

Efficacy of silicon priming and fertigation to modulate seedling's vigor and ion homeostasis of wheat (*Triticum aestivum* L.) under saline environment

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Abstract Seed preconditioning, a short gun approach to modulate the effects of abiotic stresses on crop plants, has recently gained considerable attention of the researchers to induce salinity tolerance in agronomically important crops. The present study was conducted to explore the comparative efficacy of presowing seed priming with silicon (Si) and Si fertigation to modulate the wheat growth and ion dynamics. Seeds of wheat variety, PUNJAB-11, were sown in Petri plates having nutrient solutions with (120 mM) and without NaCl. Six levels of Si (0, 10, 20, 30, 40, or 50 mM), applied as sodium silicate (Na₂SiO₃), were tested either as a seed priming agent or as a supplement in the nutrient solution. Priming of seeds with Si mitigated the adverse effects of salinity stress on germination percentage, root as well as shoot length, dry and fresh weight. Application of Si either as preconditioning of seeds or addition in the growth medium resulted in reduced accumulation of sodium (Na⁺) in wheat seedlings under saline environment. Seedling's potassium (K⁺) contents either remained unaffected or decreased whereas calcium (Ca²⁺) contents decreased at all Si concentrations except at 30 mM when Si primed seeds were grown under salt stress. Addition of Si, under salt stress, in cultivation medium exerted a positive effect on seedling's K⁺ and Ca²⁺ contents. Silicon contribution to decontamination strategies was evaluated.

Keywords Fertigation · Salinity · Seed priming · Silicon · Wheat

Introduction

A large portion (approximately 800 million hectares) of world's arable land is salt affected where 397 Mha considered saline while 434 Mha being sodic (FAO 2008). In Pakistan, about 8.6 Mha salt effected soil patches exist throughout the country, and about 24 % of the wheat belt is anticipated to be affected by salt stress up to 2050. Salinity has deleterious effects on germination, seedling vigor, crop establishment, and yield of agronomically important crops (Zhu et al. 2004). The osmotic effects, after the exposure of plants to salinity stress, cause the reduction in cell division and cell expansion as well as stomatal closure (Flowers 2004). Long-term exposure to salts causes nutritional imbalances and deficiencies (K⁺ and Ca²⁺) leading to senescence of leaves reducing photosynthetic area necessary to maintain the optimum growth (Cramer and Nowak 1992). Plant tolerance to environmental stresses generally involves responses at both cellular and entire plant levels (Ashraf and Harris 2004). The complexity of interactions between plant physiological processes and environmental stresses alters plant growth and development, ultimately affecting the productivity (Zhu 2002). In plants, absorption, translocation, and toxicity of cations like Na⁺ are also affected by interaction with other mineral nutrients as well as quasi-essential elements like silicon (Si).

Silicon, an important bioactive element, is present in most plant species (Richmond and Sussman 2003). The favorable effects of silicon include improved leaf morphology, enhanced root penetration into soil, increased plant height, increased resistance to pathogens, and tolerance to abiotic stresses (Fauteux et al. 2005; Pilon-Smits et al. 2009). Furthermore,

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silicon application enhances the stiffness and strength of plant cell wall, reduces apoplastic flow as well as absorption of toxic minerals due to the formation of silicon depositions in roots, and also minimizes water loss by cuticular transpiration (Ma 2004). These characteristics made Si a beneficial bioactive element to improve plant fitness against environmental stresses. Silicon amendments are generally alkaline in nature due to the capability of silicate ions (SiO_3^{2-}) to protonate (Rijkenberg and Depree 2010). At high dose, Si amendments could promote polymerization of SiO_3 -slag which is a potential chelating agent for toxic elements (Sommer et al. 2006).

Although Si-mediated suppression of salt stress has been thoroughly investigated in crops, yet the comparative efficacy of various application methods has not been well established in terms of their possible role for decontamination of saline soil/environment. Therefore, the present work was aimed to draw parallels among exogenous Si application, either as seed priming agent or as Si fertigation, on seedling growth and salt distribution into the plant. Also, Si contribution to salinity reclamation was evaluated.

