Effect of Silicon on Salt Tolerance Improvement For Some Cultivars of Wheat Pllants Grown on Hydroponic Media

Amal H. Mahmoud

Laboratory for Saline and Alkaline Soil Research, Institute of Soil, Water and Environment Research,
Agricultural Research Center, Pacus, Alexandria

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ABSTRACT

Using hydroponic technique, the effects of silicon application (0 and 2 mM Si) on the salt tolerance of some wheat genotypes grown under various levels of salt stress (0, 50 and 150 mM NaCl)) were investigated for three cultivars of wheat (Sakha 93, Gernmaiza 9 and Giza 168). Silicon application improved the dry weight of shoots and roots of Sakha 93 and Gemmaiza 9 under all levels of salinity. The roots of Giza 168, at 0 and 50 mM NaCl, slightly decreased the shoot dry weight of Giza 168. In plant shoots of all tested seedlings, sodium concentration increased and potassium slightly decreased with increasing salinity and more Na increments and K decrements were observed with Si application except at 150 mM NaCl where Si application to Sakha 93 reduced Na in shoot by 22.7% than in those of non Si-treated shoots. In plant roots, Na concentration highly decreased while K concentration increased in seedlings of Sakha 93 with NaCl salinity increase and vice versa in Gemmaiza 9 and Giza 168 seedlings. Silicon application to Sakha 93 caused more significant reductions in Na concentration by root with higher levels of NaCl salinity. In comparison with other cultivars, Sakha 93 showed a defense mechanism(s) by decreasing Na⁺, increasing K⁺ and increasing K⁺/Na⁺ ratio in root system and application of Si improved these mechanisms. At high levels of salt stress (150 mM NaCl), presence of Si decreased Na" uptake by shoots of Sakha 93 which reflects defense mechanism against N⁺ translocation to shoot under high salt stress. In general, the statistical analysis of results of growth parameters and Na+ and K+ concentrations in wheat plants showed that wheat genotypes had major role in salt tolerance and Si application improved Sakha 93 and Gemmaiza 9 to maintain high shoot and root dry weights under salt stress and enhanced the defense mechanisms of Sakha 93 for salt tolerance. The results confirmed that Sakha 93 genotypes is considered a salt tolerant and both Gemmaiza 9 and Giza 168 could be salt sensitive cultivars.

Key Words: salt tolerance, wheat genotyes, silicon, hydroponic

INTRODUCTION

Salinity is considered a major factor in limiting plant growth and crop productivity. The salinisation of irrigated and surrounding areas in the arid soils has not been diminished. On the contrary, it continues to increase in arid and semi-arid regions (Rus et al., 2000). It is estimated that about a third of the world's cultivated land is affected by salinity (Perez-Alfocea et al., 1996). Salinity poses several problems especially for glycophytes, by inducing physiological dysfunction (Shannon et al., 1994). The relationships between salinity and mineral nutrition of horticultural crops are extremely complex and a complete understanding of the indicate interactions involved would require the input from a multidisciplinary team of scientists (Grattan and Grieve, 1999). Salinity can be minimized with reclamation but the cost of engineering and management is very high. Increasing costs of water and energy emphasizes the need for an alternative strategy (Shannon, 1984). An alternative strategy for overcoming the negative effects of salinity on the plant growth and yield could be to attempt to supplement silicon where irrigation water is known to be or may become saline.

Silicon is the second most abundant mineral element in the soil after oxygen and comprises 31%

of the earth's crust which occurs in the soil solution at 0.1-0.6 mol m⁻³ as Si(OH)₄ (Epstein 1999). However, the role of silicon in plant biology is still poorly understood (Epstein 1999; Liang et al. 2003; Zhu et al. 2004). Although silicon has not yet been considered a generally essential element for higher plants, numerous studies have demonstrated that silicon is one of the important elements for plants, and plays an important role in tolerance of plants to environmental stresses [Epstein, 1999 and Savant et al., 1999]. Relatively more attention has been paid to the roles of silicon in controlling disease [Raid et al., 1992 and Rodrigues et al., 2003] and pest [Pan et al., 1979 and Elawad et al., 1985], alleviation the toxicity of heavy metals [Neumann and Nieden, 2001 and Hodson, A.G. Sangster, 2002] and salt stress (Liang et al., 1996; Liang, et al., 2003; and Zhu et al., 2004).

