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ORIGINAL PAPER

Beneficial Effects of Silicon Application in Alleviating Salinity Stress in Halophytic *Puccinellia Distans* Plants

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Abstract

Little is known on the impact of silicon (Si) nutrition in halophytes. Accordingly, response of Si accumulating halophyte *Puccinellia distans* to Si nutrition was investigated. The experiment was carried out as factorial in a completely randomized design. Plants were hydroponically raised for six weeks under two salinity (0 and 200 mmol L^{-1} NaCl) and Si (0 and 1.5 mmol L^{-1} Na_2SiO_3) levels. Si improved plant dry weight and water relations under salinity. Salinity decreased the plant relative water content (RWC) but Si increased this parameter. Transpiration rate and stomatal density however, declined by salinity and Si even intensified these salt effect. Si affected salt tolerance mechanisms in *P. distans*. Thus, +Si plants had greater soluble sugars and amino acids and lower Na^+ but increased cellulose and lignin and Na^+ secretion from leaves. These possibly indicate more efficient osmotic adjustment and better operation of either salt exclusion and / or excretion mechanisms. In congruence, Si greatly enhanced the activity of H^+-ATPase in both roots and shoots. +Si plants had reduced stress symptoms evidenced by lower proline and reduced electrolyte leakage indicating better membrane functioning. Altogether, Si application led to better performance of *P. distans* plants under saline conditions.

Keywords ATPase activity · Halophyte · *Puccinellia distans* · Salinity · Silicon

1 Introduction

Salt stress in plants is a major problem that retards their growth and results in reduction of natural vegetation. More than 800 million hectares of arable lands worldwide are faced with salinity [1]. Excess salt reduces the water potential of soil solution and decreases plant ability for water uptake. Furthermore, the inevitable excessive absorption and accumulation of salts in plants experiencing salinity causes ion imbalance and toxicity [2, 3]. Salinity stress in plants is also associated with many secondary physiological changes such as reduction of enzyme activity, increase of oxidative stress, membrane dysfunction, reduced photosynthesis and other morpho–anatomical alterations [3, 4]. The ability of plants for tolerating saline conditions varies among species. Halophytes are specially adapted to saline area and their growth is even improved by low levels of salt thus, they can regulate the uptake and sequestration of Na^+ and Cl^- predominantly in their vacuoles and / or secretion of extra salts through salt glands [5, 6]. As a result of maintaining the cytoplasmic K^+ and Mg^{2+} concentrations at levels required for proper essential enzyme activities, these plants can achieve osmotic adjustment through the synthesis of osmoprotectants and prevent buildup of toxic levels of reactive oxygen species (ROS) which generally occurs under saline conditions [5]. In many salt tolerant species the increased contents of amino acids such as proline and also soluble sugars and the increased activity of ATPases involved in Na^+ exclusion and secrtion have been reported as resistance mechanism to salinity [5, 6].

Silicon (Si) is an abundant element of the earth crust although its availability for plants is low in many soils [7]. While it is not considered as an essential nutrient for plants but several taxa including grasses and horsetails, may accumulate Si up to very high levels [8]. The ameliorative effects of Si on the adverse effects of salinity have been shown in several glycophytes including rice, barley, wheat,

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canola and maize [9–13]. In rice plants, Si deposition in the exodermis and endodermis reduces the uptake of sodium from roots [9, 14]. Deposition of Si in cell walls in the forms of amorphous silica and opal phytolins following Si nutrition may reduce the translocation of salts to shoot parts [15–17]. Other frequently reported positive effects of Si on salinity-stressed plants such as rice, canola, *Arabidopsis* and sorghum includes increased competence of antioxidant metabolism for ROS scavenging, prevention of lignin deposition, optimal ion balances, increased chlorophyll preservation, enhanced polyamine biosynthesis and delayed senescence [9, 12, 18, 19]; which altogether bring about better plant growth and performance.

