon was applied in well watered condition T_2 (17.69 g) which was about 9% more as compared to where silicon was not applied T_1 (16.30 g). Least shoot fresh weight was observed in drought without silicon T_3 (8.53 g) that showed 48% decreased shoot fresh weight when compared with well watered condition T_1. This decreased weight of shoot was ameliorated about 17% when silicon was applied in drought condition T_4 (9.95 g) as compared to drought. The results showed about 2 times more effect of silicon in drought in comparison to the well watered. Data regarding root fresh weight also showed significant results. Silicon in well watered condition showed maximum root fresh weight T_2 (9.18 g) and it promoted the root fresh weight up to 10% as compared to only well watered where silicon was not applied T_1 (8.35 g). Root fresh was greatly decreased i.e. 48% in drought condition (4.33 g) while it was increased up to 24% in case when silicon was applied to drought condition (5.34 g). In both the cases, either it was well watered condition or drought silicon application significantly increased the root fresh weight of the plants but in drought its effect was more than 2 folds higher as compared to well watered. Maximum shoot dry weight was recorded in well watered due to silicon application and it promoted shoot dry weight up to 4% T_2 (1.168 g) as compared to where silicon was not applied T_1 (1.124 g). A decrease of 48% was recorded due to drought condition T_3 (0.580 g) but shoot dry weight was increased up to 14% when silicon applied in drought T_4 (0.660 g). The increase in shoot dry weight with silicon application in drought was about 3 times higher than well watered. Comparative study of mean values showed that all the treatment means differ significantly in affecting the root dry weight. Maximum root dry weight was recorded under silicon application in well watered condition in T_2 (1.202 g) that showed 9% increase in root dry weight as compare to well watered condition without silicon application T_1 (1.100 g). The least root dry weight was reported in drought without silicon T_3 (0.544 g) while application of silicon to drought condition enhanced root dry weight up to 31% in T_4 (0.706 g) when compared with drought. Effect of silicon in drought condition was about 3 folds higher as compared with the well watered. Comparison of mean values revealed that treatments means were significantly different from one another. Maximum of root shoot ratio were reported where silicon was applied either in well watered T_2 (1.028) or drought condition T_4 (1.040). Least root shoot ratio was recorded where silicon was not applied in drought condition T_3 (0.938) while application of silicon ameliorated it and root shoot ratio was increased where silicon was applied in drought T_4 (0.51). The root shoot ratio decreased about 4% by creating drought but with silicon application in drought root shoot ratio enhanced 11%, which was more than doubled where silicon was applied in well-watered (5%).

Comparison of means (Table 2) showed that addition of silicon greatly influenced the relative water contents and varied from 46.68% to 86.65%. Maximum relative water contents were reported in well watered condition along with silicon T_2 (86.65%), it showed an improvement of 8% relative water contents with respect to the well watered without silicon application T_1 (80.59%) and it decreased where drought condition was provided in T_3 (46.68%). Addition of silicon to drought condition increased relative water contents up to 16% T_4 (54.18%). Silicon doubled the relative water contents when applied in drought as compared with the well-watered. Comparison of mean values for chlorophyll contents showed significant differences among the treatment means that varied from 1.234 mg dm^{-2} to 1.592 mg dm^{-2}. Maximum chlorophyll contents were recorded where silicon was applied in well-watered condition in T2 (1.592 mg dm^{-2}) as compared to well-watered condition without silicon applicationT1 (1.472 mg dm-^{2}). Application of silicon increased chlorophyll content up to 8% in well watered. In drought conditions chlorophyll contents decreased to minimum (1.234 mg dm^{-2}) and it showed a 13% increase when silicon was applied to drought condition i.e. (1.386 mg dm^{-2}). Comparative study of mean

Table 1
Effect of Si on morphology of wheat both under drought and well-watered conditions at $p \leq 0.05$
(The values are means of three replicates)

