Improvement Growth, and Yield of Wheat Plants Grown Under Salinity Stress by Using Silicon

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Abstract: The present study aims to improvement of wheat production under saline conditions using silicon (Si) treatment. Salinity caused significant reduction in the growth parameters, photosynthetic pigments and yield components of wheat plants. The magnitude of reduction increased by increasing salinity level. Significant increases were observed in activities of superoxide dismutase (SOD), peroxidase (POX), catalase (CAT) and glutathione reductase (GR) in shoots of salt stressed plants. Silicon treatment in absence and presence of NaCl had great changes on most of the assayed parameters. The adverse effects of salinity as regards the growth characters, photosynthetic pigments and yield components were significantly mitigated by Si supplement. Application of Si caused great variations in the activities of antioxidant enzymes. Under normal (Non-saline) condition, addition of Si, especially at 1 mM, markedly increased the activity of both SOD and CAT, however activity of both POX and GR was significantly decreased. Addition of Si markedly reduced the increases in the activities of SOD, POX, CAT and GR were observed in salt stressed plants. Great variations were also observed as regards the contents of endogenous phytohormones in response to Si, NaCl and their interactions. Generally, it could be concluded that Si have (to more extent) a beneficial regulatory role in plants grown under salt stress conditions.

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1. Introduction

Salinity stress negatively impacts agricultural yield throughout the world affecting production whether it is for subsistence or economic gain. (Yokoi et al, 2002). In Egypt, salinity is one of a major environmental problems which affects agricultural land. Salt accumulation in soils from natural processes and irrigation adversely affects seed germination, seedling growth as well as related metabolic processes of plants. . Soil salinity designates a condition in which the soluble salt content of the soil reaches a level harmful to crops through the reduced osmotic potential of the soil solution and the toxicity of specific ions. These soluble salts may be from those present in the original soil profile or transported to the profile by irrigation water containing an unusual high concentration (Ates and Tekeli, 2007).

Silicon is the second most abundant element both on the surface of the Earth's crust and in the soils. It has been proved to be beneficial for the healthy growth and development of many plant species, particularly graminaceous plants such as rice and sugarcane and some cyperaceous plants (Liang *et al.*, 2005). The beneficial effects of Silicon are particularly distinct in plants exposed to abiotic and biotic stresses (Ma, 2004). Although silicon (Si) is the second most abundant element both on the surface of the Earth's crust and in soils, it has not yet

been listed among the essential elements for higher plants. However, the beneficial role of Si in stimulating the growth and development of many plant species has been generally recognized. Silicon is known to effectively mitigate various abiotic stresses such as manganese, aluminum and heavy metal toxicities, and salinity, drought, chilling and freezing stresses. However, mechanisms of Si-mediated alleviation of abiotic stresses remain poorly understood (Liang et al., 2007). This study was designed to investigate the effect of Si, NaCl and their interactions on growth, yield and some metabolic activities including certain antioxidant enzymes hopping to elucidate the role of Si in alleviating salt stress on wheat plants.

2. Materials and Methods

This study was carried out in Botanical farm, Fac. of Sci., Al-Azhar Univ. Cairo, Egypt. The grains of wheat plant (*Triticum aestivum* var. Giza 168) were obtained from Agricultural Research Centre, Ministry of Agriculture, Giza, Egypt. A pot experiment was designed as follows:

A homogenous wheat grains were sown in pots (30 cm in diameter) containing 7.0 K.g. of clay soil. Si was applied to the soil at rates of 1 and 2 mM from potassium silicate (K_2SiO_3) and incorporated into the soil (Two liter for each pot) before salinity treatments. The pots were divided into nine groups

representing the following treatments, control, 1mM K₂SiO₃, 2mM K₂SiO₃, 50mM NaCl, 100mM NaCl, 50mM NaCl + 1mM K₂SiO₃, 50mM NaCl + 2mM K₂SiO₃, 100mM NaCl + 1mM K₂SiO₃ and 100mM NaCl + 2mM K₂SiO₃ A homogenous 10 wheat grains were sown in each pot The first three groups irrigated with tab water, the second three groups irrigated with saline water (50mM NaCl) and the later three groups irrigated with saline water (100mM NaCl). After 15 days from sowing, the five uniform seedlings were left in each pot. Two plants from each pot were used for biochemical analysis (100 day after sowing) and the remaining three plants were left to grow for studying the effect of different treatments on the yield. Yield components of the different treatments were recorded after 135 days from sowing. In addition, soluble carbohydrate and soluble protein contents were analyzed in the harvested grains.