Alkaligrass, *Puccinellia distans* (jacq.) parl, is a salt tolerant C_3 species that grows naturally in salt affected area [20]. It has been used for saline depressions land reclamation in north of Golestan province, Iran [21]. Alkaligrass improves productivity of these saline lands and has been used as a forage plant for livestocks nutrition [22, 23]. Halophyte grasses employ two different mechanisms to tolerate salt including osmoregulation via the accumulation of metabolically compatible organic osmolytes and salt secretion achieved by differentiated salt glands. Most of the wetland grasses are recognized as Si accumulators and therefore Si may be involved in their salt tolerance mechanisms. In the only studied halophytic grass i.e. *Spartina densiflora*, Si improved photosynthetic apparatus, water-use efficiency and mineral nutrient balance of plants under salinity stress [24].

The impacts of Si under salinity have mostly been studied in glycophyte crop plants and the subject is nearly neglected in plants not typically regarded as crop, especially halophytes which are adapted to saline environments. As the information on the impact of Si nutrition on the osmoregulation and water relation, salt excretion and membrane function of halophytes under saline conditions are lacking, in the current investigation, growth and some physiological and biochemical traits of alkaligrass (*Puccinellia distans*) grown under saline conditions in the presence and absence of Si were compared to examine the role of Si in salinity tolerance of this species.

2 Materials and Methods

2.1 Plant Culture and Growth Conditions

All experiments were carried out in a greenhouse at Golestan University. Seeds of alkaligrass (*Puccinellia distans* (jacq.) parl) were procured from Agriculture and Natural Resources Research Center of Golestan province. Seeds were sterilized with a 2.5% NaOCl solution and sown into pots filled with acid washed sand. The resulting seedlings of equal size with three leaves were subsequently transplanted in black plastic containers (7.0 L) filled with Hoagland nutrient solution and aerated continuously [25]. The experimental plan was factorial in a completely randomized design with at least five replicates. The first factor was salinity in two levels of 0 and 200 mmol L^{-1} NaCl and the second factor was Si nutrition in two levels of 0 and 1.5 mmol L^{-1} Na_2SiO_3. The salinity levels were selected based on our pervious experiments (Supplementary material 1) [26]. Silicon solubility limits are around 2 mM at 25 °C [14, 27] and the concentration range of 0.8- 2 mM is routinely used in other studies [12, 14, 15, 18, 19, 44]. Furthermore, the efficiency of Si in alleviating salinity stress is generally reduced at concentration less than 1.5 mM (Supplementary material 1) [28], accordingly in this research Si was applied to plants at 1.5 mM concentration. Plants were treated one week after the transfer of seedlings into hydroponic culture. The adjustment of pH in the nutrient solution and other growth conditions were the same as those described earlier [12]. Plants were harvested by six weeks after the start of treatments and before reaching the reproductive growth phase analyzed for growth parameters and biochemical and physiological traits.

2.2 Concentration of Si and Na^+

The plant sodium content was determined by flame photometry (Model Jenway, PEP–7 Essex, UK) after digesting 50 mg of plant material which had been dried in oven with a solution composed of HNO_3 and $HClO_4$ (3:1; v / v) at 175 °C. The dried plant material was also digested with H_2O_2 and NaOH in an autoclave and the released Si was determined spectrophotometrically according to Elliot and Synder [29].

2.3 Determination of Sugars, Amino Acids, Soluble Proteins and Phenolics

The extraction of soluble sugars, amino acids and phenolics from the plant material was carried out by using 80% (V/V) ethanol as solvent. Soluble sugars were quantified by Anthron as described by McCready et al. [30]. Soluble protein were extracted with 0.1 mol L^{-1} phosphate buffer at pH 6.8 and quantified according to the Bradford method [31]. Total phenols were quantified according to Lavid et al. [32]. Amino acids were measured by Ninhydrin reagent according to the method described by Yemm and Cocking [33]. The tissue insoluble sugar content was determined with phenol – sulfuric acid method as described by Kochert [34]. Proline content was quantified according to Bates et al. [35].

2.4 Determination of Cell Wall Components

The tissue cellulose content was extracted based on the method of Kokubo et al. [36]. Cellulose was quantified by Anthron after dissolving in 67% H_2SO_4 according to Correa–Aragunde et al. [37]. The extraction and quantification of lignin was carried out as has been mentioned in detail by Zimmer [38].