Materials and methods

Seeds of wheat variety (PUNJAB-11) were grown in nutrient solution (Eliasson 1978) with 0 or 120 mM NaCl, respectively. Silicon was applied as Na_2SiO_3 (0, 10, 20, 30, 40, or 50 mM) either directly to the seeds (as priming treatment for 8 h) or added in the growth medium. The silicate supply generally causes medium basicity due to the capability of silicate ions (SiO_3^{2-}) to protonate. Therefore, the nutrient solutions were initially neutralized with HCl, and Na was replenished with NaCl to minimize the medium basification as well as sodium effects originating from Na_2SiO_3 addition. For the seed-priming study, an experiment was carried out in a completely randomized design (CRD) by using Petri plates lined with single layer of filter paper having three replicates for each treatment. The Petri plates were placed in a growth chamber (Sanyo Versatile Environmental Test Chamber) with relative humidity of 60 %, a light period of 10 h at 25 °C, a dark period of 14 h at 20 °C, and a light intensity of approximately 150 W m^{-2} (Philips Master HPI-T Plus, 400 W). After 21 days of plant cultivation, the seedling material was harvested, oven-dried at 105 °C, and was digested according to Wolf (1982). Briefly, 100-ml flasks having 5 ml of conc. H_2SO_4 were incubated overnight at 25 °C. Next day, a 0.5 ml H_2O_2 (35 %) was poured, and the flasks were placed over a hot plate (350 °C) until no fumes were produced. Afterward, these were removed from the hot plate, allowed to cool, a 0.5 ml of H_2O_2 was added, and were again placed over a hot plate. The step was repeated until a transparent digestion mixture was achieved. The contents were diluted up to 50 ml in volumetric flasks, filtered, and stored at 4 °C till further elemental analysis.

Flame photometer (Jenway PEP7, UK) was used for determination of plant Na^+ , K^+ , and Ca^{2+} concentrations. The collected data were subjected to analysis of variance (ANOVA) using computer software CoStat version 6.2. The mean values were compared using the least significant difference at $p \leq 0.05$.

Results and discussion

The results indicated that silicon application did not enhance germination percentage under nonsaline condition. Under salt stress, Si application reduced the negative saline effects on seed germination (Fig. 1). Silicon fertigation with 30 mM proved to be more beneficial as compared with the other levels. Addition of 50 mM Si in the growth medium, under both nonsaline and saline conditions, significantly reduced the germination percentage.

Our results are in agreement with the findings of Al-aghabary et al. (2004) who concluded that Si-induced beneficial effects are more pronounced under saline environment as compared with normally grown plants. Germination has been considered as one of the most salt-sensitive growth stages and is severely inhibited with increasing salinity (Pervaiz and Satyawati 2008). Application of Si had been reported to increase germination percentage (Parveen and Ashraf 2010) and plant water status (Romero-Aranda et al. 2006) and mitigated specific ionic effects of salts (Tahir et al. 2006). Furthermore, seed soaking can regulate seed germination percentage in a short time (Zhu 2002), particularly under abiotic stresses (Parveen and Ashraf 2010). Our study confirmed that seed priming with Si increases wheat germination.

Under nonsaline condition, root length significantly decreased with increasing Si concentrations. The applied salt stress reduced the root length, but Si application recovered the plants from this stress but only with 10 and 20 mM Si treatments either as seed priming agent or addition in growth medium (Fig. 1).

When seeds were grown without stress, Si addition increased the shoot length, and the effect was more significant at 10, 20, and 30 mM (Si was used as priming agent) or at 10 and 20 mM (Si addition was in growth medium) (Fig. 1). On the other hand, the supplementation of growth medium with 50 mM Si, under nonsaline condition, significantly reduced the shoot length. The imposition of salinity stress reduced the shoot length, but application of Si, either way, reduced salt effect particularly at 10, 20, and 30 mM treatments.

Exogenous application of Si, under both nonsaline and saline conditions, increased the fresh weight. Presowing seed soaking with 20 mM Si was more effective in enhancing seedling's fresh weight. However, as for other growth attributes, 50 mM Si treatment significantly reduced the fresh weight.