Treatments	Shoot length, cm		Root length, cm		Shoot fresh weight, g		Root fresh weight, g		Shoot dry weight, g		Root dry weight, g		Root shoot ratio	
	Means	% change	Means	% change	Means	% change	Means	% change	Means	% change	Means	% change	Means	% change
T_1 = Well watered	80.59 b	0.00	43.35 b	0.00	16.30 b	0.00	8.354 b	0.00	1.124 b	0.00	1.100 b	0.00	0.976 b	0.00
T_2 = Well watered + Silicon	86.65 a	7.52	47.96 a	10.63	17.69 a	8.52	9.180 a	9.89	1.168 a	3.91	1.202 a	9.28	1.028 a	5.16
T_3 = Drought	46.68 d	-42.08	24.52 d	-43.45	8.530 d	-47.67	4.330 d	-48.17	0.580 d	-48.39	0.544 d	-50.54	0.938 c	-4.14
T_4 = Drought + Silicon	54.18 c	-32.78	29.73 c	-31.43	9.952 c	-38.95	5.348 c	-35.99	0.660 c	-41.28	0.706 c	-35.81	1.040 a	6.52

Table 2
Effect of Si on plant water relations, chlorophyll contents, Si concentration and dry matter yield of wheat both under drought and well-watered conditions at $p \leq 0.05$ (The values are means of three replicates)

Treatments	Relative water contents		Chlorophyll contents, $\mu g\ g^{-1}$ of Fresh weight		Water potential, MPa		Shoot Si, mgg^{-1}		Root Si, mgg^{-1}		Total dry matter, g/plant	
	Means	% change	Means	% change	Means	% change	Means	% change	Means	% change	Means	% change
T_1 = Well watered	80.59 b	0.00	1.47 b	0.00	-0.618 b	0.00	1.146 b	0.00	1.164 c	0.00	2.224 b	0.00
T_2 = Well watered + Silicon	86.65 a	7.52	1.59 a	8.15	-0.534 a	-13.59	4.620 a	303.14	4.770 a	309.79	2.370 a	6.56
T_3 = Drought	46.68 d	-42.08	1.23 d	-16.16	-1.254 d	102.91	0.986 b	-13.96	0.980 d	-15.81	1.124 d	-49.46
T_4 = Drought + Silicon	54.18 c	-32.78	1.38 c	-5.84	-1.058 c	71.19	4.470 a	290.05	4.342 b	273.02	1.366 c	-38.58

values of leaf water potential revealed significant differences among the treatments means that varied from -1.254 Mpa to -0.534 Mpa. Maximum leaf water potential was recorded in well-watered condition with silicon application T_2 (-0.534 Mpa) as compared to well-watered condition T_1 (-0.618 Mpa) where silicon was not applied. In drought conditions minimum leaf water potential was recorded T_3 (-1.254 Mpa) but when silicon was applied to drought condition it increased the leaf water potential up to 15% i.e. T_4 (-1.058 Mpa).

Discussion

Drought is one of the major abiotic factors that limit agricultural crop production (Nemeht et al., 2002; Lea et al., 2004 and Ramachandra et al., 2004). There are several approaches to counteract the effect of water stress together with silicon application. The current work shows that silicon is deposited in shoot as well as root of wheat plants. In the present study, the increase in silicon in the leaf and root was observed where silicon was applied in either well watered or drought condition indicating the ability of wheat to uptake the silicon. The results were in accordance with the findings of Korndorfer et al. (1999), Gali and Smith (1992) and Heine et al. (2005). High root silicification is associated with higher drought resistance (Lux et al., 2002) resultantly promoting the crop growth (Filho et al., 2005; Matichenkov and Calvert, 2002; Gong et al., 2003; Rodrigues et al., 2001; Ahmed et al., 1992). Therefore it is obvious in present work that a positive correlation exists between Si uptake and dry matter produced by plants (Table 1, Figure 1) and the effect of silicon was about 2 to 3 times more in drought in comparison to the well watered as obtained by Marshner et al. (1990) in tomato and cucumber.

In the presented work, in both either well watered condition or drought, silicon application significantly increased the root fresh and dry weights of the plants but in drought its effect was more than 2-3 folds higher as compared to well watered. Filho et al. (2005) reported that root growth is also improved by silicon application. Similar trends were also observed by (Dakora and Nelwamondo, 2003; Liang et al., 1996). However, the findings of Ahmed et al. (1992) was in contradiction to the present results as he reported that root dry weight was not affected by silicon application. It is clear from current study that root shoot ratio was smoothed by silicon application as depicted from the findings of Hattorie et al. (2005) who suggested that silicon supplied sorghum had a lower shoot root ratio that indicated the facilitation of root growth over shoot growth.

