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The effect of silicon on germination and some growth characteristics of salt-stressed canola seedling

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Abstract

The effects of salinity and supplemental silicon on germination and some seedling traits of canola, cv. Talayeh was investigated in a complete randomized block design. The first factor was 5 levels of potassium silicate (0, 1, 2, 3 and 4 g/l) and different levels of salinity as second factor (including 0, 60, 180, 120, and 250 mM NaCl). Results showed that radicle length, plumule length, radicle dry weight and plumule dry weight influenced by salinity and silicon. The highest germination percentage, germination ratio and the lowest mean germination time were observed in non saline treatment. Application of 2 g/l treatment of silicon showed the highest value for germination percentage and germination ratio and the lowest one for mean germination time compared to other treatments. Moreover, 2 g/l treatment of Si with 60 mM concentration of NaCl led to highest length of radicle and plumule. Salinity had significantly negative effect on the all studied characteristics, but application of supplemental Si improved those traits. The lowest means among treatments was in 180 and 250 mM concentration of salinity in non-Si treatment. application of Si showed no significant effect at higher concentration.

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Introduction

Salinity is an important environmental limiting factor affecting over 800 million hectares of cultivated lands (Munns, 2002). In many plant types, germination and seedling growing phase is very sensitive to salt stress. In general, the highest germination percentage occurs in non-salty conditions and it decreases depending on the ascending salt concentrations. (Khan *et al.*, 2000). Silicon is not considered an as essential element for plants growth. Results of various studies showed that silicon application significantly affects plants growth in normal (Agurie *et al.*, 1992) and biotic and abiotic stress condition (Ma, 2004). Silicon in a chemically combined form is ubiquitous in nature. The Si content of soils can vary dramatically from <1 to 45 % dry weight (Summer *et al.*, 2006), and its presence in the form of silicate acid [Si(OH)4] (or its ionized form, $Si(OH)_3O_2$, which predominates at pH > 9)] allows its uptake by plants (Epstein, 1994). Silicon deficiency usually leads to structurally weak plants subjected to abnormal growth, development and reproduction behaviors. Silicon is only element that its high levels of accumulation doesn't damage plants (Epstein, 1999). Some possible mechanisms, such as improving water content (e.g. Water content improvement), photosynthesis enhancement, stimulation of antioxidative systems, and reduction of toxic ions effects by reducing sodium uptake and increasing potassium uptake and/or H-ATPase activity are attributed to silicon that increase resistance to salinity (Liang *et al.*, 2005).

Brassica napus, is one of the three important species of Brassica family which largely cultivated in Iran (Alyari *et al.*, 2000). Although, rapeseed recognized as a salt tolerant crop, but saline soil or irrigation with saline water may reduce yield potential of canola (Shekari *et al.*, 2000). It is reported seed germination, seedling emergence and early survival of the seedling may represent salt sensitivity of a crop (Shekari *et al.*, 2000 ; 2009). In general, quality and quantity of a crop plant is depending on percentage, ratio and uniformity of emergence. Therefore, germination stage is critical and significant phase which appropriate establishment of seedlings will result in enhanced crop yield.

The aim of this study was to investigate the influence of salt-stress on germination and seedlings growth of canola, and to assess the enhanced effect of silicon on stress tolerance.

Materials and methods

Germination response of *canola, cv. Talayeh* to salinity and silicon supplementation regimes was evaluated in a completely randomized experimental design with three replicates at Library in November of 2011. Seeds of canola Talayeh, a winter and high yielding cultivar, were obtained from Seed and Plant Improvement Institute, Karaj, Iran. The first factor included 5 levels of potassium silicate (0, 1, 2, 3 and 4 g/l) and the second one included different levels of sodium chloride salinity (include 0, 60, 180, 120 and 250 mM). 50 seeds were surface sterilized in 5% Sodium hypochlorite solution. Then, they were laid between two layers of filter papers (Whattman No. 1) in 10 cm diameter sterilized Petri dishes. 5 ml of desired solution was added to each Petri dish. Petri dishes were placed within an incubator holding the constant temperature at 20 °C. The first count of germinated seeds (seeds were considered to be germinated when they retched to 5 mm length of radicle and plumule at least) was started 24 hours after starting experiment and continued at regular one day intervals for 7 days .The *counting* process lasted for until all seeds were germinated or no other seeds were able to germinate.