Under nonsaline conditions, application of Si (as priming agent or in growth medium) reduced the plant dry weight,

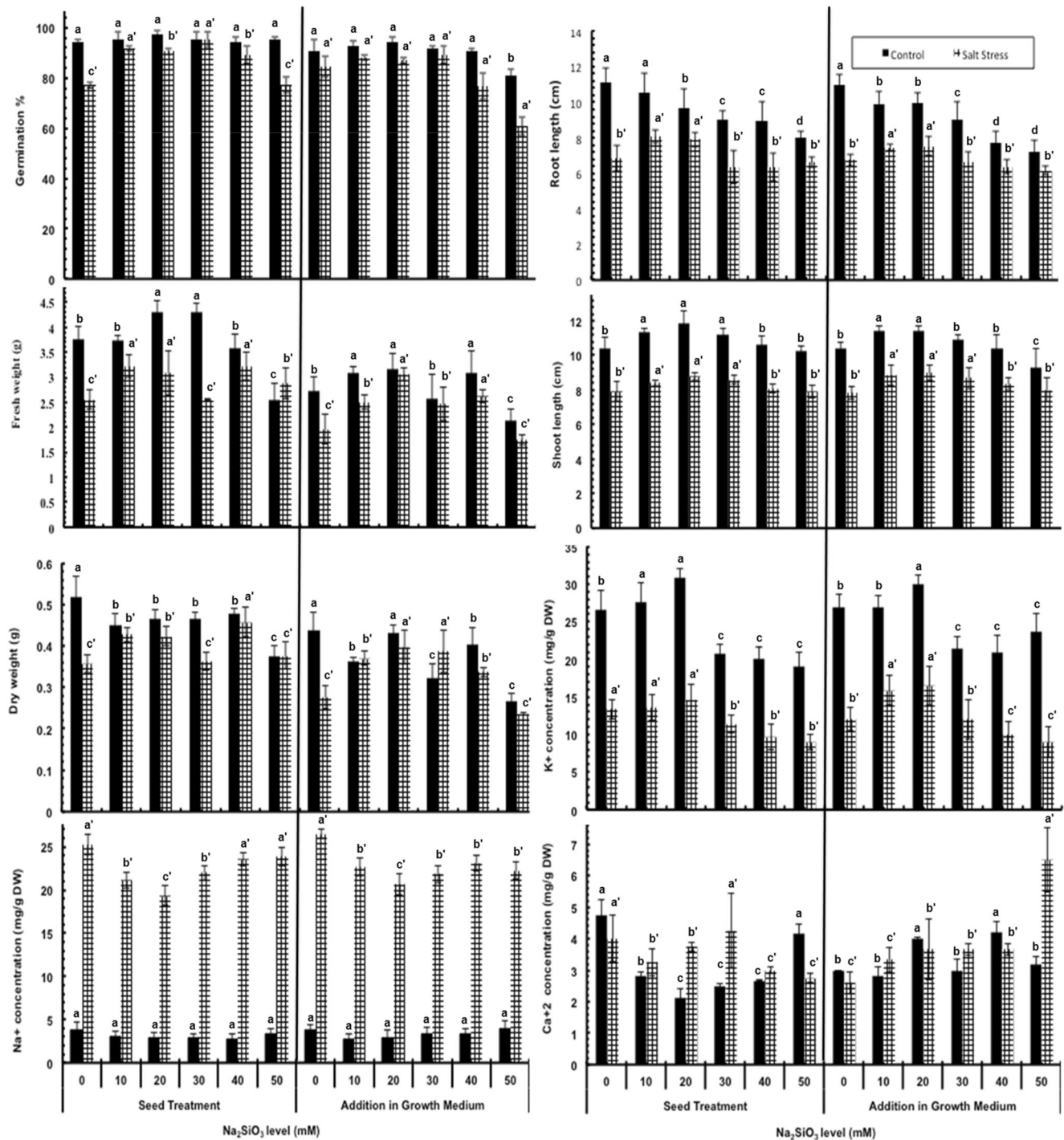


Fig. 1 Effect of silicon (Si) seed priming and Si fertigation on growth and Na⁺, K⁺, and Ca²⁺ accumulation in wheat seedlings grown under nonsaline and saline conditions. Letters *a–d* and *a'–c'* represent

significant effect of Si treatments on wheat growth and ion accumulation under nonsaline and saline conditions, respectively (*n*=3, means±SE)

significantly at 50 mM treatment. Under saline condition, seed priming with 20 and 30 mM Si resulted in the highest value for dry biomass. The proton pump (H⁺-ATPase) is one of the mechanisms responsible for plant growth. Silicon treatment under salt stress has been reported to increase plasma membrane H⁺-ATPase activity in barley shoots