Phytochemical contents

Photosynthetic pigments were estimated using the method of Vernon and Selly (1966). Contents of soluble carbohydrates were measured according to the method of Umbriet *et al.* (1969). Contents of soluble proteins were estimated according to the methods of Lowery *et al.* (1951).

Assay of enzymes activities

Protein enzymes were extracted according to the method of Mu Kherjee and Choudhuri, (1983). Super oxide dismutase (SOD) activity was measured according to the method of Dhindsa *et al.* (1981). Peroxidase (POX) activity was assayed using the method of Bergmeyer (1974). Catalase (CAT) activity was assayed according to the method of Chen *et al.* (2000). Glutathione reductase (GR) activity was assayed according to the method of Karni *et al.*(1984).

Endogenous phytohormones

Levels of endogenous gibberellic acid (GA₃), indole acetic acid (IAA), abscisic acid (ABA) and cytokinin (CK) were determined for all treatments and the controls using HPLC according to the method of Lee *et al.* (1989).

Statistical Analysis

All the obtained results were statistically analyzed using least significant difference test (L.S.D) and (T. Test) at 5% level of probability Snedecor and Cochran (1982).

3. Results and Discussion Growth parameters

The results presented in table 1 showed a retarded growth in salt-stressed plants. Plant height,

fresh and dry weight of both shoots and roots and the number of leaves/plant were significantly decreased under saline condition. Decreases in the aforementioned characters were increased with increasing the level of salinity .The reduction in root and shoot development may be due to toxic effects of the NaCl used as well as unbalanced nutrient uptake by the plants competitors between Na+ and Cl- and further anions and cations may result in a reduced plant growth and yield (De pascale et al., 2005, keutgen and Pawelzik,2008). Many studies have shown that biomass partitioning between roots and shoots is strongly influenced by the most limiting resource under stress growth conditions, and resource deficiency is often ameliorated by increasing the biomass allocation to the part of the plant responsible for acquiring the most limiting resource (Hutchings and John, 2004).

Results of the present study (Table 1) revealed that, most if not approximately all, the growth characters of salinized and non salinized wheat plants were increased under the application of silicon. The adverse effects of salinity as regards the growth parameters were significantly alleviated by Si supplement. The improvement of the growth was more pronounced at the second level of Si (2mM). These results are in agreement with those of Tuna et al. (2008), reported that supplementary silicon resulted in significant increases in dry matter of wheat plants grown under saline condition. Silicon treatment had positive effects on most of observed growth parameters of maize plants compared with the control (Vaculika et al., 2009). In the analysis of the beneficial effect of Si under saline growth conditions, it is important to consider the role of Si in plant water status because the initial reduction of plant growth after salt imposition is due to the osmotic effect of salts. The beneficial effect of silicon has been related to the depression of excessive loss of water by transpiration (Savant et al., 1999) or with silicate crystals deposition beneath the epidermal cells of leaves and stems (Trenholm et al., 2004), which may reduce water loss through the cuticles. From a whole-plant perspective, the interplay between stomatal regulation and cuticular water permeability is of decisive importance to reduce water loss to a minimum under conditions of soil and atmospheric water stress (Burghardt and Riederer, 2003).

Photosynthetic pigments

As shown in table 2, contents of chlorophyll a ,b as well as contents of clorophyll a+b in leaves of wheat plants were significantly decreased in response to saline conditions. The decrease in chlorophyll contents was increased with increasing salinity level. On the other hand, the obtained results showed that carotenoid content in leaves of wheat plants was significantly increased under salt stress conditions (Table 2). Several investigators confirmed that salinity adversely affect the photosynthetic pigments of different plants (Almodares *et al.*, 2008 on sorghum and Abeer, 2009 on cowpea).