The role of silicon in the alleviation of salinity stress in plants has been observed in wheat (Ahmad et al., 1992), barley (Liang et al., 2003, 2005), tomato (Alaghabary et al., 2004), rice (Match et al., 1986; Yeo et al., 1999), cucumber (Zhu et al., 2004) and maize (Shu and Liu 2001). However, the mechanism (s) for these effects of silicon is still unclear. Match et al. (1986) suggested that silica deposition in the leaf decreased transpiration and therefore decreased salt accumulation. Ahmad et al. (1992) suggested that silicon complexed sodium in

the root, therefore decreasing sodium transport to the shoot, but no direct evidence was presented. Applied silicon increased antioxidant defence in barley (Liang et. al. 1996 and Liang et al., 2003), cucumber (Zhu et al. 2004) and tomato (Al-Aghabary et al., 2004). Yeo et al. (1999) indicated that salt-resistance is attributed to silica particles deposited in apoplast spaces that contributed to the block of Na into cells and inhibitions of sodium transport in apoplast. Silicon deposited in cell walls interlaces with organic macromolecules (including cellulose, pectin, glycoprotein, and lignin) to form amorphous colloidal complexes with absorption surfaces.

Using silicon in the forms of calcium silicates (Amador et al., 2004), sodium silicate (Tuna et al., 2008 and Yeo et al., 1999), potassium silicate (Hattori et al., 2005) or silicic acid (Tamai and Ma, 2003) is considered one of the important techniques to alleviate salt stress and improve the yield of various crops such as legumes and cereals. In the present study, this technique is used for enhancing the salt tolerance in some wheat genotypes.

MATERIALS AND METHODS

Seeds of three wheat cultivars (Sakha 93, Gemaiza 9 and Giza 168) were surface sterilized with $\rm H_2O_2$ (10%) for 15 min (Miché and Balandreau, 2001), rinsed thoroughly with distilled water and germinated on moist filter paper for 2 days in an incubator at 25°C. After germination, the seeds were sown in quartz sand in the laboratory at room temperature. When the second leaf was emerged, uniform seedlings were transplanted into 1L plastic pots containing continuously aerated nutrient solution Table (1).

Each pot was covered with a polyethylene lid through which five plants were supported over the nutrient solution. The nutrient solution was prepared using de-ionized water and renewed every other day. After ten days of the growth, in nutrient solution, seedlings were transferred into another nutrient solution containing 0, 50 or 150 mM NaCl

Table 1: Composition of nutrient solution

Macronutrients compounds final concentration (mM)		Micronutrients compounds			
		final concentration (uM)			
K2SO4	0.75	Fe-EDTA	0.1		
MgSO4	0.65	Н3ВО3	1.0		
KH2PO4	0.25	MnSO4	1.0		
Ca(NO3)2	2.00	ZnSO4	1.0		
		CuSO4	0.1		
		(NH4)6Mo7O24	0.005		

(Zou et al., 2001)

and presence of 0 or 2 mM silicon in the form of sodium meta-silicate (Na₂SiO₃5H₂O), giving 18 different treatments and each treatment was replicated 3 times. The pots were subjected to a completely randomized design

After 35 days of growth, the weights of fresh plants were measured and the plants were then divided into shoots and roots and the weight of each part was recorded. The plants were washed in detergent solution to remove any dust on leaf surfaces, soaked in 0.5M HCl for 20 s, followed by three to four rinses in distilled water and then dried at 70 °C for 48 h to constant weight (Jones and Case, 1990). The dried shoots and roots were weighted then ground to powder using a pestle and mortar and stored in polyethylene bottles. Ground samples were ashed at 550 °C for 6 h. The white ash was taken up in 2M hot HCl, filtered into a 50mL volumetric flask and made up to 50mL with distilled water. Sodium and potassium ions were determined in these solutions using a flame photometer (Chapman and Pratt, 1982).