2.5 Plant Water Relations

The plant relative water content was calculated using the equation

$$RWC = \frac{F_w - D_w}{T_w - D_w} \times 100$$

where Fw is the fresh weight (g) of plants immediately after harvest, Tw is turgid weight (g) of the plants after floating for 16 h in de–ionized water at 25 °C and Dw is the dry weight (g) of plants.

Transpiration of whole plant was measured gravimetrically using 2 dm^3 polythene pots filled with fresh nutrient solution. Pots were completely sealed with solid paraffin to limit weight losses solely due to plant transpiration. Evaporative water loss was quantified during 5 h under continuous light.

Stomata density was quantified on strips from leaf abaxial surface (epidermis) peeled off from three mature leaves using an Olympus microscope (model BX51TRF) (magnification 100).

2.6 Assessing Membrane Permeability, Salt Secretion and Rhizosphere Acidification

Membrane permeability of the excised leaves was determined at the end of the experiment as described by Lutts et al. [39]. Briefly, four healthy leaves were separated from each replicate plant, washed in distilled water, segments (n = 4) each with an area of 1cm^2 were cut and immersed in distilled water (10 mL). Samples were shaken for 24 h at 30 ºC, and then the conductivity of the solution was measured with a conductivity meter (EC_1) (HI 8633, Hanna Instruments Co. Ltd). Samples were also boiled for 1 h and their conductivity was measured again in the solution that had been cooled down to room temperature (EC_2). The percentage of electrolyte leakage was calculated by the following equation.

$$E_c = \frac{C_1}{C_2} \times 100$$

The secreted salt through hydathodes was quantified by dissolving the excreted salt from shoots in 10 mL distilled water and measuring the amount of sodium in this solution [40].

Rhizosphere acidification in intact roots of alkaligrass plants was identified by agar technique as described by Romheld [41]. Roots from treated plants for six weeks were carefully washed with distilled water and placed on a large petri plate and then incubating agar solution was added to immerse roots completely without being damaged. The agar gel (0.75%; w/v) consisted of 0.06 g bromocresol purple, 2.5 mmol K_2SO_4, and 1 mmol $CaSO_4$ per liter adjusted to pH 6. The accuracy of color gradient of this solution was attested using standard pH buffers. The incubation for revealing rhizosphere acidification was carried out at day time in greenhouse for 1 h. Proton extrusion along the roots was visualized by the color shift of the pH indicator (bromocresol purple) from purple to yellow.

Assay of H^+–ATPase activity was determined after spectrophotometric quantification of released phosphate according to Ames [42]. The detail of the protocol has been explained recently by Geirvani et al. [43], which has been modified from Janicka–Russak and Kabała [44].

2.7 Statistical Analyses

All data were subjected to ANOVA and a comparison of the means was carried out using a least significant difference (LSD) test.

3 Results

3.1 Si Improves P. Distans Growth Under Salinity

Under salinity plant growth was compromised and older leaves displayed burnt tips. However, Si application increased plant growth under both saline and non-saline conditions (Supplementary material 1).

Salt treatment resulted in significant reduction in both shoot and root dry weights of plants but Si nutrition increased dry weight of salt treated plants significantly (at $P \leq 0.05$) and especially their roots (Fig. 1). Si application increased total dry weights of plants by about 32% and 29% in 0 and 200 mmol L^{-1} NaCl treatments, respectively. Salinity also induced marked decrease in shoot / root ratio and Si application counteracted this reduction under saline conditions.

3.2 Differential Impacts of Salinity and Si on Plant Water Relations and Osmolytes Accumulation

The relative water content of plants decreased under salinity (Fig. 2). In contrast, supplemental Si caused significant increase in this parameter under both saline and control conditions.