The results presented in table 2 revealed that the reduction in photosynthetic pigments caused by NaCl was relieved, to high extent ,by the application of silicon. Results revealed also that addition of Si markedly reduced the increases in the carotenoid contents were observed in salt stressed plants. In this concern, It has been reported that Si improves photosynthesis rates in barley and cucumber (Adatia and Besford, 1986), which was related with leaf ultra-structure, chlorophyll content, and ribulose biphosphate carboxilase activity. Al-Aghabary *et al.* (2004) showed that Si decreased salt-induced production of H_2O_2 and improved photochemical efficiency of PSII of salinized tomato plants. Tuna *et al.* (2008) found that supplementary Si resulted in significant increases in chlorophyll contents of wheat plants grown at high NaCl.

treat	ments		F.wt. shoot	D.wt.	F.wt. root	D.wt. root /	No. of leaves
NaCl	Si	Plant height (cm)	/ plant (g)	shoot /	/ plant	plant (g)	/ plant
(mM)	(mM)			plant (g)	(g)		
	0.0	57.11	16.21	1.93	12.17	2.04	27.03
	1.0	61.20**	18.07 **	2.72 **	14.25 **	2.39 **	31.27 **
0.0	2.0	60.07 *	18.13 **	3.01 **	17.36 **	2.89 **	30.09 **
	0.0	53.13**	14.41 **	1.51 **	11.14 **	1.86	21.24 **
50.0	1.0	59.08 *	17.18	2.35 **	15.07 **	2.54 **	30.51 **
	2.0	60.13 **	18.72 **	2.60 **	16.32 **	2.72	29.13 **
100.0	0.0	41.20**	11.39 **	1.09 **	8.06 **	1.63 **	21.27 **
	1.0	45.14**	14.21 **	2.14 *	11.24 **	1.97	29.51 **
	2.0	60.91 **	15.42 *	2.64 **	11.61 **	1.98 *	29.32 **

Table (1): Effect of Si, NaCl, and their interactions on growth characters of wheat plants.

Each value is a mean of seven replicates.

* Significant at 5% confidence level.

** Significant at 1% confidence level.

Table (2): Effect of Si, NaCl, and their interactions on photosynthetic pigments (mg./g. F.wt.).

		Photosynthetic pigments (mg/g. F.wt.)				
Treatments		Chl. a	Chl. b	Chl. a+b	Carotenoids	
NaCl	Si					
(mM)	(mM)					
	0.0	3.42	0.89	4.31	0.91	
0.0	1.0	3.81 *	1.11 **	4.92 *	0.95	
	2.0	3.56	1.81 **	5.37 **	1.02 *	
	0.0	3.01 *	0.75 **	3.76 *	1.31 **	
50.0	1.0	3.41	0.94	4.35	0.97	
	2.0	3.52	0.89	4.41	0.91	
	0.0	2.87 **	0.73 **	3.60 **	1.89 **	
100.0	1.0	3.01 *	0.93	3.94 *	1.01 **	
	2.0	3.21	0.81	4.02	0.95	

Each value is a mean of three replicates.

* Significant at 5% confidence level.

** Significant at 1% confidence level.

Yield and yield components

Table 3 showed that NaCl significantly reduced number of both tillers and spikes per plant, number &weight of grains per plant and weight of pigments and assimilates. Therefore, translocation of 1000-grains. The magnitude of reduction was increased with increasing salinity level. Reduction in grain yield of stressed wheat plants might be attributed to the rapid reduction in leaf photosynthetic assimilates from stem to grains is the main source as well as limiting factor for growth and development of grain. According to Munns (2002), salinity reduces plant productivity first by reducing plant growth during the phase of osmotic stress and subsequently by inducing leaf senescence during the phase of toxicity when excessive salt is accumulated in transpiring leaves.

Results of the present investigation (Table 3) revealed that addition of silicon resulted in increasing the yield and its components of the tested plants. This was the case in plants grown in either absence or presence of NaCl. The obtained results are in agreement with the work of Mercedes *et al.* (2006) on tomato and Tuna *et al.* (2008) on wheat.

In the present investigation, contents of soluble carbohydrates as well as soluble proteins in the yielded grains of wheat plants were significantly decreased with increasing salinity level (Table 2). Similar results were observed by other investigators (Koyro and Eisa, 2008, Abeer, 2009).

The obtained results (Table 2) showed that silicon supplementary resulted, in most cases, in a significant increase as regards the contents of soluble carbohydrates and proteins in the yielded grains of salinized and non-salinized plants. In this respect, Zhu *et al.* (2004) reported that Si significantly increased leaf soluble protein content of salt-stressed cucumber plants.