The Obtained data were analyzed using a Costat ANOVA program and statistically different groups were determined by Duncan's test (P < 0.01) (.Cohort, 1986)

RESULTS AND DISCUSSION

Wheat Seedlings Growth

In the absence of added sodium chloride, silicate enhanced the dry weight of the shoots of Sakha 93 and Gemaiza9 seedlings and the root of all tested genotypes (Fig. 1). However, growing the seedlings in the NaCl stressed nutrient solution slightly reduced the shoot dry weight of all seedlings but did not affect the root dry weight. Application of sodium silicate improved the shoot dry weight of Sakha 93 and gemmaiza 9 particularly under high rate of salinity stress (150 mM NaCl). The dry weight of seedling roots significantly increased in the presence of silicate under the growth in 50 mM NaCl for all tested genotypes (Fig. 1). Similar results were found by Tuna et al. (2008) who studied the growth of wheat seedlings for 65 days in complete nutrient solution in presence of 0 and 100 mM NaCl with or without silicate (0.25 and 0.5mM Na₂SiO₃) and found about 39 and 45% reduction in total plant dry weight with NaCl treatment. Silicon significantly improved dry biomass when added to saline stressed plants. On the other hand, in hydroponic experiments, Gong et al. (2006) indicated that application of Si to rice seedlings grown in normal nutrient solution increased both shoot and root dry weight by about 33.96 and 15.05 %, respectively while the effect of Si was lower under the growth in presence of 50 mM NaCl. Yeo et al. (1999) found that silicon application in the form of sodium silicate to three rice genotypes did not significantly improve the shoot and root dry weights under both non-saline

and saline growth conditions. Match et al. (1986) mentioned that silicon did not enhance rice plant growth without salt.

Sodium and Potassium Accumulation in Wheat Seedlings

As shown in figures 2-4, under non saline conditions, silicon application did not affect the sodium concentration in the shoot of wheat seedlings for all the tested cultivars and in the root of Gemaiza 9 and Giza 168 while decreased Na concentration in the root of Sakha 93. Under NaCl stress, the concentration of Na significantly increased in the shoots of Gemaiza 9 and Giza 168 (in both stress levels 50 and 150 mmol NaCl/L) and Sakha 93 (in 50 mmol/L NaCl treatment) while Na concentration decreased in the shoot of Sakha 93 seedlings grown under 150 mmol/L NaCl stress.

Sodium concentration in roots of Gemaiza 9 and Giza 168 increased as NaCl stress increased in the absence and presence of silicon, but application of Si to 50 mmol/L NaCl-treated seedlings decreased Na concentration comparing to those grown in the absence of Si for both cultivars (Figs. 3 and 4). In contrast, Na concentration in the root of Sakha 93 decreased as NaCl stress increased and more decrements were observed in the presence of Si (Fig. 2).

Potassium concentration in the seedlings shoot decreased as NaCl stress increased in all the tested varieties of wheat. (Figs.2-4). More decrements were recorded in K concentration in the presence of Si in all seedlings. Under various stress levels of NaCl Gemmaiza 9 maintain high levels of K in the shoot (110.77 – 94.86 umol K/g dry weight of shoot in the absence of Si and 103.75 – 92.45 umol K/g DW in the presence of Si) comparing with the other tested seedlings.

In the absence or presence of Si, potassium concentration in the seedlings root of Sakha 93 increased as NaCl salinity increased while its concentration decreased with increasing of NaCl in the roots of both other cultivars (Figs. 2-4). Only under non-saline conditions, application of Si to Sakha 93 seedlings increased the root K concentration more than its concentration in the absence of Si. Dramatic reduction concentration in the roots of Gemmaiza 9 and Giza 168were observed in the presence of Si (Figs3and 4)

Figures 2-4 showed that K/Na ratio in the shoot was highly decreased with increasing the stress of NaCl. This ratio was greater in Si non-treated seedlings than plants grown with Si application. Similar trend was observed in the K/Na ratio of the roots of Gemmaiza 9 and Giza 168. In contrast, K/Na ratio of Sakha 93 seedlings root increased with increasing the stress with or without applied Si. Under non-saline and 50 mM NaCl growth conditions, the ratio was greater in silicate treated

seedlings than plants grown without Si application (Fig. 2).

