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RESEARCH ARTICLE

Interactive Effects of Silicon and Potassium Nitrate in Improving Salt Tolerance of Wheat

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Abstract

Adequate regulation of mineral nutrients might be effective to ameliorate the deleterious effects of salts and help to sustain crop productivity, particularly in glycophytes, under salt stress. In this study, laboratory and greenhouse experiments were carried out at Agricultural and Natural Resources Research Centre in East Azerbaijan, Iran, to investigate the interactive effects of silicon and potassium nitrate in alleviating NaCl induced injuries in wheat (*Triticum aestivum* L.). In the laboratory experiment, three winter wheat cultivars Pishgam, Afagh and Alvand were grown on sterile filter paper moistened with 20, 40, 60, 80, and 100 mmol L^{-1} NaCl solution. Results revealed that wheat cultivars were significantly different in their growth response to different concentrations of NaCl and Pishgam was found to be the most tolerant to NaCl stress, and used in the second part of study. In the greenhouse experiment, Pishgam was grown in a hydroponic system subjected to different NaCl levels (20, 60 and 100 mmol L^{-1}) and treated by silicon (0, 2 and 4 mmol L^{-1}, final concentration in nutrient solution using K_2SiO_3) and potassium nitrate (0, 0.5, 1, and 2 mmol L^{-1}, foliar application). The experimental design was factorial based on a completely randomized design with three replications. It was found that NaCl stress significantly increased proline accumulation and sodium content in the plant tissues while decreased potassium uptake and accumulation by plants. Moreover, plant weight, 100-seed weight, relative water content, chlorophyll content, and photosynthesis were also significantly affected by varying levels of NaCl. However, exogenous application of silicon and potassium nitrate reduced sodium uptake, increased potassium and consequently improved plant weight, 100-seed weight, seed yield, ear length, and photosynthesis rate. This study suggested that utilization of the salt-tolerant cultivar (Pishgam) combined with proper foliar application of potassium nitrate (2 mmol L^{-1}) and silicon (4 mmol L^{-1}) at the wheat booting stage might be a promising approach to obtain higher grain yield on saline lands.

Key words: germination, grain yield, foliar application, photosynthesis, proline, relative water content

INTRODUCTION

Salinity is the major stress factor, (Rueda-Puente *et al.* 2007) and is one of the most serious environmental problems influencing crop growth and production (Lopez *et al.* 2002). Excessive soil salinity, resulting from natural processes or from crop irrigation with saline water, occurs in many semi-arid to arid regions of the world where it affects plant growth and yield through osmotic effects, nutritional imbalances, oxidative damage, and/or specific ion toxicities (Mohsen *et al.* 2013). To sustain food security and the well-being of humankind, a priority should be given to minimize the detrimental effects of salt stress. Attempts to improve tolerance to salinity through physiological selection criteria has increased substantially to improve the probability of success by

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making empirical selection more efficient (Noble and Rogers 1992). Researches have shown that physiological activities such as photosynthesis and respiration (Alian *et al*. 2000), water balance, turgor pressure, degeneration of cell membranes (Kaya *et al*. 2002), efficiency of enzymes, mineral nutrients uptake, synthesis, storage of assimilates, and metabolites such as proline are directly affected by salt stress (Alia *et al*. 2001; Jamil *et al*. 2007). Plants have developed complex mechanisms that contribute to acclimate to salinity induced osmotic and ionic stresses (Meloni *et al*. 2004). Among different techniques, proper management of mineral nutrients plays a crucial role in increasing plant tolerance to salinity (Marschner 1995). A number of studies have shown that silicon may increase salinity tolerance in wide variety of plants through different mechanisms including reduced Na^+ uptake and translocation, improved plant water status (Romero-Aranda *et al*. 2006), increased photosynthetic activity and ultra structure of leaf organelles (Shu and Liu 2001), stimulation of antioxidant system (Zhu *et al*. 2004), and alleviation of specific ion effect by H-ATPase dependent enhancement of potassium in shoots (Liang *et al*. 2005). In addition, silicon is reported to reduce the effect of salinity on wheat and other crops. Saqib *et al*. (2008) have reported that silicon decreased plant sodium uptake and shoot:root sodium distribution of a salt-resistant as well as a salt-sensitive wheat genotype. They found that silicon increased cell-wall sodium binding, concentration of glutathione and antioxidants under saline conditions (Horst and Marschner 1978).

Silicon existing in the Earth's crust is classified as the most abundant element following oxygen (Epstein 1994). It is most commonly found in soils in different forms including silicon in soil solution as silicic acid ($Si(OH)_4$), and its absorption occurs directly in this form (Chen *et al*. 2010; Sharifnabi and Nili 2011; Bybordi 2012). This element is also one of the most abundant mineral elements in plant tissues (Richmond and Sussman 2003; Zhu *et al*. 2004). Although, silicon is not considered as an essential element for plant growth, it is designated as an element with a beneficial influence on plant growth and development as well as crop yield (Liang 1999; Ma 2004), and required in large amounts for Gramineae crops, in particular. Ample evidence indicated that silicon, when readily available to plants, had positive effects on plant growth, particularly under biotic and abiotic stresses. In addition, studies have shown that supplementing the soil with silicon can improve salt tolerance of barley (Liang 1999; Liang *et al*. 1999), maize (Shu and Liu 2001), rice (Kraska and Breitenbeck 2010), and wheat (Ahmad *et al*. 1992). Trenholm *et al*. (2004) have suggested that silicate crystals deposited in epidermal cells form a barrier that reduced water loss through the cuticles.