Table (3): Effect of Si, NaCl, and their interactions on yield characters and contents of soluble carbohydrates (mg./g. D.wt.) and soluble proteins (mg./g. D.wt.) in the yielded grains.

treat	ments	No. of		No. of	weight of		Soluble	
NaCl (mM)	Si (mM)	tillers / plant	No. of spikes/ plant	grains/ plant	grains/ plant(g. (g)	weight of 1000 grains (g)	proteins mg/g.	Soluble carbohydrates mg/g.
	0	5.23	4.11	128.03	5.13	40.37	17.24	43.21
	1	6.14 **	4.32 **	155.61**	7.03 **	45.21 **	21.05 **	52.18 **
0	2	6.07 **	4.07	166.73**	7.26 **	42.07 **	21.73 **	51.36 **
	0	5.32	3.92 *	135.13*	5.06	37.21 **	17.05	37.69 **
	1	6.74 **	4.11	119.04**	5.64 **	42.05 **	19.03 **	47.03 **
50	2	6.81 **	4.71 **	146.34**	6.31 **	41.03 *	23.21 **	44.31 *
	0	4.97	3.17 **	125.00	4.38 **	32.52 **	11.17 **	34.50 **
	1	5.11	4.02	129.78	5.02 *	38.92 **	17.32	39.30 **
100	2	5.08	4.13	127.17	5.41 **	39.81	18.14 **	40.11 **

Each value is a mean of three replicates.

* Significant at 5% confidence level.

** Significant at 1% confidence level.

Antioxidant enzymes

Even under optimal conditions many metabolic processes produce ROS. The production of toxic oxygen derivatives is increased as a result of all types of abiotic or biotic stresses. Plants posses efficient systems for scavenging active oxygen species that protect them from destructive oxidative reactions Foyer et al. (1994). As part of this system, antioxidant enzymes are key elements in the defense mechanisms. In the present study, significant increases were observed in the activities of SOD, APX, CAT and GR in the shoots of wheat plants under salt stress conditions. Moreover, it was found that the activities of the assayed antioxidant enzymes were increased with increasing salinity level. Changes in the activities of the antioxidant enzymes under saline conditions has been reported by several investigators, increased in the case of salt-tolerant cotton Meloni et al. (2003), shoot cultures of rice Fadzilla *et al.* (1997), cucumber Lechno *et al.* (1997), wheat shoot Meneguzzo *et al.* (1999) and pea Hernandez *et al.* (1999), but decreases in wheat roots Meneguzzo *et al.* (1999), or is unaffected as in the case of SOD in cucumber Lechno *et al.* (1997). In this respect, the effects of salt stress on the antioxidant enzymes are very complex and depend on the treatment time, plant species and genotypes (Zhu *et al.*, 2004).

In the present experiment, application of Si caused great variations in the activities of the measured antioxidant enzymes (Table 4). Under normal (Non-saline) conditions, addition of Si, especially at 1mM, markedly increased the activity of both SOD and CAT in shoots of wheat plants, however the activity of both POX and GR was significantly decreased. Under saline conditions, addition of Si markedly reduced the increases in the activities of SOD, POX, CAT and GR were observed

in salt stressed plants (Table 4). Similar results were obtained in the work of Shen et al. (2010) on soybean plants, found that Si application significantly reduced the membrane damage. Catalase (CAT), peroxidase (POD), superoxide dismutase (SOD) and hydrogen peroxide were observed under stress conditions. Silicon also increased leaf and root superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and glutathione reductase (GR) activities and the glutathione (GSH) concentration but suppressed the malondialdehyde (MDA) concentration in barley under salt stress and stimulated root H-ATPase and H- PPase activity in the plasma membranes, tonoplasts and mediated membrane fluidity, suggesting that Si may affect the structure, integrity and functions of plasma membranes by influencing the stress-dependent peroxidation of membrane lipids (Liang *et al.*, 2006). This hypothesis (Liang *et al.*, 2003) that Si decreased lipid peroxidation in salt-stressed plants via enhancing antioxidant enzyme activity and non-enzymatic antioxidants has recently been corroborated in experiments with cucumber (Zhu *et al.*, 2004) and tomato (Al-Aghabary *et al.*, 2004). However, Liang *et al.* (2003) found an increase in SOD activity in salt-stressed barley leaves and increases in SOD, GPX, CAT and GR activity in salt-stressed barley roots. A significant increase in antioxidant enzymes of salt-stressed leaves by Si addition suggest that Si may be involved in the metabolic or physiological activity in cucumber exposed to salt stress (Zhu *et al.*, 2004).

Table (4): Effect of Si, NaCl, and their interactions on enzyme activities (unit/g./hour).