The results of Na and K accumulation in the shoot and root of tested varieties of wheat seedlings indicate that the presence of different strategies which play a role to control the entrance and translocation of Na in the root and shoot systems, respectively. The results showed that the seedlings of Sakha 93 have different mechanism than seedlings of Gemmaiza 9 and Giza 168 for resisting the salinity stress. In the root system of Sakha 93, Na decreased as sodium ions increased in the nutrient solution. This result reflects a defense mechanism (s) against Na⁺ entrance to the roots or Na+ excluding system working under salt stress susceptibility and it can identify this system by three indicators: (i) decreasing of Na⁺ (Na concentration was reduced from 20.67 umol Na/g DW of root under 0 mM NaCl to 13.29 and 4.71 umol Na/g DW root under 50 and 150 mM NaCl, respectively), (ii) increasing of K+ (K+ concentration in root increased from 2.44 umol K/g DW root under 0 mM NaCl to 8.24 and 11.79 umol K/g DW root under 50 and 150 mM NaCl, respectively) and (iii) increasing the K⁺/Na⁺ ratio in the root tissues. These defense systems to tolerate the salinity stress are enhanced or improved by application of Si. In contrast, for both other cultivars (Gemmaiza 9 and Giza 168), the results indicate the absence such mechanisms in the root system. The previous recent studies on the evaluating salt tolerance of local wheat genotypes ranked - using multiple growth and yield parameters - Sakha 69 and Sakha 93 as salt tolerant and Giza 168 and Gemmaiza 7 as sensitive genotypes (El-Hendawy et al., 2005). In wheat, salt tolerance is related to the enhanced K/Na selectivity (Dajic, 2006) and the maintenance of higher K⁺/Na⁺ ratios, especially in young growing and recently expanded tissues, appeared to be important characteristics of salt tolerance in barley cultivars (Wei et al., 2003). On the other hand, the achievement of a high K+:Na+ ratio is more simply maintaining important than concentrations of sodium ions (Maathuis and amtmann, 1999). So, the growth under salt stress of NaCl solution fostered Sakha 93 only to enhance K/Na ratio in roots for resisting the stress and these results confirm that Sakha 93 is considered salt tolerant genotype comparing to Gemmaiza 9 and Giza 168.

Under the highest level of NaCl salinity (150 mM), Na concentration in the seedlings shoot reduced only in Sakha 93 in presence of Si (from 3002.263 to 2322.16 umol Na/g DW of shoot; about 22.7%) which could support the suggestion of existence an inhibition mechanism of sodium translocation from root to shoot. Gong et al. (2006) concluded that silicate application to rice seedlings grown under saline conditions (50 mM NaCl) caused a decrease in net Na transport to the shoot by

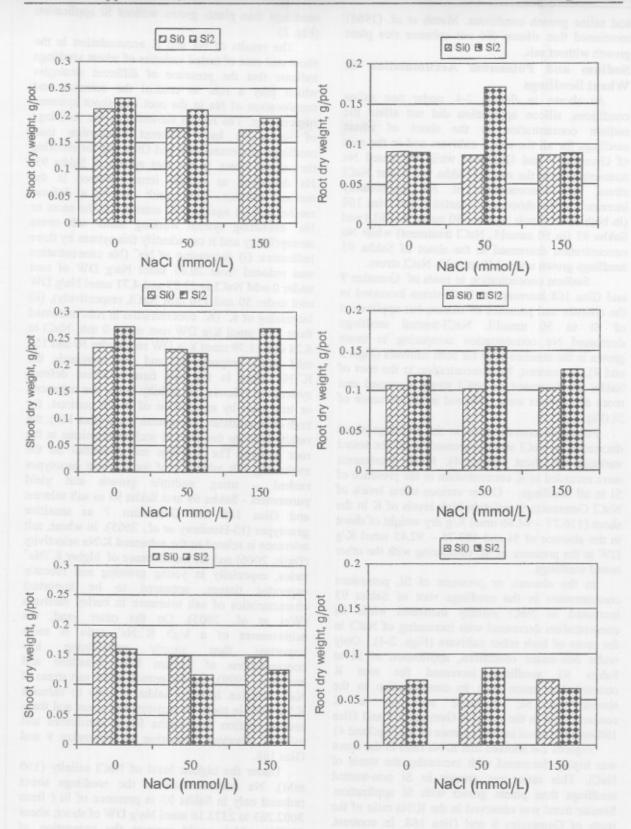


Fig. 1: The relation between NaCl treatments and silicon application on the shoot and root dry weights of 35-day old wheat seedlings