Romero-Aranda *et al*. (2006) have suggested that silicate crystals deposited in the epidermal cells form a barrier that reduces water loss through cuticle, which in turn, contributes to salt dilution, mitigating salt toxicity effects in tomato. More recently, Si benefits on salt tolerance of barley and cucumber have been related to antioxidant enzyme activity (Liang *et al*. 2003; Zhu *et al*. 2004).

Among the mineral nutrients, potassium is known to be very dynamic and a major contributor to the organic structure and metabolic functions of the plant. Potassium contents in plant tissues progressively decrease with increasing salinity. Therefore, maintenance of adequate level of K^+ is essential for plant survival under salt stress.

Potassium has substantial effect on enzyme activation, protein synthesis, photosynthesis, stomatal movement, and water relation in plants (Marschner 1995). Increased application of potassium has been shown to enhance photosynthetic rate, plant growth and yield in different crops under salinity water stress conditions (Egilla *et al*. 2001). For example, exogenous application of potassium ameliorated adverse effects of salt stress in wheat (Akram *et al*. 2007), rice (Bohra and Doerffling 1993), tomato (Lopez and Satti 1996), cucumber, and pepper (Kaya *et al*. 2001).

The aim of this study was to evaluate the effect of different concentrations of NaCl on plant growth and yield and the contribution of silicon and potassium nitrate foliar application to salt tolerance of wheat.

RESULTS AND DISCUSSION

Germination test

The results of analysis of variance on germination indexes are given in Table 1. There are significant differences between cultivars and NaCl stress levels. Comparisons of means showed that, irrespective of cultivar, increase in NaCl concentrations had deleterious effect on germination pace, germination percentage, radicle and shoot length as well as radicle and shoot weight (Table 2). Among cultivars, Pishgam cultivar showed the highest germination pace and percentage at all levels of NaCl. The results also indicated that Pishgam cultivar produced the highest radicle and shoot length and radicle and shoot weight. Therefore, Pishgam cultivar was selected as the most salt tolerant cultivar for further studies in the second part of the experiment.

Potassium nitrate, silicon and NaCl stress treatments had a significant effect on all studied traits (Table 3).

Proline accumulation

Comparison of means revealed that interaction between potassium nitrate, silicon and NaCl stress was significant on proline accumulation in Pishgam. Experimental treatments caused a significant increase in proline accumulation and the highest proline accumulation was observed when 4 mmol L^{-1} silicon and 2 mmol L^{-1} potassium nitrate was applied under mild and severe stress conditions (Table 4). On the other hand, the lowest proline

Table 1 Analysis of variance on wheat germination indexes affected by cultivar and salinity

Source of variation	*df*	Germination pace	Germination percentage	Radicle length	Shoot length	Radicle weight	Shoot weight
Cultivar	2	**	**	**	**	**	**
Salinity	4	**	**	**	**	**	**
Cultivar×Salinity	8	**	**	**	**	*	**
Error	30	2.46	0.35	8.84	10.69	0.00002	0.0009
Coefficient of variation (%)		2.97	0.87	10.44	6.86	8.20	15.57

* and **, significance at 0.05 and 0.01 probability levels, respectively. The same as below.

Table 2 Interaction between salinity and cultivar on wheat germination indexes

Cultivar	Salinity (mmol L^{-1})	Germination pace	Germination percentage (%)	Radicle length (mm)	Shoot length (mm)	Radicle weight (g)	Shoot weight (g)
Pishgam	20	90.83 a	99.16 a	84.21 a	84.93 a	0.17 a	0.92 a
	40	79.50 bc	96.50 b	30.5 d	71.16 b	0.08 c	0.12 c
	60	59.50 e	62.50 e	20.48 e	40.38 d	0.04 e	0.08 cdef
	80	41.50 g	50.50 h	14.45 fg	31.50 ef	0.02 g	0.04 efg
	100	25.16 i	39.50 k	8.33 h	53.41 c	0.01 hi	0.02 fg
Afagh	20	81.83 b	98.83 a	78.16 b	80.50 a	0.16 b	0.70 b
	40	71.50 d	95.50 c	29.21 d	38.16 d	0.07 d	0.10 cd
	60	49.50 f	61.50 f	18.58 ef	30.50 f	0.03 ef	0.08 cdef
	80	39.50 g	49.50 i	12.50 gh	52.50 c	0.02 g	0.03 fg
	100	23.50 i	37.50 l	7.28 h	36.45 de	0.006 i	0.02 g
Alvand	20	78.16 c	98.16 a	68.98 c	54.60 c	0.16	0.67 b
	40	59.50 e	93.50 d	20.65 e	26.16 f	0.06 d	0.09 cde
	60	39.50 g	59.50 g	14.51 fg	51.48 c	0.03 f	0.06 defg
	80	30.50 h	47.50 j	11.41 gh	37.50 d	0.01 gh	0.02 g
	100	20.16 j	22.50 m	7.50 h	25.68 f	0.01 hi	0.006 g

Values within the each column and followed by the same letters are not different at $P<0.05$ by an ANOVA protected Duncan's multiple range test. The same as below.

