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The effect of exogenous silicon on seed germination and seedling growth of wheat cultivars under salt stress conditions

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Wheat Silicon Salinity Germination Seedling growth ABSTRACT- Seed germination and early seedling growth are critical stages for plants establishment and production, particularly under salinity conditions. Exogenous application of silicon (Si) can enhance germination as well as seedling growth. In this experiment, the effect of priming with Si (0, 0.75, 1.5 and 2.25 mM sodium silicate) on seed germination and seedling growth under NaCl (0, 100 and 150 mM) conditions was studied in two wheat cultivars of Kavir (salt tolerant) and Shiraz (salt sensitive). The experiment was designed as a factorial based on completely randomized design with three replications in the cereal laboratory of college of Agriculture, Shiraz University, in 2012. Results showed that seed priming by Si improved germination percentage, germination rate, vigor index, shoot and root length and seedling dry weight in both stress and non-stress conditions. Moreover, Si increased K⁺ uptake and K⁺/Na⁺ ratio and decreased Na⁺ content of cultivars with the effect of 2.25 mM being more pronounced. On the contrary, salt stress reduced the above traits and K⁺ uptake and K⁺/Na⁺ ratio and increased mean germination timeand Na⁺ uptake in both cultivars with the negative effects of 150 mMNaCl being more severe. However, the tolerant cultivar (Kavir) accumulated less Na⁺ and more K⁺ and had greater K⁺/Na⁺ ratio compared to nontolerant cultivar (Shiraz). Although the salinity adversely affected seed germination and seedling growth in both cultivars, Kavir (tolerant cultivar) was less affected. It was concluded that priming with Si may promote germination and subsequent seedling growth of wheat cultivars under salinity conditions by reducing Na⁺ in favor of K⁺ accumulation.

INTRODUCTION

Wheat (Triticum aestivum L.) is an important food crop grown widely in the world where its yield might be affected by adverse environmental conditions(Borjian and, Emam 2001; Emam, 2011). Of the relevant factors in wheat establishment, salt is the main cause of severe yield reductions. Soil salinity is the major problem in arid and semi-arid regions of the world where out of 270 million ha of irrigated lands, 110 million ha are located in this region (Smedema and Shiati, 2002). High concentration of soluble salts in upper soil layers prevents or reduces germination of seeds, thereby affecting crop establishment. Most crop species are quite susceptible to salt injury at germination and the stand establishment stages (He and Liu, 2002; Wang et al., 2009). High germination rate of seeds would result in better establishment of seedlings particularly under stress conditions. Improving salt tolerance of crop plants may decrease the detrimental effects of soil salinity on germination of seeds (Zhu, 2000).

Saline agriculture technology is one approach for effective utilization of salt affected soils, which involves the cultivation of salt tolerant plants (Ahmad et al., 2007; TaleAhmad and Haddad, 2011; Ahmed et al., 2011; Ahmed and Khurshid 2011). This approach is often not suitable for crops with few salt tolerant cultivars. Breeding of salt tolerant crops is another option to tackle this problem. However, the classical screening method for salt tolerance based on yield response to salt is very expensive, space consuming and slow (Munns and James, 2003). In contrast, pre-sowing seed treatment is an easy, low cost and low risk technique and an alternative approach recently used to overcome agricultural salinity problems. Many molecules such as silicon (Si), jasmonic acid, salicylic acid and polyamines have been suggested as signal transducers and messengers which may have profound effects on plant growth and development (Hattori et al., 2005;

Krantev et al., 2008; Tale Ahmad and Haddad, 2011; Ahmed and Khurshid, 2011).