To test seedling growth, after sterilizing worktable with alcohol, 50 seeds of each treatment were arranged with a distance of 7 cm from the edge in paper towel, covered with another paper towels. These towels were put in 1000 ml beakers and add 100 ml of each treatment solution. Towels then were covered with a plastic bag. The treatments then were kept in growth chambers holding 25 °C for 7 days. After this period, all paper towels were opened and normal seedlings were counted. After removing the cotyledons, radicle and plumule length were measured immediately. Fresh weight of radicle and plumule were measured. The radicle and plumule of each treatment were conditioned in paper bags and oven-dried at 72 °C for 24 h and were weighed to obtain dry weight. Germination percentage (equation No. 1), mean time to germination (equation No. 2) and germination ratio (equation No. 3) determined as Ellis & Roberts equation (1980):

Germination percentage $(\mathrm{GP}) = 100\left(\frac{\mathrm{ni}}{\mathrm{N}}\right)$ (equation No. 1)

Mean time to germination $(\mathrm{MTG}) = \frac{\sum(\mathrm{ni}*\mathrm{di})}{\mathrm{N}}$ (equation No. 2)

Germination ratio $(\mathrm{GR}) = \frac{\mathrm{N}}{\sum(\mathrm{ni}*\mathrm{di})}$ (equation No. 3)

Where; *d* is the day on which germination count was made, *n* is the number of seeds germinated on the day of d and *N* is total number of germinated seeds. MTG and GR are inversely proportional. The analysis of variance was run using the software SAS (90) and MSTATC. The means were compared using Duncan's multiple range tests and graphs were plotted by Excel Microsoft Office.

Results and discussion

Salinity and silicon had significant effects on all studied characteristics. The Simple and interaction effects of radicle length, plumule length, radicle dry weight, plumule dry weight as well as simple effects of germination percentage, germination ratio and mean time to germination were significant (Table 1).

Germination percentage

The non-Saline treatment differed significantly from other treatment and had highest germination percentage. The lowest germination percentage was found in 250 mM NaCl treatment (table 2). Application of 2 g/l Si increased germination percent, but this treatment had not significant difference with 1 g/l Si treatment. 4g/l Si treatment showed the lowest GP (table 2). It has been reported that in response to environmental stress, plants represents various resistances with respect to their phonologic stages. The early growth stage is considered as most sensitive stage among all phonological stages (Eslami *et al.*, 2009). Since, weak germination and reduced seedling growth leads to poor establishment of seedling and/or completely break down of it. Therefore, it is vital for plants in this stage to be resistant to salinity (El-Keblawy and Al-Rawai, 2005 Soltani *et al.*, 2006). This stress lead to reduced seeds germination through restriction of water uptake, reduction in decomposition of seed storage material and impairment in storage protein synthesis process (Ashraf and Foolad, 2005; Voigt *et al.*, 2009). Furthermore, Na and Cl toxicity are important factor in decreasing seed germination indexes (Hanslin and Eggen, 2005). It has been suggested that salinity stress causes induced dormancy and reduced GP due to reduced water requirement for imbibitions and toxicity effects of Na and Cl ions (Zia and Khan, 2004; Sosa *et al.*, 2005; Ghars *et al.*, 2009). In addition, the accumulation of toxic ions in cytoplasm may result in accumulation and toxicity of specific ion which lead to metabolism disruption of essential elements required for seedling growth and availability to these growth elements restrict (Gorham, 1996). It is reported that, application of 1, 2, 3 and 5 mM concentration of silicon on *Momordica charantia* significantly increased germination rate, germination index, vigor index, malondialdehyde content, superoxide dismutase, catalase and peroxidase activities both under salt stress and normal conditions compared to non application of Si. (Moussa, 2006).

Germination ratio

GR in Non-salinity treatment was high and showed significant difference compared to other treatments. Moreover, the lowest GR was recorded in 250 mM concentration of salinity (table 2). The results revealed that 2 g/l treatment of Si had maximum GR and had not significant difference compared to 1 g/l Si (table 2). Jamil *et al.*, (2008) studies on

Brassica species (canola, cabbage and cauliflower) and Al-Harbi *et al.*, (2008) studies on tomato cultivars showed that GR markedly reduced in high concentration of salinity compared to control conditions.

Table 1. Mean squares for salinity and silicon treatment of canola.