(Liang et al. 2006). The H⁺-pumping activity of H⁺-ATPase enzyme is the main contributory parameter of the enzyme in cell wall acidification, which in turns leads to expansion growth. The increase in plant growth even under salt stress might be attributed to role of Si in the improvement of plant water status (Romero-Aranda et al. 2006). Other researchers

(Al-aghaby et al. 2004; Gong et al. 2006) had also reported the considerably enhanced growth and dry matter production in response to Si treatment under saline or nonsaline environment. The observed relatively small changes in growth attributes in the present study might be due to the use of Na_2SiO_3 , where sodium itself is likely to affect plant tolerance against salt stress.

Under nonsaline condition, Si treatment resulted in either increased (at 10 and 20 mM) or decreased (30, 40, and 50 mM) K content (Fig. 1). The Ca content also decreased at all Si treatments (except 50 mM) while Na content remained unchanged irrespective of Si levels.

The salt stress reduced the plant K concentration. Silicon treatment, as priming agent under stress condition, either did not effect K content (at 10 and 20 mM) or further reduced the K content (at 30, 40, and 50 mM) (Fig. 1). Contrary to this, addition of Si in growth medium, under saline condition, either improved the K content (at 10 and 20 mM) or had no effect (at higher Si treatments, i.e., 30, 40, and 50 mM). The observed Si-induced stimulation of K^+ uptake under salt stress during the present study might be concerned with the activation of H^+ -ATPase (Tuna et al. 2008) as Liang et al. (2003, 2006) reported increase in plasma membrane H^+ -ATPase activity in response to Si application under saline condition. The electrochemical H^+ gradient activates voltage-dependent K^+ inward-rectifying channels (Perrot-Rechenmann 2010) which in turn contributes to the generation of an increased turgor pressure and enables the cell wall to expand. This seems likely as the plant biomass was found to be increased during the present study.

The plant Na concentration significantly increased under stress condition when no Si was applied. The priming of seeds with Si under saline condition either decreased (at 10, 20, and 30 mM) or had no effect (40 and 50 mM) on Na content (Fig. 1). The addition of Si in growth medium resulted in reduced Na content as compared to plants that were grown under saline condition but without Si treatment. The reduced Na uptake by wheat seedlings grown from Si primed seeds might be due to biological dilution as evident from increased plant fresh weights at lower Si treatments. With increasing absorption area, the tissue concentration diminishes, and the plant may compete for the uptake of Na as reported earlier for other cations (Greger 1999; Javed and Greger 2011). Reduction in plant Na content, when Si was added in growth medium, may be due to medium basification, an effect arising from the protonation of silicate ions (SiO_3^{2-}). Furthermore, reduction of plant Na level might be due to the inhibitory effect of Si on the transpiration rate (Ahmad et al. 1992) or due to deposition and polymerization of Si in endodermal and exodermal layers inhibiting the apoplastic Na uptake by plant roots under salt stress (Yeo et al. 1999; Gong et al. 2006).

The plant Ca concentration was decreased when seeds were subjected to salt stress. However, the Ca content either

decreased (10, 20, 40, or 50 mM) or remained unchanged (30 mM) when Si primed seeds were used (Fig. 1). The addition of Si in growth medium resulted in increased Ca contents except 10 mM treatment. In salt stressed environment, impaired cell membrane permeability had often been linked with decrease in Ca level (Mengel and Kirkby 1987; Tuna et al. 2008). Thus, by increasing Ca concentration, Si supplementation seems to play an important role to maintain the permeability of membrane, which ultimately resulted in better survival and stimulated wheat growth even under salt stress.

We concluded that both methods of exogenous Si application exerted similar beneficial effects for all studied attributes except seedling's emergence. The better seedling's emergence was observed under Si seed priming while Si fertigation resulted in reduced Na content and improved the K as well as Ca nutrition of the seedlings. The results are important and should be the part of long-term programs involving Si to boost wheat productivity under saline conditions and/or reclamation of salt affected soils in arid and semi-arid regions. For example, cotreatment of salt stress along with beneficial elements like Si would result in stabilization of sodium in saline environments.

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