Table 3 Analysis of variance on some traits of salt tolerant wheat cultivar affected by salinity, silicon and potassium nitrate

Sources of variation[1)]	*df*	Proline	Sodium	Potassium	Silicium	Plant weight	Seed yield	100-seed weight	Ear length	Relative water content	Chlorophyll	Photosynthesis
B	2	**	*	**	**	**	**	**	**	**	**	**
K	3	**	**	**	**	**	**	*	**	**	**	**
S	2	**	**	**	**	**	**	**	**	**	**	**
N	2	**	**	**	**	**	**	**	**	**	**	**
K×S	6	ns	*	**	**	**	*	**	ns	**	**	ns
K×N	6	ns	**	**	**	**	ns	*	ns	**	**	ns
S×N	4	ns	**	**	**	**	*	**	**	**	**	ns
K×S×N	12	*	**	**	**	**	ns	**	ns	**	**	ns
Error	70	0.39	4.95	0.76	0.45	0.0005	1.07	0.05	0.16	3.79	0.40	5.27
Coefficient of variation (%)		5.14	7.47	2.28	4.66	4.22	3.31	6.16	6.72	3.90	1.50	13.28

[1)] B, block; K, potassium nitrate; S, silicon; N, NaCl.

ns, no significance.

Table 4 Interaction between potassium nitrate, silicon and salinity on some traits of salt tolerant wheat cultivars

Potassium (mmol L^{-1})	Silicon (mmol L^{-1})	Salinity (mmol L^{-1})	Proline (mg g^{-1} FW)	Sodium (mmol g^{-1} DW)	Potassium (mmol g^{-1} DW)	Silicium (mmol g^{-1} DW)	Plant weight per plant (g)	100-seed weight (g)	Relative water content (%)	Chlorophyll (mg g^{-1} FW)
0	0	20	8.20 q	0.24 k	0.30 lm	0.04 k	0.83 ef	4.20 efg	65.15 h	47.48 ij
		60	8.94 pq	0.45 f	0.26 n	0.06 j	0.55 lm	3.6 hijkl	35.14 op	37.51 o
		100	9.91 mnop	0.73 a	0.24 o	0.05 j	0.34 stu	2.89 op	20.18 v	17.85 v
	2	20	9.91 mnop	0.14 no	0.31 l	0.15 i	0.93 ab	4.60 bcde	75.1 1ef	55.48 ef
		60	10.51 lmn	0.29 j	0.28 mn	0.16 hi	0.64 j	3.83 ghij	44.1 1kl	44.51 l
		100	11.91 ijk	0.59 d	0.24 o	0.17 fgh	0.45 pq	3.29 lmno	25.18 st	24.33 t
	4	20	11.85 ijk	0.08 qrs	0.32 k	0.17 defg	0.74 h	4.80 bc	82.15 abc	57.51 cd
		60	13.21 fg	0.19 lm	0.20 p	0.18 bcdef	0.47 op	3.84 ghij	51.03 i	47.48 ij
		100	16.17 ab	0.39 gh	0.16 q	0.17 defg	0.26 wx	3.59 hijklm	3.11 w	27.51 r
0.5	0	20	9.14 opq	0.21 klm	0.38 hi	0.06 j	0.86 de	4.30 def	70.37 g	49.44 h
		60	9.21 opq	0.40 g	0.29 mn	0.06 j	0.56 lm	3.79 ghijk	38.11 no	39.48 n
		100	11.27 jkl	0.69 b	0.34 k	0.07 j	0.35 st	2.99 nop	21.11 uv	22.46 u
	2	20	11.27 jkl	0.11 opqr	0.54 c	0.16 hi	0.96 a	4.70 bcd	77.25 de	56.48 de
		60	12.01 hijk	0.241 k	0.39 gh	0.17 defgh	0.46 op	3.99 fgh	47.18 jk	45.48 kl
		100	13.11 fgh	0.53 e	0.37 ij	0.18 cdefg	0.68 i	3.39 jklmn	27.51 rs	25.51 s
	4	20	13.22 fg	0.07 rst	0.54 c	0.18 cdefg	0.76 gh	5.54 a	84.11 ab	58.48 bc
		60	13.52 ef	0.13 op	0.36 j	0.19 bcd	0.47 op	3.77 ghijk	52.14 i	48.48 hi
		100	16.48 ab	0.33 i	0.24 o	0.19 bc	0.29 vw	3.56 hijklm	34.14 p	28.41 r
1	0	20	9.01 pq	0.19 lm	0.49 d	0.06 j	0.88 cd	4.46 cde	72.14 fg	52.44 g
		60	10.23 lmno	0.36 hi	0.34 k	0.06 j	0.58 kl	3.77 ghijk	40.46 mn	42.53 m
		100	11.72 ijk	0.63 c	0.44 f	0.06 j	0.38 rs	3.30 lmno	22.13 tuv	24.50 st
	2	20	12.20 ghij	0.09 pqrs	0.53 c	0.17 gh	0.69 i	4.67 bcd	79.16 cd	57.50 cd
		60	12.20 ghij	0.22 kl	0.40 g	0.18 cdefg	0.42 qr	3.89 fghi	49.22 ij	46.51 jk
		100	13.50 ef	0.52 e	0.37 ij	0.18 cdefg	0.21 y	3.38 jklmn	30.11 qr	25.16 st
	4	20	13.64 def	0.05 st	0.66 b	0.18 cdefg	0.79 fg	4.98 b	85.18 a	59.44 b
		60	14.67 cd	0.12 opq	0.39 gh	0.18 bcde	0.50 no	3.98 fgh	52.14 i	49.16 h
		100	15.54 bc	0.21 klm	0.29 mn	0.19 b	0.31 uv	2.08 q	34.18 p	30.46 q
2	0	20	9.60 nop	0.17 mn	0.54 c	0.06 j	0.91 bc	4.48 cde	73.16 fg	54.46 f
		60	10.86 klm	0.36 hi	0.36 j	0.06 j	0.61 jk	3.98 fgh	42.14 lm	44.51 l
		100	12.27 ghij	0.68 b	0.46 e	0.06 j	0.41 qr	3.18 mno	24.14 tu	25.50 s
	2	20	11.40 jkl	0.09 pqrs	0.65 b	0.17 efgh	0.70 i	4.78 bc	81.11 bc	58.48 bc
		60	12.88 fghi	0.21 klm	0.47 e	0.18 cdefg	0.46 op	3.84 ghij	51.66 i	47.25 j
		100	14.81 c	0.50 e	0.30 lm	0.18 bcdef	0.24 xy	3.49 ijklm	37.81 no	24.51 st
	4	20	14.56 cde	0.03 t	0.70 a	0.18 bcdef	0.81 f	4.88 bc	83.11 ab	62.44 a
		60	16.05 ab	0.011 opqr	0.34 k	0.19 b	0.53 mn	3.35 klmn	51.14 i	51.50 g
		100	16.81 a	0.19l m	0.20 p	0.26 a	0.32 uv	2.58 p	33.11 pq	32.10 p