Treatments		Enzyme activities (unit/g.F.wt./hour)					
NaCl (mM)	Si (mM)	Super oxide dismutase (SOD)	Peroxidase (POX)	Catalase (CAT)	Glutathione reductase (GR)		
	0.0	54.22	61.61	43.05	41.24		
0.0	1.0	67.31**	53.07 **	45.13 **	40.70 *		
	2.0	51.42 *	50.50 **	45.71 **	37.37 **		
	0.0	48.51 **	70.94 **	46.24 **	44.24 **		
50.0	1.0	54.06	68.42 **	45.31 **	38.61 **		
50.0	2.0	60.17 **	61.16	45.27 **	39.05 **		
	0.0	61.24 **	73.31 **	51.80 **	49.10 **		
100.0	1.0	57.13 *	61.05	45.13 **	40.38 **		
	2.0	56.17	66.20 **	47.14 **	41.92 *		

Each value is a mean of three replicates.

* Significant at 5% confidence level.

** Significant at 1% confidence level.

Endogenous phytohormones

Results of the present investigation (Table 5) showed that salinity greatly affected the activities of the endogenous phytohormones in shoots of wheat plants. Under the first level of salinity (50Mm NaCl), contents of both ABA and CK were markedly increased than that of the control ones. At the same time, contents of GA₃ and also contents of IAA were significantly decreased in response to the first level of NaCl. At the second level of NaCl (100Mm). significant decreases were observed in the contents of GA3, IAA and CK while contents of ABA were found to be highly significantly increased. In this regard, El-Khallal et.al. (2009) found that salt stress led to sharp decrease in the levels of IAA, GA₃ and Zeatin, while ABA level greatly increased in maize shoots. These results appeared that salt stress led to sharp changes in the balance of endogenous hormones which associated with the accumulation of ABA and

decrease in the level of IAA, GA₃ and cytokinins. Thus, reduction in shoot growth of maize plants (El-Khallal et.al., 2009) is probably related to hormonal signals generated in response to salt stress as suggested by Ghanem et al. (2008). The decrease in CK may be the result of reduced transport of root synthesized CK, and/or increased breakdown of CKs (Werner, et.al., 2006, Havlová et.al., 2008). On the other hand, Roitsch et.al. (2003) suggested that the strong decrease observed in zeatin content under saline conditions can also may be explained by an induction of cytokinin oxidase activity. Results of the present study (Table 5) revealed that the adverse effects of salinity as regards the endogenous phytohormones were significantly alleviated by Si treatment. Si supplementary resulted in increasing the contents of GA₃, IAA and CK. This was the case in plants grown in either saline or non-saline conditions. On the other hand, insignificant increases in the contents of ABA were observed in plants grown in non salinized conditions due to application of Si. However, in salinized plants, application of Si resulted in reducing the increases in contents of ABA were observed. This was the case throughout the two applied levels of salinity.

Conclusion

From the outcome of the obtained results, it could be concluded that the application of Si greatly improving most of the growth characteristics of wheat plants grown in either saline or non saline conditions. This may be due to that, these treatment participate in different metabolic processes. In this concern, Liang *et al.* (2007) concluded that Si is not inert, but acts as a physical or mechanical barrier in

plants. It is not only deposited in the cell walls, but is also actively involved in the metabolic and/or physiological activities, especially in plants subject to multiple stresses. Finally, it is worth to mention that the role of Si in increasing tolerance of plants to salt stress advocate the need of additional research for elucidating the close and direct relationships among Si and different metabolic and physiological activities in plants.

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Table (5): Effect of Si, NaCl, and their interactions on Endogenous phytohormones (ug./100 g. Fwt.).

Treatments		Endogenous phytohormones (ug./100 g. Fwt.)					
NaCl (mM)	Si (mM)	Abscisic acid (ABA)	Indole acetic acid (IAA)	Gibberellic acid (GA ₃)	Cytokinin (CK)		
	0.0	18.20	17.24	39.05	41.15		
0.0	1.0	20.31	23.15 **	45.23 **	51.73*		
	2.0	19.42	29.52 **	45.74 **	47.81 **		
	0.0	24.51 **	14.37 **	33.27 **	36.27**		
50.0	1.0	19.06	19.44 **	41.21 **	45.36 **		
	2.0	15.17 *	16.34	39.23 **	42.78 **		
	0.0	28.24 **	13.84 **	31.90 **	35.24 **		
100.0	1.0	21.13 **	13.75	34.13 *	42.19 **		
	2.0	19.17 *	16.30 **	33.14 **	40.93 *		

Each value is a mean of three replicates.

* Significant at 5% confidence level.

** Significant at 1% confidence level.

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