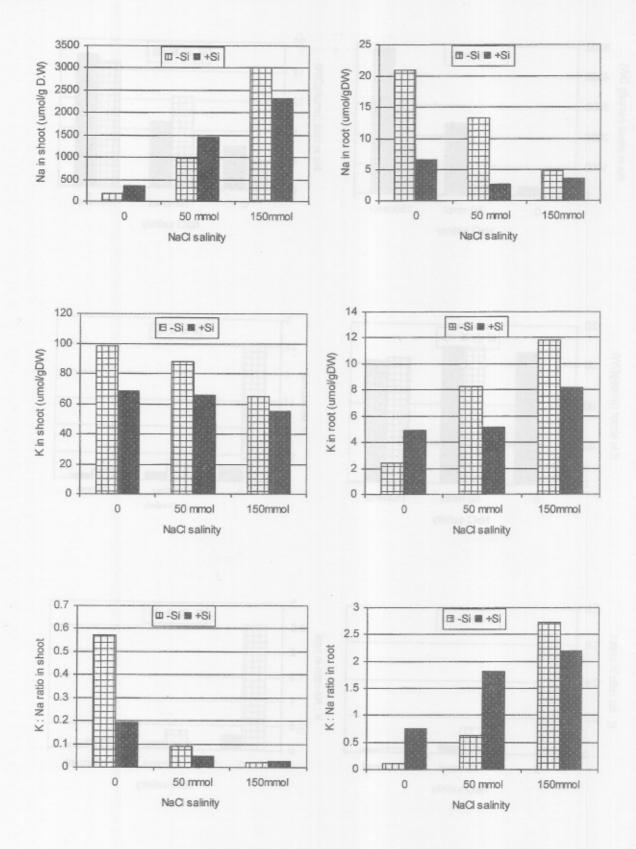


Fig. 2: Effect of silicon addition to 35-day old wheat seedlings (Sakha 93) on sodium and potassium ion distribution in plant parts grown under different levels of NaCl salinity stress

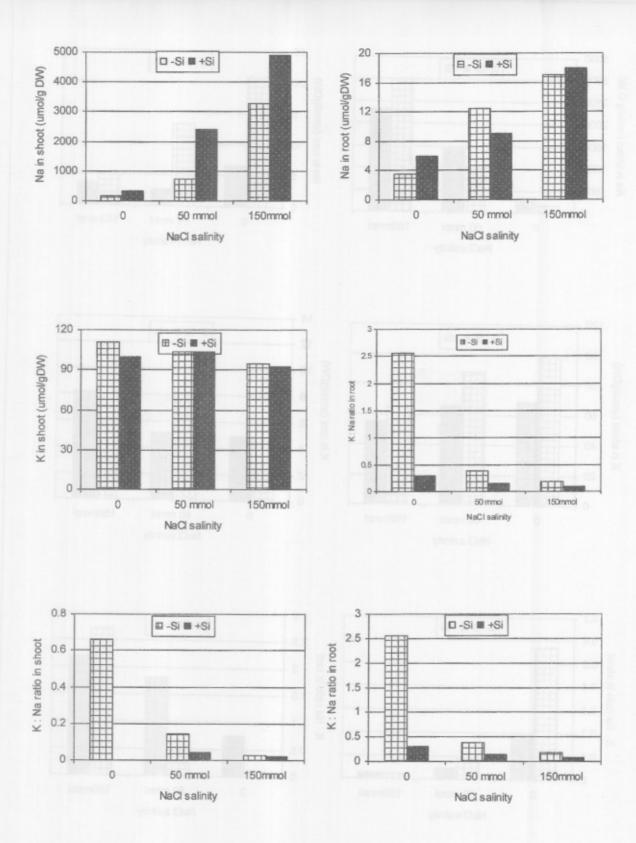


Fig. 3: Effect of silicon addition to 35-day old wheat seedlings (Gemaiza 9) on sodium and potassium ion distribution in plant parts grown under different levels of NaCl salinity stress