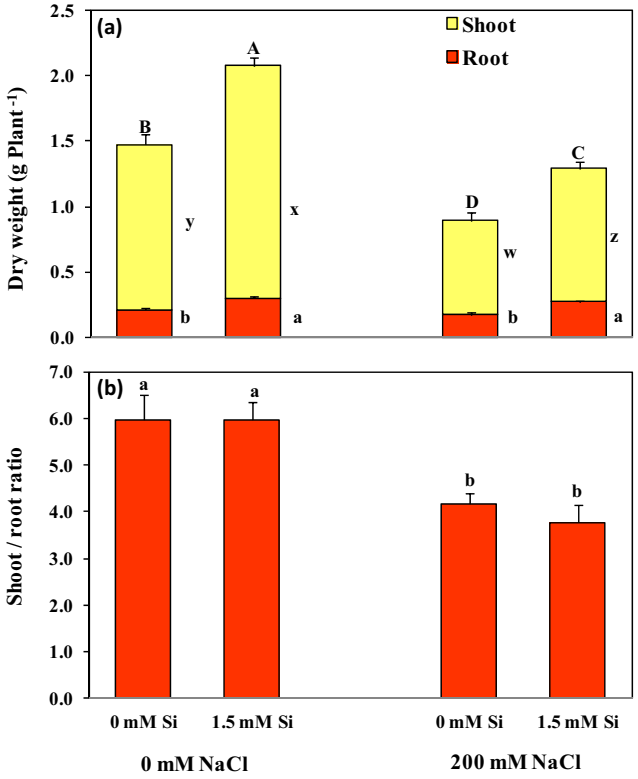

Fig. 1 Effects of salinity (0 and 200 mmol L^{-1} NaCl) and Si (0 and 1.5 mmol L^{-1} Si) on the root and shoot dry weights (**a**) and shoot / root ratio (**b**) of *P. distans* plants. Error bars represent the standard error. Different letters (a–c for root, x–z for shoot and total A–C dry weights) on histograms represent statistically significant differences at P≤0.05

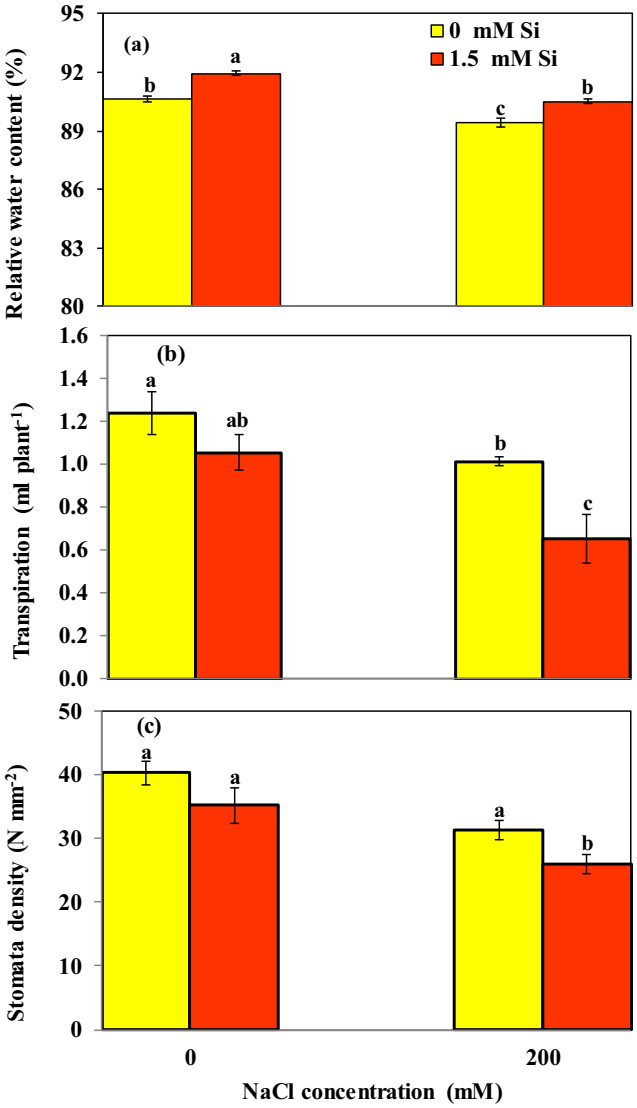

Fig. 2 Changes in relative water content (**a**) transpiration rate (**b**) and stomata density of *P. distans* plants in the presence or absence of 1.5 mmol L^{-1} Si under salinity. Error bars represent the standard error. Different small letters on histograms represent statistically significant differences at P≤0.05

The rate of transpiration was also reduced after plant exposure to salinity and Si application reduced transpiration rate irrespective of salinity (Fig. 2). Thus, the figures in Si–fed plants were less by about 15 and 38% under control and saline conditions, respectively, compared to those not supplied with Si.