Si is the second most abundant element in the earth's crust (Epstein, 1999; Ahmed et al., 2011; Ahmed and Khurshid, 2011). It is in plants, especially in grasses, in amounts equivalent to those of macronutrient elements such as calcium, magnesium, and phosphorus (Ahmed et al., 2011; Ahmed and Khurshid, 2011). Si in the form of mono silicic acid is actively absorbed by the root system in several plant species and its uptake and transportation is well characterized in plants (Ma and Yamaji, 2006). Silicon subsequently accumulates as

insoluble silicon compounds, primarily in cell walls, and it is also deposited on the plant surfaces after transpiration. Moreover, Si has been shown to be able to promote the growth and development of plants under both biotic and abiotic stresses such as water and salt stresses (Epstein, 1999; Gong et al., 2005; Hattori et al., 2005). Therefore, Si application might be used to improve early growth and establishment of wheat plants.

The formation of Si-organic complexes was reported in rice shoots (Munns and James, 2003; Inanaga and Okasaka, 1995). Mera and Beveridge (1993) suggested that Si can modify the cation-binding properties of cell walls. Different mechanisms for Si-mediated stress alleviation have been proposed by researchers. Si deposition in leaves was reported to be able to decrease transpiration (Hattori et al., 2007) and so, alleviating salt stress. The most widely reported mechanism was that Si might decrease the oxidative damage in plants subjected to environmental stresses (Remus-Borel et al., 2005). Gunes et al. (2007) reported that Si alleviated sodicity and boron toxicity in spinach (Spinaciao leracea L.) and tomato plants by reducing oxidative membrane damage. Reduced oxidative damage due to the addition of Si under saline conditions was also reported in barley (Liang et al., 2003; Liang et al., 2005) and cucumber (Zhu et al., 2004). In maize, the addition of Si increased water use efficiency by reducing leaf transpiration and the water flow rate in the xylem vessel (Ghoulam et al., 2002). Hattori et al. (2005) suggested that Si could facilitate water uptake and transport in sorghum under drought conditions. In potted wheat, Si alleviated oxidative stress by regulating the activities of antioxidant enzymes under drought stress conditions (Hattori et al., 2005). Seed germination and stand establishment in wheat farms areoften very poor due to the high levels of irrigation water salinity in Iran. Therefore, the aim of this investigation was to study the effect of seed pre-sowing treatment with Si on the germination and seedling growth of two wheat cultivars under salinity stress conditions.

MATERIALS AND METHODS

The experiment was carried out in Cereal Laboratory of College of Agriculture, Shiraz University, Shiraz, Iran in 2012. The experiment was set as a factorial based on completely randomized design with three replications. Seeds of two wheat cultivars differing in salt tolerance, Kavir (tolerant) and Shiraz (non-tolerant) (Emam, 2011) were used in the present study. To eliminate seed born microorganisms, seeds were sterilized with sodium hypochlorite solution (10%) for five minutes, and then washed three times with distilled water and surface dried by being placed between paper towels for 30 minutes at room temperature.

Seeds were primed with 0, 0.75, 1.5 and 2.25 mM silicon solutions (Sodium silicate) for 3h in the dark at 25 0 C, distilled water was also used as control. After priming, seeds were washed with tap water for two minutes, rinsed with distilled water and then dried

between two filter papers (22^oC and 60% relative humidity). Seeds were then germinated in Petri dishes on double layers of filter paper. Before this, 15 ml of different saline solutions (0, 100 and 150 mMNaCl) were added to the Petri dishes.

The germination test was carried out in darkness in a temperature controlled by the incubator and held at 23 ^oC. Seeds were considered as being germinated according to ISTA and the germination data were recorded for a continuous period of seven days. Germination percentage, mean germination time and germination rate were calculated according to equations of Belcher and Miller (1975) and Ellis and Roberts (1981), respectively:

Germination percentage =
$$\left(\frac{N_i}{N_t}\right) \times 100$$

Mean time germination = $\frac{\sum Dn}{\sum n}$

Mean time germination= Germination rate $=^{l}$ */Mean time germination*

in which N_i is the total number of germinated seeds for a continuous period of seven days and N_t is the total number of seeds, n is the number of seed germinated on day D, and D is the number of days counted from the beginning of germination.