accumulation was found in plants which were not treated with silicon. Proline is an important osmolyte which synthesized in many micro organisms and plants which protect them against salinity and drought stress. Proline accumulation in plants exposed to salinity stress is due to low activity of oxidant enzymes (Sudhakar 2001). Increasing potassium nitrate concentration also increased proline content under NaCl stress (Table 4). Potassium nitrate induced proline accumulation under NaCl stress might due to an increase of ornithine amino transferase activity in ornithine pathway (Cao and Wei 2010).

Sodium, potassium and silicium contents

Sodium, potassium and silicium contents were affected by all experimental treatments. The highest sodium content was observed when severe NaCl stress was applied without any potassium or silicon treatment (Table 4). Silicon application especially at 4 mmol L^{-1} concentration significantly decreased sodium content in the plants. The effect of potassium nitrate varied from treatment to treatment. Regarding potassium content, the highest potassium content was observed in non stressed plants, receiving 2 mmol L^{-1} potassium nitrate and 4 mmol L^{-1} silicon (Table 4). Silicium content increased due to high level of silicon and potassium nitrate application (Table 4). The lowest silicium content was obtained under weak stress conditions without using silicon or potassium nitrate (Table 4). In an experiment with wheat (Ahmad *et al*. 1992), the addition of silicon to the solution cultures decreased the sodium content. In this study, added silicon was also found to significantly decreased sodium content under salt stress (Table 4). However, the mechanism by which silicon stimulated potassium uptake but inhibited sodium uptake remains poorly understood. As has been known, sodium is passively taken up by the plants and the uptake process is affected mainly by the transpiration rate (Marschner 1995). The reduced uptake of sodium by plants in this study can be explained at least partly by the inhibitory effect of silicon on the transpiration rate (Ahmad *et al*. 1992). While, potassium uptake and transport is an active process associated with ATP-driven hydrogen pump in the plasma membranes (Marschner 1995). One possible mechanism for stimulating effect of silicon on potassium uptake by plants under salt stress is the activation of HC-ATPase in the membranes.

Plant weight

The highest plant weight was obtained when 2 mmol L^{-1} silicon and 0.5 mmol L^{-1} potassium nitrate were applied on plants subjected to weak NaCl stress (Table 4). While, the lowest plant weight values were observed under severe NaCl stress along with 4 mmol L^{-1} silicon and 0 or 0.5 mmol L^{-1} potassium nitrate and also 2 mmol L^{-1} silicon and 1 or 2 mmol L^{-1} potassium nitrate (Table 4). The oxidative stress caused by salt stress can lead to a decrease in dry weight of plant (Bonilla and Tsuchiya 1998). It has been reported that wheat dry matter weight was significantly increased by the addition of a small amount of soluble silicon (Ahmad *et al*. 1992). Silicon supplementation resulted in improved plant growth and yield due to significant reduction in sodium content in the rice and barley shoots (Gong *et al*. 2005). Kaya *et al*. (2006) showed that application of silicon increased corn fresh and dry weight and improved grain yield. Potassium nitrate at 0.5 mmol L^{-1} increased plant weight; increasing potassium nitrate concentration in nutrient solution with NaCl has improved the possibility of potassium absorbance, and therefore relieves the adverse saline effects.