		Mean squares								
S.V	df	Radicle length	Plumule length	Radicle Fresh weight	Plumule Fresh weight	Radicle Dry weight	Plumule Dry weight	GP	MTG	GR
R	2	ns 3.50	** 3.03	ns0.0007	ns 0.56	ns 0.00002	ns 0.0009	ns 209.91	** 1.76	** 0.078
Si	4	** 15.97	** 12.24	** 0.041	** 2.01	** 0.00027	** 0.00065	** 802.34	** 1.23	** 0.074
Nacl	4	** 9.96	** 3.31	** 0.012	** 0.40	** 0.00004	* 0.0001	** 2103.10	** 2.83	** 0.226
Si*Nacl	16	** 2.74	** 1.30	** 0.0069	ns 0.079	** 0.00002	** 0.00016	ns 151.96	ns 0.13	ns 0.019
error	48	1.13	0.45	0.0015	0.079	0.000007	0.00003	94.32	0.19	0.008
CV		13.03	14.40	14.48	14.27	15.38	12.75	16.30	19.45	19.14

*,**,Significant at 0.05, 0.01 probability , ns = Non-significant;

Table 2. Mean comparison for different levels of salinity and silicon.

T	MTG	GR	GP (%)	T	MTG	GR	GP (%)
	Silicon levels				Salinity levels		
0	2/45 a	0/44 b	56/04 b	0	1/54 c	0/68 a	77/93 a
1	1/90 b	0/ 55 a	67/09 a	60	2/31 ab	0/45 b	60/82 b
2	1/96 b	0/557 a	67/84 a	120	2/24 ab	0/44 b	57/31 b
3	2/43 a	0/42 b	53/40 b	180	2/39 b	0/44 b	56/52 b
4	2/47 a	0/41 b	53/37 b	250	2/73 a	0/36 b	45/15 b

Note. Means followed by a common letter are not significantly different at 1 % level by DMRT

Mean time to germination

250 mM concentration of NaCl had maximum MTG and this treatment didn't show significant difference compared to 60, 120 and 180 mM treatments. The lowest MTG was recorded in control treatments (table 2). The lowest MTG was recorded in 1 g/l Si treatment. This treatment had no significant difference with 2 g/l treatment of Si (table 2). MTG increased markedly by increasing salt concentration. Karagüzel (2003) expressed mean germination time in many plant species significantly increased by increasing salt concentrations. According to Ozcoban and Demir (2006) findings, salinity had more influence on MTG than GP, implies that high level of salt increases the MTG. Jeannette (2002) has reported that MGT increased by increasing in NaCl levels for most species of the genus *Phaseolus* and this increase was greater in higher levels of NaCl.

Radicle and plumule lengths

The highest length of plumule was observed in 60 mM salinity and 2 g/l of Si. This treatment had significant difference compared to other treatments. The minimum radicle length was recorded in 250 mM NaCl and non- Si treatment (Table 3).

The highest radical length was found in 2 g/l liter treatment of Si in 60 mM concentration and showed no significant difference compared to non-saline and 2 g/l Si treatment. Minimum length of radicle was observed in 250 mM and non-Si treatment

(Table 3). 120 mM NaCl with 4 g/l Si treatment had maximum dry weight of plumule. The seedlings which exposed to 250 mM NaCl and without any application of Si had the lowest dry weight of plumule (Table 3). Shekari *et al.* (2000, 2009) showed radicle and plumule length and dry weight was decreased by increasing salt levels.

Table 3. Means comparison of interaction effect of growth parameters affected by different levels of salinity and Si.