Final length of shoot and root was measured 8 days after germination andthen vigor index was calculated according to equation of Abdul-Baki and Anderson (1970), in which GP is germination percentage and MSH is the sum of shoot and root length.

$$Vigor\ index = \frac{GP \times MSH}{100}$$

Seedlings were weighted using an analytical balance (ALE-40 SM- Shimatzu) and were then dried at 60 °C for 24 hours and finally weighted. The oven-dried shoots were finely ground to pass through 2 mm sieve. The dried materials were digested in a digesting mixture (sulphuric acid -hydrogen peroxide) according to the method suggested by Wolf (1982). The Na⁺ and K⁺ concentrations in digests were determined with a flame photometer (Jenway, PFP7).The data collected for each attribute were subjected to SAS *v*.9.1 software for analysis of variance. The Duncan's Multiple Range test (P \leq 0.05) was used to determine significant differences between the treatment means.

RESULTS AND DISCUSSION

Salinity treatment at 100 and 150 mMNaCl levels reduced germination percentage by 5% and 9% innontolerant cultivar (Shiraz), respectively. Whereas NaCl did not affect seed germination percentage in wheat tolerant cultivar (Kavir), however, seed priming by silicon (Si) improved germination percentage under both salt stress conditions in non-tolerant cultivar (Table 1). In both cultivars, germination rate was reduced by salinity stress. In addition, compared to control (0 NaCl), 100 and 150 mMNaCl levels reduced germination rate by 7% and 34% in tolerant cultivar and by 11% and 48% in non-tolerant cultivar, respectively (Table 1). On the contrary, Si increased germination rate under both stress and non-stress conditions in both cultivars and the effect of 2.25 mM silicon was more severe whereas, in Shiraz cultivar (non-tolerant), it was effective under both levels of salt stress (Table 1).

Mean germination time for tolerant and non-tolerant cultivars was increased by salinity stress (Table 1). The response of two cultivars to interactive effect of Si and salinity was different for mean germination time. Shiraz cultivar was more responsive to 2.25 mM Si than Kavir at 100 mMNaCl level. However, the application of Si reduced mean germination time for both cultivars under stress and non- stress conditions (Table 1). The response of two cultivars to salt stress was also different for vigor index. 100 and 150 mMNaCl levels reduced vigor index by 53% and 58% in tolerant cultivar and by 57% and 63% in non-tolerant cultivar, respectively (Table 1). Vigor index was improved under both stress and non-

stress conditions by Si, especially at 2.25 mM in both cultivars. Kavir cultivar was more responsive to 2.25 mM Si than Shiraz in terms of vigor index at 100 mMNaCl level (Table 1).

Compared with control (0 NaCl), 100 and 150 mMNaCl levels reduced shoot length by 14% and 23% in tolerant cultivar (Kavir) and by 49% and 60% in non-tolerant cultivar (Shiraz), respectively (Table 1). Moreover, the response of two cultivars to Si application was different for shoot length. Si at 2.25 mM increased shoot length of Kavir by 22% and 19% and shoot length of Shiraz by 24% and 30% at 100 and 150 mMNaCl levels, respectively (Table 1).

Root length was significantly affected by salinity and Si. Increasing salinity decreased root length, while priming by Si improved this trait. However, the longest root length was observed at 2.25 mM Si under control conditions (0 mMNaCl) in tolerant cultivar (Kavir). In contrast, minimum root length was found at 0 mM Si under 150 mMNaCl conditions in non-tolerant cultivar (Table 1).

 Table 1. Mean values of germination percentage, germination rate, mean germination time, vigor index, shoot length, root length and seedling dry weight of two wheat cultivars under interactive effects of silicon (Si) and salinity (NaCl).