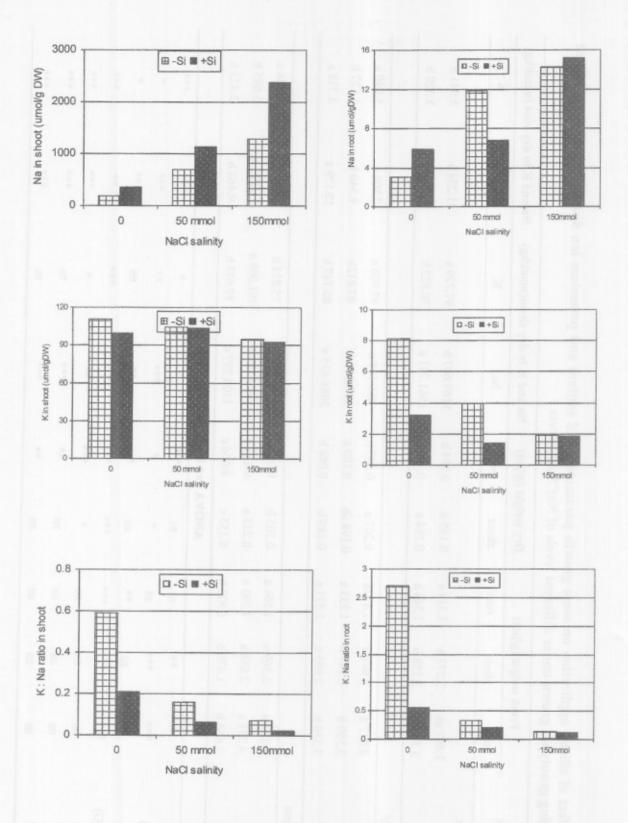


Fig. 4: Effect of silicon addition to 35-day old wheat seedlings (Giza 168) on sodium and potassium ion distribution in plant parts grown under different levels of NaCl salinity stress

Table 2: Effect of silicon application on some growth parameters and sodium and potassium ion distribution in three wheat seedling genotypes grown under different levels of NaCl salt stress

Factors	Fresh weight (g/pot)		Dry weight (g/pot)		Na and K in dry shoot(umol/kg)		Na and K in dry root (umol/kg)		
	total	shoot	root	shoot	root	Na	K	Na	К
Silicon									
without Si	3.687 ab	2.373 a	1.314 a	0.195 a	0.084 b	1169.607 b	97.729 a	11.254 a	5.941 a
with Si	3.502 b	2.158 b	1.344 a	0.194 a	0.107a	1561.741 a	76.822 b	8.329 b	3.329 b
Salinity									
0 mM	4.017 a	2.601 a	1.417a	0.207 a	0.087 b	266.188 с	97.920 a	7.806 c	5.040 a
50 mM	3.5 8 0 b	2.247 b	1.333 a	0.198 ab	0.100 a	915,314 b	87.812b	9.340 b	4.127 b
150 mM	3.386 b	2.055 с	1.331 a	0.189 b	0.088 b	3088.635 a	80.747 ъ	12.179 a	4.738 a
Genotypes				<u> </u>		<u></u>			
Sakha 93	4.133 a	2.569 a	1.564 a	0.205 b	0.095 b	1382.011 b	75.834 c	8.722 b	8.788 a
Gem 9	4.251 a	2.658 a	1.593 a	0.233 a	0.113 a	1779.829 a	101.008 a	11.190 a	3.695 b
Giza 168	2.600 b	1.676 b	0.923 b	0.155 с	0.066 с	1108.297 c	89.637 b	9.462 b	3.423 b
				ANOVA	and F sign	ificance			
Silicon (Si)	*	**	ns	ns	***	*	*	***	***
Salinity (Sa)	***	***	ns	*	*	***	**	***	*
SaxSi	ns	ns	**	ns	***	**	ns	***	*
Genotypes (G)	***	***	***	***	***	***	***	**	***
GxSi	ns	ns	ns	*	***	**	*	***	***
GxSa	ns	ns	ns	ns	**	***	ns	***	***
GxSaxSi	ns	ns	ns	ns	**	***	ns	***	***

restriction (reducing bypass flow) of Na entry to the xylem or enhanced retranslocation in the phloem.

Regardless the role of silicon for improving salinity stress only in Sakha 93, it had positive effect in improving the dry weight in both shoot and root of Sakha 93 and Gemmaiza 9. Further researches are needed to explore the specific effects of silicon application to plants grown in saline soils and/or irrigated with saline water.

The analysis of variance (Table 2) showed that wheat genotype is the dominant factor affecting on growth parameters and ion distribution between shoot and root. Thus the effects of NaCl salinity are highly effective in fresh weight and sodium ion distribution and the presence of silicon played a significant role in decreasing the harmful effect of salt stress on fresh and dried biomass of shoot and root of young wheat plants.