Stomata density reduced due to salinity and this reduction was intensified by Si application (Fig. 2, Supplementary material 2). At 1.5 mmol L^{-1} Si treatment, the plant stomata density was about 13 and 23% less in control and 200 mmol L^{-1} NaCl, respectively, compared to those grown without supplemental Si (Fig. 2).

Under salinity, Na^+ concentration increased significantly in both shoots and roots (Fig. 3). The application of Si under salinity prevented buildup of Na^+ in roots and shoots, as a result at 1.5 mmol L^{-1} Si treatment the tissue Na^+ contents were about 34% and 25% less respectively, compared to - Si conditions (Fig. 3).

Soluble sugar contents in both roots and shoots increased due to salinity. While Si nutrition decreased the content of soluble sugars in roots under salinity, it remained unaffected in shoots (Table 1). Application of Si however, caused significantly greater soluble sugar content in shoots under control conditions.

The level of insoluble sugars remained unaffected by salinity in plants. Roots of plants supplied with 1.5 mmol L^{-1} Si had greater insoluble sugars. The insoluble sugars of roots under saline and control conditions were greater by about 34 and 50% respectively, in Si supplied plants compared to those without supplemental Si. In shoots, on the contrary, Si nutrition decreased the contents of insoluble sugars by 76% and 32% under saline and control conditions, respectively (Table 1).

The proline content increased only in roots under salt treatment but it remained unchanged in shoots (Table 1).

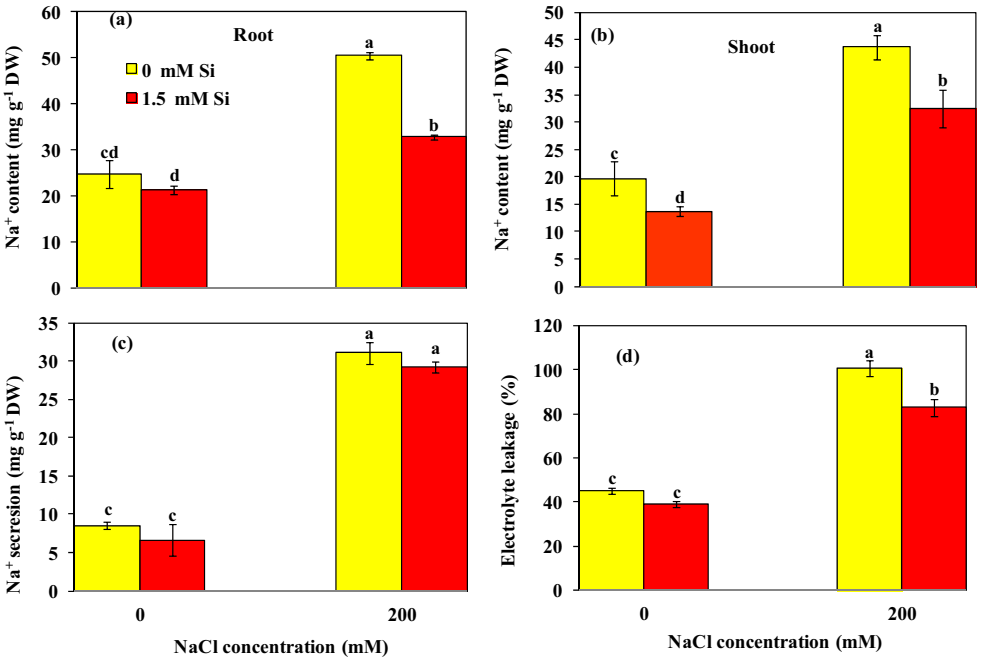

Fig. 3 Comparative effects of salinity (0 and 200 mmol L^{-1} NaCl) and Si (0 and 1.5 mmol L^{-1} Si) on the Na^+ contents in roots (**a**) and shoots (**b**), excreted Na^+ from shoots (**c**) and electrolyte leakage of roots (**d**) of *P. distans* plants. Error bars represent the standard error. Different small letters on histograms represent statistically significant differences at $P \leq 0.05$

Si nutrition decreased the proline content of roots under salinity while in shoots no significant changes occurred in the proline content after the application of Si.