Cultivar	NaCl (mM	Si (mM)	Germination Percentage	Germination Rate (1/day)	Mean germination time(day)	Vigor Index	Shoot length (cm/plant)	Root Length (cm/plant)	Seedling Dry Weight (mg/plant)
Shiraz	0	0	100a	0.74f	1.35de	18.68c	8.93c	9.75c	0.12e
	0	0.75	100a	0.76f	1.30e	18.43c	9.96b	12.36b	0.12e
	0	1.50	100a	0.83d	1.20ef	20.54b	9.96b	14.55a	0.19cd
	0	2.25	100a	0.90b	1.10f	20.65b	10.65b	14.66a	0.20c
	100	0	95b	0.66h	1.50d	8.07f	4.64f	4.03g	0.09g
	100	0.75	100a	0.71g	1.40d	8.66f	4.93f	4.65f	0.10f
	100	1.50	100a	0.86c	1.15f	9.03ef	4.87f	5.76e	0.11f
	100	2.25	100a	0.86c	1.15f	10.41e	6.10e	6.50e	0.11f
	150	0	91c	0.341	2.55a	7.00g	3.96g	3.16g	0.05h
	150	0.75	100a	0.45k	2.20ab	8.76f	4.66f	3.21g	0.06h
	150	1.50	100a	0.49j	2.01ab	8.34f	4.71f	3.63g	0.09g
	150	2.25	100a	0.58j	1.70c	9.30e	5.65e	4.50f	0.09g
Kavir	0	0	100a	0.86c	1.15f	19.06bc	9.41bc	8.28d	0.21c
	0	0.75	100a	0.88bc	1.13f	22.91ab	10.54b	8.46cd	0.24b
	0	1.50	100a	1.00a	1.00f	25.77a	11.20a	10.48c	0.29a
	0	2.25	100a	1.00a	1.00f	25.43a	11.84a	11.23b	0.30a
	100	0	100a	0.80gh	1.25d	9.06f	8.10c	4.13f	0.19cd
	100	0.75	100a	0.80e	1.25e	11.08de	8.43c	4.20f	0.20c
	100	1.50	100a	0.86c	1.15f	11.60d	9.83b	4.15f	0.20c
	100	2.25	100a	0.86c	1.15f	12.05d	10.30b	4.31f	0.25b
	150	0	100a	0.57j	1.85b	8.09f	7.26d	3.32g	0.13e
	150	0.75	100a	0.57i	1.75bc	8.17f	7.96c	4.10fg	0.15d
	150	1.50	100a	0.86c	1.15f	10.28e	8.10c	4.23f	0.16d
	150	2.25	100a	0.86c	1.15f	10.85e	8.94c	4.23f	0.19cd

In each column, means with different letters are significantly different (DMRT P<0.05).

NaCl severely affected shoot dry weight in both cultivars. The relative reductions under 100 and 150 mM levels were 10% and 39% in Kavir (tolerant cultivar) and 25% and 60% in Shiraz (non-tolerant cultivar), respectively. Si improved seedling dry weight in both cultivars. The relative increases in seedling dry weight for Shiraz cultivar at 2.25 mM level of Si were 32%, 19% and 45% at 0, 100 and 150 mMNaCl levels, whereas in Kavir cultivar, the relative increases were 30%, 26% and 32%, respectively (Table 1).





Fig. 1. Interactive effect of NaCl and silicon (Si) on accumulation of Na⁺ in non-tolerant (Shiraz) and tolerant (Kavir) cultivars. For each cultivar, means with different letters are significantly different (DMRT P<0.05).</p>

Moreover, NaCl increased accumulation of Na⁺ and decreased K⁺ in both cultivars (Fig. 1 and 2). The highest Na concentration was found in the 0 mM Si under 150 mMNaCl level and that of K⁺ was observed in the 2.25 mM Si under 0 mMNaCl (control) in both cultivars (Fig. 2). Compared with the corresponding control (0 NaCl), the relative increase in Na⁺ uptake in non-tolerant cultivar (Shiraz) was higher than that of tolerant cultivar (Kavir) (Fig. 1). Moreover, the relative K⁺ uptake was greater in tolerant cultivar (Fig. 2).