The growth depends upon the water content retained by the plants under stressful conditions that could be improved by silicon application by Epstein (1999). The current research indicated that addition of silicon greatly influenced the relative water contents under normal and water-stressed conditions that varied from 46.68% to 86.65%. Therefore, a positive correlation was observed between Si uptake and relative water content of plants under both conditions (Figure 2). The decrease in relative water contents of plant due to induction of drought condition is also similar to the results of Lawlor and Cornic (2002) and Gong et al. (2003) advocated an increase in water status of plant by silicon application. The water status of plants can also be measured in terms of water potential. It is significantly affected by the decrease in water supply in root zone (Molnar et al., 2002). The decreased leaf water potential can be enhanced via silicon application (Matoh et al., 1991 and Agarie et al., 1998; Gong et al., 2003). According to the recent work, applied silicon to drought condition increased the leaf water potential up to 15%. Therefore, it is obvious that a positive correlation exists between Si uptake and Water potential of plants under drought conditions (Figure 3).

Any change in the chlorophyll content affects the photosynthesis, which in turn influences the crop growth. The de-

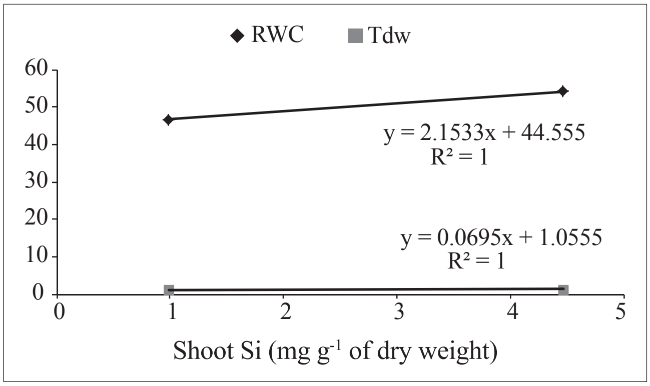

Fig. 1. Correlation between Si, Relative Water content and total dry weight of wheat under drought conditions

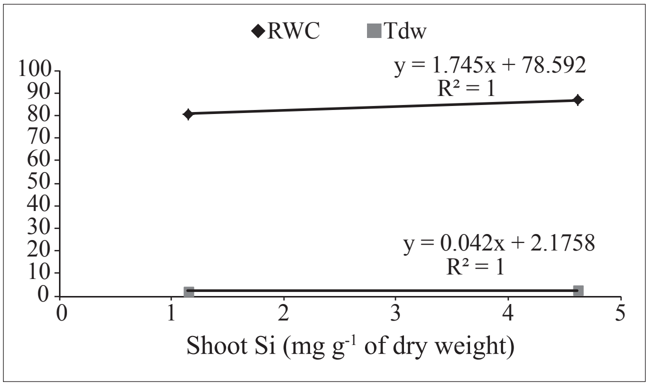

Fig. 2. Correlation between Si, Relative Water content and total dry weight of wheat under well watered conditions

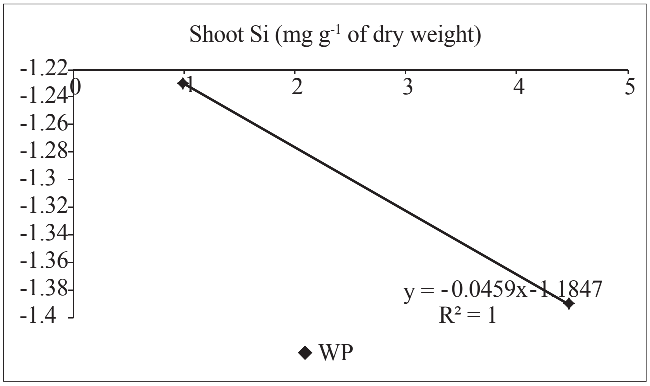

Fig. 3. Correlation between Si and water potential of wheat under drought conditions

crease in chlorophyll content owing to water stress can be enhanced by silicon application (Reynolds et al., 2005; Ladjal et al., 2000; Amador et al., 2005; Al-agharaby et al., 2004; Tamai 2008; Yang 2007; Ali et al., 2012). The current findings showed a 13% increase when silicon was applied under drought condition.

Conclusion

It was concluded from current discussion that the use of Si is beneficial to mitigate the water stress. The possible mechanism involved in the enhancement of growth was the increased water status of plants which in turn increased the chlorophyll contents that was responsible to expedite the photosynthetic process ultimately improving crop growth and yield.

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