Si	Nacl	Radicle Length (cm)	Plumule Length (cm)	Radicle dry weight (g)	Plumule dry weight (g)	Radicle fresh weight (g)	Plumule fresh weight (g)
0	0	8/39 bcdef	4/43 cde	0/ 016cdefg	0/044 cde	0/278 cdef	1/66 defg
	60	8/23 bcdefg	4/12 cde	0/014 defg	0/035 def	0/208 efg	1.644 fgh
	120	6/5 defg	3/61 de	0/011 fg	0/034 def	0/208 efg	1/54 fgh
	180	5/74 fg	3/86 cde	0/01 g	0/032 ef	0/146 g	1/35 h
	250	5/ 51 g	3/54 e	0/01 g	0/029 f	0/143 g	1/363 h
1	0	8/02 bcdefg	4 /18 cde	0/018 bcdef	0/035 def	0/257 ef	1/673 defg
	60	7/54 cdefg	4/16 cde	0/015 cdefg	0/054 abc	0/239 efg	1/761 defgh
	120	7/16 cdefg	3/56 de	0/014 defg	0/045 cde	0/257 ef	1/872 cdefgh
	180	7/66 cdefg	3/54 e	0/014 defg	0/041 cdef	0/23 efg	1/669 defgh
	250	6/02 efg	3/58 de	0 /013 efg	0/034 def	0/19 g	1/309 h
2	0	10/56 ab	6/27 b	0/023 b	0/042 cdef	0/281 cdef	2/019 abcdefgh
	60	11/93 a	8/45 a	0/031 a	0/045 cde	0/375 abc	2/57 a
	120	9/29 bcd	5/62 bc	0/024 bc	0/051 abc	0/36 abcd	2/366 abcde
	180	7/3 bcefg	4/80 bcde	0/018 cdef	0/051 abc	0/267 def	2/289 abcde
	250	7/21 cdefg	4/67 bcd	0/021 bc	0/060 ab	/268 def	2.179 abcdef
3	0	8/96 bcd	5/33 bcd	0/02 bcde	0/044 cde	0/294 bcde	1/96 abcdefgh
	60	9/36 bc	5/32 bcde	0/018 bcde	0/044 bcd	0/307 bcde	2/609 a
	120	9/12 bcd	5/55 bc	0/018 bcde	0/048 cde	0/28 cdef	2/395 abcde
	180	8/26 bcdef	5/28 bcde	0/016 cdefg	0/045 cde	0/275 def	2/309 abcde
	250	8/42 bcdef	4/47 cde	0/016 cdefg	0/043 cdef	0/253 ef	1/863 bcdefgh
4	0	8/46 bcdef	4/65 bcde	0/017 bcdef	0/045 cde	0/266 def	2/142 abcdef
	60	8/16 bcdefg	4/51 cde	0/016 cdefg	0/045 cde	0/283 cdef	2/185 abcdef
	120	8/87 bcd	5/01 bcde	0/02 bcd	0/063 a	0/38 ab	2/291 abcde
	180	8/87 bcd	4/63 bcde	0/021 bcd	0/061 ab	0/432 a	2/472 abc
	250	8/63 bcde	4/24 cde	0/015 cdefg	0/043 cdef	0/249 ef	2/071 abcdef

Note. Means followed by a common letter are not significantly different at 1 % level by DMRT

The results of studies on seed germination of crop plants suggested that by increasing salt levels plumule and radicle length as well as dry weight of seedlings, decreased significantly. One possible reason for this is the reduction of nutrients or non-transferability of these nutrients from cotyledons to embryos. Reduced seed water uptake in stress condition is another reason leads to decreased enzymes activities, turgid pressure, hormones *secretion* and subsequently seedling growth impairment (kafi *et al.*, 2005). Okio *et al.* (2005) and Hanafy *et al.* (2008) reported that the effect of Si on shoot lengths of wheat plant may be due to inductive effect of Si on *cell development* and cell division through affecting the RNA, DNA synthesis. These results are *in accordance with* the Parvin and Ashraf (2010) findings for significant effect of Si on the shoot length of maize cultivars under saline regimes. It is recorded that application of 100 ppm of Si could increase shoot length in rose (Hwang *et al.,* 2008 and Reezi *et al.*, 2009) and wheat (Hanafy Ahmed *et al.*, 2008).

Plumule and radicle fresh weight

The highest radicle fresh weight was observed in 180 mM NaCl and 4 g/l Si treatment. This treatment showed no significant differences compared to 120 mM salinity and 4 g/l Si and 60, 120 mM salinity with 2 g/l Si treatments. Minimum radicle dry weight was recorded in 250 mM and non-Si treatment. This treatment showed no significant difference with all non-Si 60, 120 and 180 mM. The highest value of plumule fresh weight was obtained in 60 mM salinity with 3 g/l Si. This treatment showed no significant difference compared to all levels of salinity in 2 g/l treatment of Si and to all levels of salinity in 4 g/l treatment of Si. Lowest mean for fresh weight of plumule was obtained in non-Si treatment of 180 and 250 mM (Table 3). Hashemi et al. (2010) reported that salinity reduced growth parameters such as fresh and dry weight of seedlings and this reduction was accompanied by increased lignin content, sodium accumulation and increased lipid peroxidation. Osmoregulation can reduce growth sensitivity to water and salinity stress or leads to slight increase in growth rate under water stress by maintaining turgor. So, reduced fresh weight and subsequent changes in fresh weight of seedling could be due to turgor pressure adjustment (Bassirirad et al., 1992). Reduced fresh weight can be explain by reduction in water content of seedling tissues by decreasing water uptake of seedlings, that is in accordance with Sharma et al. (2004) findings concerned fresh weight reduction due to reduced water content of tissues under increased salinity.