However, under salt stress conditions, 2.25 mM Si significantly reduced Na⁺ uptake by 46% in Kariv and 27% in Shiraz cultivars (Fig. 1) and increased K⁺ uptake by 13% in Shiraz and 20% in Kavir (Fig. 2). However, NaCl decreased K⁺/Na⁺ ratio in both cultivars. K⁺/Na⁺ ratio under 100 and 150 mM levels of NaCl was 30% and 39% respectively, which was greater in tolerant cultivar (Kavir), compared to non-tolerant cultivar, (Shiraz) (Fig. 3). However, under 100 and 150 mM levels, 2.25 mM Si significantly increased K⁺/Na⁺ ratio by 38% and 49% in tolerant cultivar and 31% and 32% in non-tolerant cultivar, respectively (Fig. 3).



Fig. 2. Interactive effects of NaCl and silicon (Si) on accumulation of K^+ in non-tolerant (Shiraz) and tolerant (Kavir) cultivars. For each cultivar, means with different letters are significantly different (DMRT p<0.05).

Overall, results of this experiment showed that the tolerant cultivar (Kavir) accumulated less Na^+ and had a greater K^+ and K^+/Na^+ ratio than the non-tolerant cultivar (Shiraz). A lower uptake of Na^+ and a higher uptake of K^+ is an important mechanism for salt tolerance found in many crop species (Liang et al., 1996; Wang et al., 2010). In addition, salt stressed plants treated with Si accumulated lower Na^+ and higher

 K^+ with greater K^+/Na^+ ratio in both cultivars. These features are associated with salt tolerance in most crop species (Gong et al., 2006). Some possible mechanisms through which Si may increase salinity tolerance in plants include: immobilization of toxic sodium ion, reduced sodium uptake in plants and enhanced potassium uptake (Liang et al., 2005) and higher potassium, sodium selectivity (Gong et al., 2006). In rice, Si alleviated salt stress by reducing Na⁺ uptake through partial blockage of the transpiration bypass flow, a major pathway of Na⁺ uptake in this species (Gong et al., 2008). Hence, Si might play a vital role for better plant growth under salinity. According to our results, silicon showed great effects to ameliorate salinity damages.





Fig. 3. Interactive effects of NaCl and silicon (Si) on K⁺/Na⁺ in non-tolerant (Shiraz) and tolerant (Kavir) cultivars. For each cultivar, means with different letters are significantly different (DMRT p<0.05).</p>

In the present study, salinity adversely affected seed germination and seedling growth in both cultivars, however, Kavir cultivar with higher salinity tolerance was less affected. Therefore, relative reduction in germination percentage, germination rate, vigor index, shoot length, root length and seedling dry weight were found to be less in this cultivar (Kavir), compared to non-tolerant cultivar (Shiraz). Wang et al. (2009) in alfalfa, Cabuslay et al. (2002) in rice, Ghoulam et al. (2002) in sugar beet and Iqbal and Ashraf (2005) in wheat have also reported that salt stress has reduced germination percentage, vigor index and seedling growth in these crops.

The seeds pre-treated with silicon exhibited higher germination percentage, germination rate and vigor index. Similar results were reported by other researchers that confirm the positive role of silicon under salinity stress conditions (Lee et al., 2010; Saqib et al., 2008; Wang et al., 2011; Zhu et al., 2004). Indeed, Si is one of the beneficial and essential elements for plant growth under biotic and abiotic stresses. Some authors reported that Si could ameliorate salt stress depression on crop plant species (Wang et al., 2010; ,Zuccarini, 2008). There are also some reports on protective role of silicon oncropseed germination under salt stress conditions (Lee et al., 2010; Wang et al.,2010).