Seed yield

Interaction between potassium nitrate and silicon application as to seed yield is given in Fig. 1. A glance sat this Fig. reflects this fact that increase in silicon concentration increases seed yield. Moreover, potassium nitrate has significant effect on seed yield improvement so that the highest seed yield was obtained when 4 mmol L^{-1} silicon and 2 mmol L^{-1} potassium nitrate were applied. Interaction between salt stress and silicon application is shown in Fig. 2. Although, salt stress decreased seed yield, silicon application improved seed production at each level of NaCl.

Seed weight

Application of 4 mmol L^{-1} silicon and 0.5 mmol L^{-1} potassium nitrate significantly increased 100-seed weight. On the other side, the lowest seed weight was obtained when 4 mmol L^{-1} silicon and 1 mmol L^{-1} potassium nitrate were applied under severe NaCl stress conditions (Table 4). Akram *et al*. (2002) and Kamkar *et al*. (2004) reported that salinity reduced the yield primarily by a sever reduction in grain number and weight. Badr and Shafei (2002)

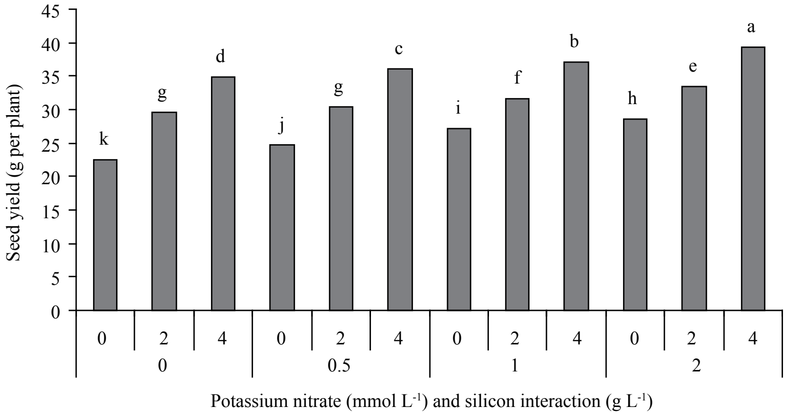

Fig. 1 Interaction between potassium nitrate and silicon on seed yield of Pishgam. Values within the each column and followed by the same letters are not different at $P<0.05$ by an ANOVA protected Duncan's multiple range test. The same as below.

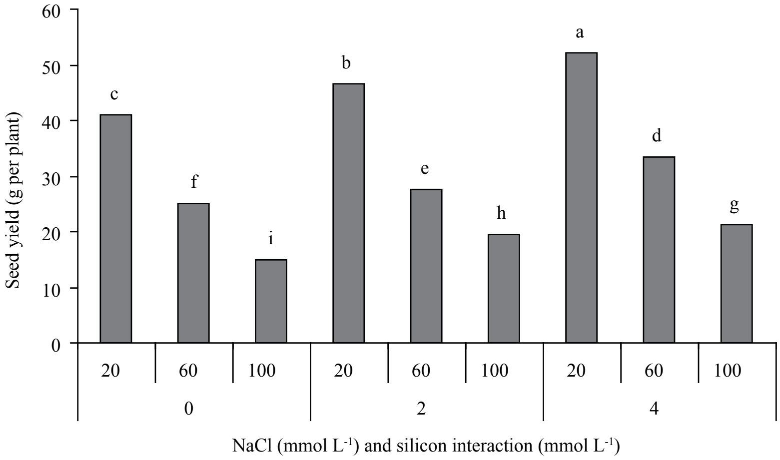

Fig. 2 Interaction between salinity and silicon on seed yield of Pishgam.

reported that increasing potassium application could be useful to overcome the adverse effect of salinity on the growth of wheat plant. It can be stated that the ability of plants to retain potassium at high sodium concentration, of the external solution, may be involved in reducing the damage associated with excessive sodium concentration in plant tissue. The results of researchers have shown that the silicon causes increase of seed weight in crops. In addition, Balastra *et al.* (1989) found that increasing of yield by the use of silicon in plants is due to increased grain weight.

Ear length

Main effect of NaCl, silicon and potassium nitrate application were significant on ear length (Table 3). As can be seen from Fig. 3, potassium application increased ear length so that increase in potassium concentration was parallel with increase in ear length. Fig. 4 shows two-way interaction between NaCl and silicon on ear length. The highest ear length was related to 4 mmol L^{-1} silicon and 20 mmol L^{-1} NaCl. It is clear from the data that increase in silicon concatenation in increases ear length linearly while increase in NaCl decreases this parameter.