Total amino acids increased in both roots and shoots due to salinity (Table 1), and Si nutrition caused significant increase in total amino acid contents of plants so that the figures in roots and shoots of +Si plants were 26 and 16% respectively, more than those grown in the absence of Si.

Irrespective to Si treatments, the protein content remained unchanged only in roots under salt treatment but it increased in shoots (Table 1). Si nutrition decreased the protein content of roots while in shoots significant increase were observed in the protein content after the application of Si.

3.3 Si Content and Cell Wall Components

Salinity increased the Si content of plants (Table 2). Also, increase in the Si content of plants occurred under both saline and control conditions when plants supplied with Si.

The amount of phenolics in shoots was markedly greater than roots (Table 2). This parameter increased in roots by salt treatment however, the applied Si did not show any effect in this organ. Phenolics decreased in shoots under

Table 1 Effect of salinity (control versus 200 mmol L^{-1} NaCl) on sugars, amino acids, proline and protein contents in roots and shoots of *Puccinellia distans* grown with or without supplementary silicon

		0 mmol L^{-1} NaCl		200 mmol L^{-1} NaCl	
		0 mmol L^{-1} Si	1.5 mmol L^{-1} Si	0 mmol L^{-1} Si	1.5 mmol L^{-1} Si
Soluble sugar (mg g^{-1} Dw)	root	11.15±1.76c	9.67±0.80c	49.65±3.29a	38.00±1.44b
	shoot	22.90±1.35c	31.84±1.06b	38.17±2.36a	42.90±1.61a
Insoluble sugar (mg g^{-1} Dw)	root	0.34±0.02b	0.52±0.03a	0.31±0.00b	0.62±0.05a
	shoot	0.77±0.09a	0.18±0.03c	0.68±0.02a	0.46±0.04b
Amino acids (mg g^{-1} Dw)	root	14.37±1.30c	16.59±2.56bc	22.85±0.85b	30.88±2.67a
	shoot	29.28 ±3.23c	33.16±2.16bc	38.91±0.84b	46.21±1.25a
Proline (mg g^{-1} Dw)	root	2.81±0.18b	2.98±0.11b	4.07±0.20a	3.25±0.22b
	shoot	4.77±1.28b	4.97±0.45b	6.46±0.56ab	7.62±0.50a
Protein (mg g^{-1} Fw)	root	5.08±0.13a	4.27±0.22bc	4.63±0.13ab	3.86±0.06c
	shoot	5.54±0.10c	5.22±0.16c	7.92±0.24b	9.39±0.57a

Data are means ±standard errors (n = 5). Different small letters in each row represent statistically significant differences at $P<0.05$

Table 2 Effect of salinity (control versus 200 mmol L^{-1} NaCl) on phenolics, lignin, cellulose and Si contents in roots and shoots of *P. distans* grown with or without supplementary Si

		0 mmol L^{-1} NaCl		200 mmol L^{-1} NaCl	
		0 mmol L^{-1} Si	1.5 mmol L^{-1} Si	0 mmol L^{-1} Si	1.5 mmol L^{-1} Si
Phenolics (µmol g^{-1} Dw)	root	0.38±0.03b	0.51±0.03b	0.72±0.05a	0.85±0.05a
	shoot	1.17±0.05b	1.59±0.04a	1.00±0.02c	1.02±0.05bc
Lignin (mg g^{-1} Dw)	root	4.81±0.26ab	5.38±0.25a	3.91±0.22b	5.24±0.53a
	shoot	2.69±0.04b	4.28±0.48a	3.14±0.14b	4.12±0.20a
Cellulose (mg g^{-1} Dw)	root	26.22±5.58c	31.88±4.80c	49.55±4.60b	64.16±1.43a
	shoot	16.38±0.47c	22.38±1.91bc	28.55±4.23b	45.11±0.61a
Silicon (mg g^{-1} Dw)	root	1.70±0.31d	3.79±0.19b	2.48±0.28c	5.67±0.42a
	shoot	2.16±0.14c	5.90±0.81b	2.50±0.11c	8.01±0.29a
Phenolics (µmol g^{-1} Dw)	root	0.38±0.03b	0.51±0.03b	0.72±0.05a	0.85±0.05a
	shoot	1.17±0.05b	1.59±0.04a	1.00±0.02c	1.02±0.05bc

Data are means ±standard errors (n = 5), Different small letters in each row represent statistically significant differences at P <0.05

saline conditions and Si application increased the phenolic content of this organ only under the control conditions.