In this experiment, Si also improved the performance of shoot length and root length under both non stress and salt stress conditions. Improvement of shoot and root growth has previously been noted by Lee et al. (2010) and Wang et al. (2010). The positive effect of Si on shoot growth of wheat seedlings under salt stress conditions has been attributed to decreased stomatal conductance, drop in leaf relative water content, as well as decreased tissues Na⁺ content (Gao et al., 2006). The application of nano silicon on soybean has also enhanced the crop growth (Lu et al., 2002). It has also been reported that Si application under salt stress could enhance such antioxidatsas SOD, POD and CAT activitywhich can play a great role to counterbalance salinity damages (Wang et al., 2011).

CONCLUSIONS

It could be concluded that exogenous application of silicon through seed priming had a positive role on seed germination and seedling growth of wheat cultivars under salt stress conditions. Also, pre-sowing seed treatment with silicon improved salt tolerance in both cultivars by reducing the uptake and accumulation of Na⁺ and promoting K⁺ uptake. Based on the results of the present study, it might be recommended that presowing seed treatment or seed priming, which is an easy and low risk technique, be used as an alternative approach to overcome agricultural salinity problems in salt prone wheat growing areas. Further research in this area is recommended.

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تاثیر کاربرد سیلیس بر جوانهزنی و رشد گیاهچه ارقام گندم تحت شرایط تنش شوری

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گندم سیلیس شوری جوانهزنی رشد گیاهچه

چکیده- جوانهزنی بذر و رشد اولیه گیاهچه از مراحل حساس در استقرار و تولید عملکرد گیاهان زراعی بویژه در شرایط تنش شوری میباشند. کاربرد سیلیس میتواند موجب بهبود جوانه-زنی و رشد گیاهچه گردد. این پژوهش با هدف بررسی تاثیر پیش تیمار بذر با سیلیس در غلظت-های مختلف (۰، ۱/۵، ۱/۵ و ۲/۲۵ میلیمولارسیلیکات سدیم) بر جوانهزنی و رشد گیاهچه دو رقم گندم کویر (مقاوم به شوری) و شیراز (حساس به شوری) در شرایط تنش کلرید سدیم (۰، ۱۰۰ و ۱۵۰ میلیمولار) به صورت فاکتوریل در قالب طرح کاملا تصادفی با سه تکرار در گلخانه پژوهشی دانشکده کشاورزی دانشگاه شیراز در سال ۱۳۹۱ به اجرا در آمد. نتایج نشان داد که پیش تیمار بذر با سیلیس سبب افزایش درصد جوانهزنی، سرعت جوانهزنی، بنیه بذر، طول ریشهچه و ساقچه و وزن خشک گیاهچه ارقام گندم در هر دو شرایط تنش و عدم تنش شوری گردید و نیز کاهش مدت زمان جوانهزنی را در هر دو شرایط به همراه داشت. بعلاوه، کاربرد سیلیس موجب افزایش غلظت پتاسیم و نسبت پتاسیم به سدیم و نیز کاهش غلظت سدیم گردید و تاثیر غلظت ۲/۲۵ میلی مولار سیلیکات سدیم مشهودتر از سایر غلظتهای به کار رفته بود. در مقابل، تنش شوری سبب کاهش صفات اندازه گیری شده گردید و افزایش مدت زمان جوانهزنی و غلظت سدیم را در هر دو رقم به همراه داشت. با این حال، رقم مقاوم به شوری (کویر)، در شرایط تنش، سدیم کمتر و نیز پتاسیم بیشتری را در مقایسه با رقم حساس به شوری (شیراز) در خود تجمع داد. اگر چه با افزایش غلظت NaCl (۱۵۰ میلی مولار) تاثیر منفی شوری بر پارامترهای اندازه گیری شده در هر دو رقم بیشتر بود، با این حال، رقم مقاوم کویر تاثیر کمتری از تنش شوری در مقایسه با رقم حساس شیراز پذیرفت. در مجموع میتوان نتیجه گیری نمود که پیش تیمار بذر با سیلیس میتواند سبب بهبود جوانهزنی شود و نیز از طریق افزایش تجمع پتاسیم و کاهش سدیم سبب رشد و در نتیجه استقرار بهتر گیاهچههای گندم تحت شرایط تنش شوری گردد.