Leaf relative water content

Leaf relative water content significantly increased due to application of 4 mmol L^{-1} silicon and 1 or 2 mmol L^{-1} potassium nitrate in plants subjected to weak salt stress. The lowest relative water content was observed under severe NaCl stress and minimum use of silicon (Table 4). Decrease in relative water content due to NaCl stress has been reported previously in lentil (Ashraf and Waheed 1993) and corn (Çarkirlar and Çiçek 2002). Kaya *et al.* (2006) found that 2 mmol L^{-1} Na_2SiO_3 increased leaf relative water content by 26.5% in corn. Furthermore, according to Gunes *et al.* (2008), silicon applied to the soil increased sunflower leaf relative water content. Li (2006) revealed that potassium application increased relative water content from 92 to 94% in tobacco leaf. Such increase with potassium application may be ascribed to improve cell turgor through osmotic adjustment.

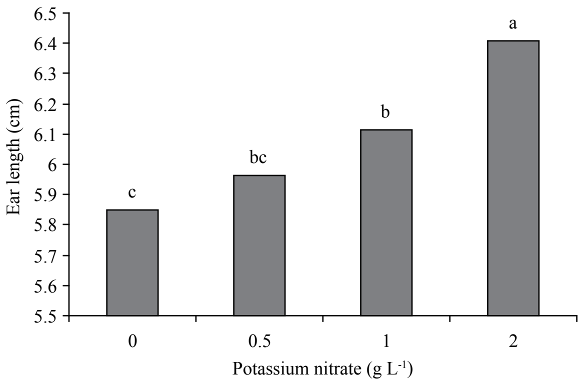

Fig. 3 Main effect of potassium nitrate foliar application on ear length of Pishgam.

Chlorophyll content

The highest chlorophyll content was observed when 4 mmol L^{-1} silicon and 2 mmol L^{-1} potassium nitrate were

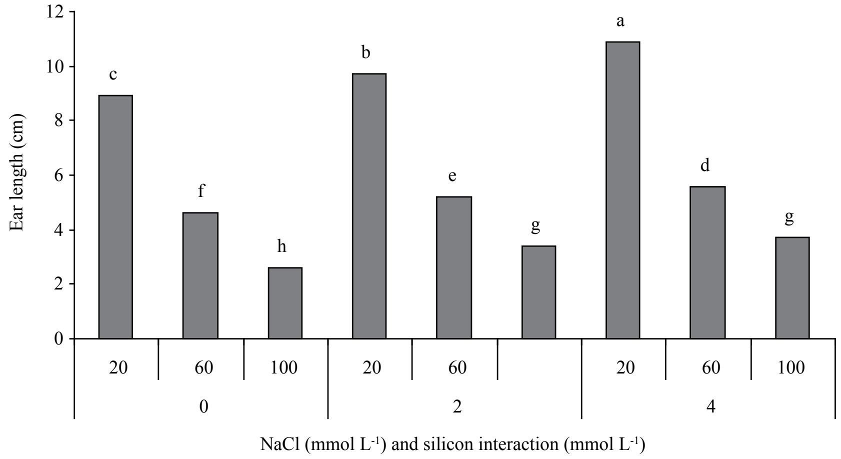

Fig. 4 Interaction between salinity and silicon on ear length of Pishgam.

applied on wheat plants grown under weak salt stress conditions. On the other hand, severe salt stress and no application of silicon along with 0 or 0.5 mmol L^{-1} potassium nitrate showed the minimum amount of chlorophyll in the plants (Table 4). There have been reports of a significant decrease in the total chlorophyll content of corn grown under salt stress (Levent Tuna *et al*. 2008; Celik *et al*. 2010). The results of the current study were in agreement with the findings of the studies that reported an increase in the total chlorophyll content after treating rice (Bonilla and Tsuchiya 1998) and barley (Liang *et al*. 2003), with silicon following salt stress treatment. Potassium is reported to improve water relations as well as productivity of different crops under environmental stresses (Islam *et al*. 2004).

Photosynthesis rate

Photosynthesis rate improved on account of potassium application (Fig. 5). Similarly, silicon application led to increase in photosynthesis rate in wheat plants so that the highest concentration of silicon showed the highest photosynthesis rate (Fig. 6). The authors have reported that added silicon increased the leaf net photosynthetic activity and depressed the uptake of sodium but stimulated the uptake of potassium by barley grown hydroponically, thereby enhancing the selectivity ratio. That silicon increases the photosynthesis of wheat plants might be associated with the increases in activities of photosynthetic enzymes, chlorophyll content and anthocyanin content. Adatia and Besford (1986) reported that the addition of silicon could increase the chlorophyll content and ribulosebisphosphate carboxylase activity in cucumber plants grown in recirculating nutrient solution. According to Jang *et al*. (1999), an accumulation of silicon in the leaves of plants in Poaceae family causes the leaves to erect, which facilitates light penetration, and promotes photosynthesis by significantly lowering the production of ethylene, which destroys chlorophyll. On the contrary, NaCl stress decreased photosynthesis dramatically (Fig. 7). Significant and positive correlation coefficient was found between proline and silicium, while significant and negative correlation was observed between proline and plant weight and proline and relative water content (Table 5). Sodium accumulation in wheat plants had significant and negative correlation with all studied traits. Conversely, potassium content showed the positive correlations (Table 5). Silicium and plant weight correlate to each other negatively, while this correlation is positive with seed yield and photosynthesis (Table 5). Plant weight indicates a significant and positive correlation with all studied traits. Similar results were obtained regarding seed yield, seed weight, ear length, relative water content, chlorophyll, and photosynthesis rate (Table 5).