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الملخص العربي

تأثير السيليكون على تحسين مستوى تحمل الأملاح لبعض أصناف القمح النامية في محاليل مغذية

أمل حسن محمود

معمل بحوث الأراضى الملحية والقلوية، معهد بحوث الأراضى والمياه والبيئة مركز البحوث الزراعية – الاسكندرية

باستخدام تقنية الزراعة في محاليل مغذية تم دراسة تأثير إضافة السليكون (بتركيزات صفر و٢ ماليمول/لتر) على درجة تحمل الأملاح (في صورة كلوريد صوديوم بتركيزات صفر ، ٥٠ ، ١٥٠ ملليمول/لتر) بواسطة ثلاثة أصناف من القمح هي سخا ٩٣ وجميزة ٩ وجيزة ١٦٨ نامية في محاليل مغذية لمدة ٣٥ يوم. بينت النتائج أن إضافة الــسيليكون أدت الــي تحــسن الوزن الجاف لكل من الجذور والأوراق لبادرات القمح من سخا ٩٣ وجميزة ٩ تحت كل مستويات الاجهاد الملحي المستخدمة كما حسنت الوزن الجاف لجذور بادرات جيزة ١٦٨ عند مستويات صفر و٥٠ ملليمول/لتر كلوريد الصوديوم فقط وأحــدثت نقص طفيف في الوزن الجاف لأوراق هذا الصنف النامية في التركيزات الأعلى من كلوريد الصوديوم. ارتفعت تركيزات أيون الصوديوم في أوراق البادرات بارتفاع تركيز كلوريد الصوديوم في المحاليل المغذية بينما انخفض في المقابل تركيــز أيــون البوتاسيوم وأدى وجود السليكون الى مزيد من الارتفاع في تركيز الصوديوم والانخفاض في تركيز البوتاسيوم باستثناء الصنف سخا ٩٣ النامي في محاليل تحتوي على ١٥٠ ملليمول/لتر كلوريد صوديوم فقد أدى وجود الــسليكون الـــي خفــض تركيـــز الصوديوم الممتص في الأوراق بنسبة ٢٢,٧% مقارنة بمحتوى الأوراق في غياب السليكون عند نفس المستوى. في جذور البادرات لوحظ أن امتصاص الصوديوم الخفض بزيادة الاجهاد الملحى المعرضة له النباتات وذلك في صنف ســخا ٩٣ فقــط وازداد بزيادة الاجهاد في باقى الاصناف المختبرة. وقد أدى إضافة السليكون الى المحاليل المغنية الى مزيــد مــن انخفــاض تركيز الصوديوم في جنور صنف سخا ٩٣ كلما زاد مستوى الاجهاد الملحي ولم يلاحظ هذا التأثير في الاصناف الأخرى. من ناحية أخرى ارتفع تركيز البوتاسيوم في جنور صنف سخا ٩٣ بزيادة تركيز كلوريد الصوديوم في المحاليل المغذية في وجود أو غياب السليكون بينما انخفض تركيز البوتاسيوم بزيادة الاجهاد الملحى في الأصناف الأخرى. توضح النتائج المتحصل عليها أن هناك آلية يملكها صنف سخا ٩٣ لمقاومة الاجهاد الملحى المتزايد أو الرفع مستوى تحمل الأملاح المحيطة بجنور النباتسات مقارنة بصنفي جميزة ٩ وجيزة ١٦٨. والدلائل الثلاثة المتحصل عليها وهي الخفاض تركيــز الــصوديوم وارتفــاع تركيــز البوتاسيوم وزيادة نسبة البوتاسيوم الى الصوديوم في جنور نباتات هذا الصنف هي دلائل تاكيدية مستخدمة للتعسرف علسي الأصناف النباتية المتحملة للأملاح. وقد أظهر صنف سخا ٩٣ بالإضافة لذلك قدرة عالية أيضا على خفض مستوى الصوديوم الممتص في الأوراق عند المستويات المرتفعة من الاجهاد الملحي (١٥٠ ملليمول/لتر كلوريد صوديوم) ولكن فقط في وجسود السليكون مما يدعم دور السليكون في تحسين مستوى تحمل الأملاح في صنف سخا ٩٣ في مستويات الإجهاد المحي المرتفعة سواء بالتربة أو بمياه الرى. وقد أوضح التجليل الاحصائي أن العامل الأكثر أهمية في تحمل الأملاح هو الصنف نفسه ويأتي السليكون في المرتبة الثانية بعد الصنف في تجسين ورفع مستوى تحمل الأملاح في الاصناف التي تملك آليات لتحمل الأملاح.