Tissue cellulose increased in both roots and shoots under saline conditions (Table 2). The cellulose content of plants under saline conditions was further increased when they were fed with supplemental Si. Thus, roots and shoots of Si treated plants maintained under saline conditions displayed 23 and 37% respectively, greater cellulose as compared to those not supplied with Si (Table 2).

The plant lignin content did not show any changes following salt treatment (Table 2), the application of Si increased the lignin content in both roots and shoots irrespective of salinity condition.

3.4 Electrolyte Leakage, Na^+ Secretion and Rhizosphere Acidification

The tissue electrolyte leakage increased following salt treatment of plants while Si nutrition reduced it in these plants by about 18% (Fig. 3).

The secretion of Na^+ from plant leaf surfaces increased under saline conditions (Fig. 3). Si nutrition could increase Na^+ secretion so that the excreted Na^+ in Si–supplied plants grown under saline condition was 25% greater than those grown without supplemental Si.

Rhizosphere acidification presumably due to H^+–ATPase activity were visualized by changing color of the bromocresol pH indicator from pink to yellow in agar gel substratum immobilized around roots. The color change was more pronounced around the younger roots. Salinity and Si treatments were both increased color shift to yellow around roots indicating higher rhizosphere acidification (Supplementary material 3).

The activity of H^+–ATPase increased significantly in roots but not shoots under salinity (Fig. 4). However, Si application increased this activity in both organs irrespective of salt treatment and even it was more efficient in stimulating the activity of shoot H^+– ATPase under saline conditions. Thus, the enzyme activity in shoots and roots of Si–fed plants increased by 90 and 38% respectively, under saline conditions.

4 Discussion

Salinity decreased total and shoot dry weights of *P. distans* plants but did not show any effect on root dry weight (Fig. 1). The decreased growth due to salinity has been reported in other Si accumulating grasses such as*Puccinellia tenuiflora, Sporobolus ioclados* and others [23, 45, 46]. The reduced shoot to root ratio in *P. distans* appears as a strategy to manage water demand under saline conditions as this trait results in reduced water loss from aerial parts and increased water absorption from roots [44, 47]. Reduction of Si nutrition alone increased dry weight of plants and furthermore mitigated harmful effects of salinity on this parameter (Fig. 1). Improvement of salt tolerance by Si nutrition has been reported in non-halophytic barley, rice and canola plants [10, 12, 14, 48] salt tolerant sorghum and kentucky bluegrass [18, 49] and halophytic grass *Spartina densiflora* [24].

Si nutrition also led to reduced amounts of soluble sugars but increased insoluble sugars in *P. distans* roots under salinity. The lower soluble sugar content of roots might be due to greater growth respiration under these conditions. In contrast, the amount of insoluble sugar decreased in

shoots (Table 1). It seems that Si nutrition through supply of more soluble sugars from shoot could sustain root growth under salinity associated with transient starch deposition. Increased starch content associated with induction of Si transporters has recently been reported in barley plants subjected to osmotic stress [50].