CONCLUSION

In general, our results indicated that silicon may act to

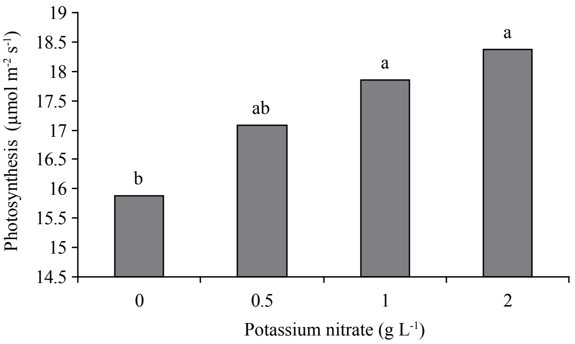

Fig. 5 Main effect of potassium nitrate foliar application on photosynthesis of Pishgam.

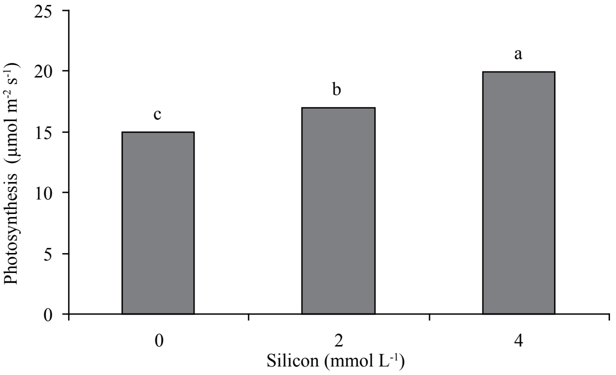

Fig. 6 Main effect of silicon on photosynthesis of Pishgam.

alleviate salt stress in wheat. Silicon partially offset the negative impacts of salt stress due to increase the tolerance of wheat to NaCl stress by enhancement of relative water content, chlorophyll content and photosynthetic activity. In addition, potassium nitrate foliar application could alleviate symptoms of the individual salt stress by improving water relations, nutrient uptake, chlorophyll content and photosynthesis.

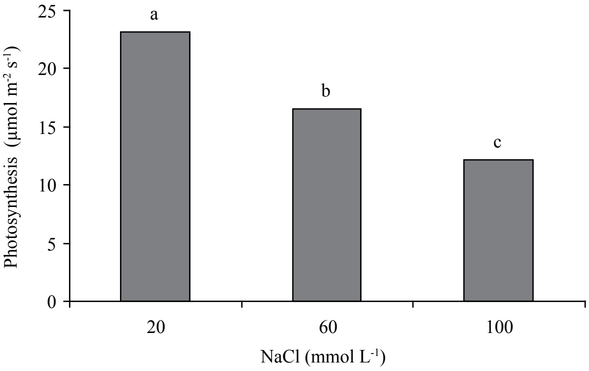

Fig. 7 Main effect of salinity on photosynthesis of Pishgam.

MATERIALS AND METHODS

Laboratory and greenhouse experiments were conducted at Agricultural and Natural Resources Research Centre in East Azerbaijan, Iran to investigate the effects of silicon and potassium nitrate application on salt tolerance of wheat (*Triticum aestivum* L.), during 2013. Initially, a factorial experiment in completely randomized design with three replications was carried out in the laboratory to assess salt tolerance of wheat cultivars. Uniform-sized seeds of three wheat cultivars Pishgam, Afagh and Alvand were surface sterilized with hydrogen peroxide for 10 min and 95% ethanol for 10 s. After that, seeds were rinsed thoroughly with sterile water for several times. 100-seed of each cultivar were placed on sterile deep dish plant germination Petri plates containing two sheets of sterile filter paper moistened with five different concentrations of sodium chloride solution (20, 40, 60, 80, and 100 mmol L^{-1}) and allowed to germinate in the dark at 24°C. The germinated seeds were counted everyday to calculate germination pace at the end of the 10-d period according to following formula:

Germination pace=$\Sigma(N_i/T_i)$

Where, N_i is the number of germinated seeds on day i and T_i is the number of days after sowing. The amount of survived germinated seeds was defined as percentage. In addition,

Table 5 Correlation coefficient between different traits of wheat affected by salinity, silicon and potassium nitrate

	Proline	Sodium	Potassium	Silicium	Plant weight	Seed yield	100-seed weight	Ear length	Relative water content	Chlorophyll	Photosynthesis
Proline	1										
Sodium	-0.15 ns	1									
Potassium	-0.08 ns	-0.43**	1								
Silicium	0.76**	-0.43**	0.01 ns	1							
Plant weight	-0.50**	-0.53**	0.53**	-0.19*	1						
Seed yield	0.004 ns	-0.83**	0.65**	0.26**	0.74**	1					
100-seed weight	-0.17 ns	-0.43**	0.47**	0.07 ns	0.70**	0.68**	1				
Ear length	-0.18 ns	-0.75**	0.66**	0.10 ns	0.83**	0.96**	0.77**	1			
Relative water content	-0.19*	-0.79**	0.68**	0.12 ns	0.79**	0.94**	0.66**	0.93**	1		
Chlorophyll	-0.14 ns	-0.86**	0.60**	0.15 ns	0.76**	0.91**	0.70**	0.90**	0.92**	1	
Photosynthesis	0.01 ns	-0.82**	0.62**	0.21*	0.60**	0.86**	0.46**	0.81**	0.85**	0.82**	1

radicle length and weight as well as shoot length and weight were also measured and average for all seeds was calculated.