It seems that one possible reason for reduced plumule weight at low water potentials is low mobility and transferability of nutrients from cotyledons to embryonic axis. It is worth noting, factors that affect growth rate of embryonic axis could influence mobility and transferability of nutrients from cotyledons to embryonic axis (Zhang *et al*., 2003).

Plumule and radicle dry weight

Maximum plumule dry weight was observed in 120 mM salinity and 4 g/l Si treatment. The lowest plumule dry weight was found in 250 mM salinity and without application of Si (table 2). Maximum radicle dry weight was observed in 60 mM salinity and 2 g/l Si treatment and showed significant difference compared to other treatments. Minimum radicle dry weight was recorded in 250 mM NaCl and non-Si treatment (table 3). Amylase, protease and phosphatase as hydrolytic enzymes of seed storage material, are necessary for germination. These hydrolyzed compounds are used for tissue production at germination stage (Soltani *et al*., 2006).Since water availability to seeds is reduced under osmotic stress (Prisco *et al*., 1992), so hydrolyzing of stored seed materials impaired and leads to reduced seedling dry weight. Studies have shown that salinity decrease rate of water and nutrients uptake of plants through root growth inhibition (Jamil *et al.,* 2006). The researches have demonstrated that plants respond to salt stress conditions by producing organic compounds such as proline and glycine and accumulating minerals for osmoregulation. Since production of these materials is an energy consuming process, reduced growth and dry weight occurred in this condition (Serraj and Sinclair, 2002). Kaori *et al*. (2011) reported by application of silicon on sorghum roots under salt stress conditions the reduction of root dry weight was less than control plants and water uptake increased significantly. Silicon application reduced root osmotic potential with no effects on water content, indicating that osmoregulation has occurred by water uptake improvement. Mohaggeg (2009) state that Si application on cucumber increased dry and fresh weight of shoot and root as well as plant height. Optimal nutrition with Si leads to increased root weight and root volume and subsequently increased elements adsorbing surface (Sun *et al*., 2005). Hashemi *et al*. (2010) reported that Si resulted in increased growth parameters, prevented lignination and sodium accumulation in foliage, decreased lipid peroxidation in root and increased chlorophyll concentration. Parveen and Ashraf (2010) showed that silicon application significantly increased root dry weights in two maize cultivars under salt stress conditions. Silicon application may contribute to improved nutrition balance in salinity condition and results in effective growth and higher yields (Tahir *et al.,* 2006). Immobilization of toxic sodium ion, reducing sodium uptake, increment of potassium uptake and potassium to sodium selectivity are among some possible mechanisms that Si may induced in plants (Liang *et al*., 2005). Ashraf (2008) reported that calcium silicate amendments significantly decreased sodium uptake and transfer in two sensitive and tolerant genotypes of sugarcane. But potassium contents of roots and shoots in two genotypes of sugarcane was accompanied by higher Na^+/K^+ ratio as a suitable index for of salt tolerances. Contents of foliage Si showed positive correlation with shoot dry matter in two genotypes. The results suggest that Si have an interaction effect with reduced Na uptake and its transfer to foliage, therefore ,stalk yield and molasses quality of two sensitive and tolerant genotypes improved. The results of Bandani and Abdolzadeh (2007) researches on *Puccinellia distans* showed that salinity led to reducing in growth rate while application of Si resulted in enhanced growth and increased dry weight. Salinity increased sodium content in root and shoot. However, Si treatment reduced sodium ion concentration. It has been assumed that reduced sodium uptake in Si treatments is due to the inhibitory effect of Si on transpiration rate in salt stress. Hashemi *et al.*, (2010) found that Si application reduced deteriorating effect of salinity on canola through reduced Na^+ content of tissues, maintaining membrane integrity of root cell by reducing lipid peroxidation, decreased oxygen scavenging function and reduced lignination.

In general, by increasing salinity levels some parameters such as germination rate and percent, root and shoot length or weight decreased. But treatment with Si led to improve the mentioned traits and decreased unfavorable effects of salinity. Among Si treatments, the highest values were found in 2 g/l potassium silicate concentration.

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