Based on the results of laboratory experiment (Tables 1 and 2), Pishgam, the most salt-tolerant cultivar, was chosen for investigating the interactive effects of silicon and potassium nitrate in salt tolerance of wheat. For this purpose, a randomized complete block design with three replicates was used. Candidate wheat seeds were surface sterilized as mentioned above and sown in plastic pots (25 cm in diameter and 30 cm in height) filled with perlite and vermiculite (1:1; v:v). The pots were placed in greenhouse subjected to natural light. 1 L of fresh half strength Hoagland nutrient solution (pH 5.6) was used everyday and full strength Hoagland nutrient solution was introduced at three-foliate stage when seedlings were reduced from ten to five plants per pot. At this time, NaCl and silicon treatments were started by adding sodium chloride (NaCl) and K_2SiO_3 to 1 L of nutrient solution. Final concentrations of sodium chloride were 20, 60 and 100 mmol L^{-1} while silicon 0, 2 and 4 mmol L^{-1} in nutrient solution. Potassium nitrate foliar treatments were applied at stem elongation and booting stage at 0, 0.5, 1, and 2 g potassium nitrate L^{-1}. The potassium sprays were prepared by diluting the appropriate amount of potassium nitrate in distilled water and adjusting to pH 5.5. One drop of Tween 20 surfactant was added to 500 mL of spray solutions. All vegetative and generative parts of the plants were subjected to different treatments of potassium nitrate. 1 L of solution was sprayed onto the plants two times successively at 20 min intervals. For all control treatments water, instead of the K solution was sprayed.

Data collecting

At seed filling stage, photosynthetic active radiation of flag leaf was recorded by a data logger (Meteodata-256, Geonica, Madrid, Spain). Data collecting was performed at 10 o'clock in the morning. Five plants in each plot were selected randomly, and flag leaves were removed to evaluate leaf relative water content, proline accumulation and chlorophyll concentration.

Relative water content was determined for detached wheat leaves using the method cited by Mata and Lamattina (2001) according to the formula below:

Relative water content (%)=(FW-DW)/(TW-DW)×100

Where, FW, fresh weight; DW, dry weight (obtained after drying the samples at 80°C for at least 48 h); TW, turgor weight which was determined by subjecting leaves to rehydration for 2 h.

Proline content of leaves was determined according to a modification of the method of Bates *et al*. (1973). Samples of leaves (0.5 g) were homogenized in a mortar and pestle with 10 mL sulphosalicylic acid (3% w/v), and then centrifuged at 18000 ×g for 15 min. Two millilitres of the supernatant was then added to a test tube, to which 2 mL glacial acetic acid and 2 mL freshly prepared acid ninhydrin solution (1.25 g ninhydrin dissolved in 30 mL glacial acetic acid and 20 mL 6 mol L^{-1} orthophosphoric acid) were added. The test tubes were incubated in a water bath for 1 h at 100°C and then allowed to cool to room temperature. Four millilitres of toluene were then added to the tubes and mixed on a vortex mixer for 20 s. The test tubes were allowed to stand for at least 10 min to allow separation of the toluene and aqueous phases. The toluene phase was carefully pipetted out into a glass test tube and its absorbance was measured at 520 nm in a spectrophotometer. The content of proline was expressed as mg g^{-1} FW.

Chlorophyll was extracted in 80% acetone from the leaf samples, according to the method of Arnon (1949). Extracts were filtrated and then absorbance of chlorophyll *a* and *b* were determined by a spectrophotometer (UV-S, Sinco 2100) at 645 and 663 nm. The content of chlorophyll was expressed as mg g^{-1} FW.

At the end of growing period, when wheat plants turned yellow, all plants were harvested and single plant weight, single plant yield, 100-seed weight, and ear length were determined. Moreover, sodium, potassium and silicium contents were assayed in whole part of the plants. Potassium and sodium were measured using a flame-photometer (JenWay PFP7, Burlington, NJ, respectively). The total silicium content was measured using an atomic absorption method (Model GBC 932).

Statistical analysis

The first and second experiments were structured in a completely randomized design arranged in 3×5 factorial and randomized complete block design arranged in 4×3×3 factorial with three replications, respectively. For all variables, analysis of variance (ANOVA) was performed to test for differences between NaCl, silicon and potassium treatments and their interactions using the GLM procedure in SAS ver. 9.1. Main and interaction effects of experimental factors were determined. Where interactions between two factors were significant, we presented the results in the form of a combination of treatments and not separately or individually. The significance of differences among treatment means was compared by Duncan's multiple range teat at the 5% probability level.

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