Salinity reduced the relative water content, transpiration and stomatal density of *P. distans* plants (Fig. 2). Decreased relative water content indicates water deficit due to reduction of water absorption under salinity, however, salinity in succulent halophytes in which salt excretion is not important for ion homeostasis, has positive correlation with relative water content [6]. The reduction of transpiration and stomata density in *P. distans*, might represent the acclimation of plants to salinity which prevents further water loss. These are in congruence with those reported for other halophytic grasses such as *Spartina densiflora* and *Sporobolus ioclados* [24, 45]. Thus, the water relation-related responses to salinity of *P. distans* plants i.e. a halophytic Si accumulator do not differ significantly from other halophytes. Si application stimulated salt-induced reduction of stomatal density and transpiration rate in *P. distans* and thus led to increased relative water contents of plants (Fig. 2). The increased relative water content has been reported in a glycophytic i.e. tomato [15] however, in a halophyte Si accumulating grass i.e.*Spartina densiflora,* Si application under saline conditions was associated with increased transpiration [24]. In rice i.e. a glycophyte Si accumulator, Si deposition as colloidal silica gel (SiO_2) in root apoplast prevents the the flux of transpired water derived from bypass flow towards the xylem vessels [9, 14]. Furthermore, the Si mediated increase of water uptake by rice roots under saline conditions is associated with enhanced expression of aquaporins [51]. These mechanisms might also be applied equally to *P. distans* plants to justify the Si mediated reduced transpiration and increased relative water contents of this species under salinity which consequently improves plant growth.

Due to salinity, the contents of amino acids, proline and soluble sugars increased in *P. distans* plants while soluble sugars displayed the greatest increase compared to amino acids and proline (Table 1). These water soluble low molecular weight compounds which do not interfere with plant metabolic processes are known as compatible solutes [2]. The increased levels of these metabolites bring about plant osmotic adjustment to encounter water deficit imposed by salinity. In halophytes including Si accumulating Poaceae, compatible solutes may act as chemical chaperones and as scavenger of hydroxyl radicals [52]. Besides compatible solutes, osmotic adjustment of *P. distans* plants might be achieved through Na^+ accumulation. Thus, a two-fold increase in the tissue sodium content occurred in plants exposed to salinity (Fig. 4). As emphasized for halophytes [5], osmotic adjustment is achieved mostly with Na^+ and Cl^- in the vacuoles, while compatible organic solutes function in the cytosolic compartment.

The increased concentration of tissue Na^+ might disturb the intracellular ion homeostasis and reduces membrane functionality. The increased Na^+ secretion from leaves of *P. distans* plants under salinity (Fig. 3) is thus a response to restore ion homeostasis. Thus, this halophyte employs the salt secretion mechanism to withstand saline conditions [6]. Salinity was also associated with increased proton extrusion and H^+-ATPase activity of roots but not shoots (Fig. 4). The increased root H^+-ATPase activity might contribute to salt exclusion mechanism at the root surfaces and probably acts in the stele to reduce salt (Na^+) transport from root to shoot [6].

Amino acids were the only organic solute which showed greater accumulation following Si nutrition under saline conditions while other organic (soluble sugars, proline) and inorganic (Na^+) solutes declined (Table 1, Fig. 3). It appears that in *P. distans* plants under saline conditions, Si attenuates stress symptoms evidenced by lower proline and soluble sugars and furthermore, stimulate amino acid and soluble protein accumulation especially in aerial parts (Table 1). Decrease of Na^+ uptake under salinity in plants supplied with Si might be related to its reduction of apoplastic transport in the cell walls and endodermis due to Si deposition [9] and / or increased efficiency of exclusion

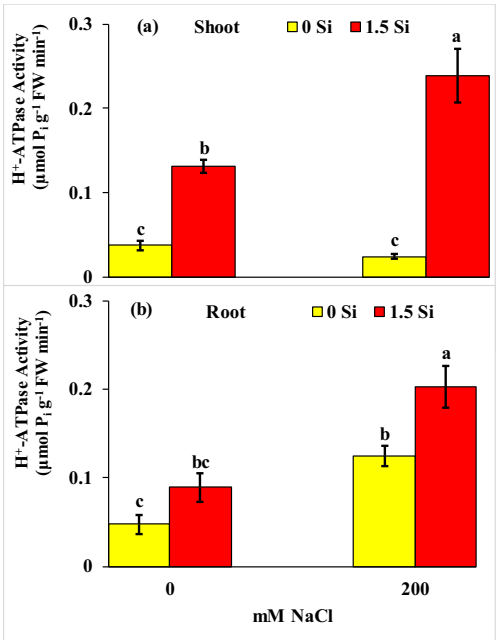

Fig. 4 Changes in ATPase activity in shoot (**a**) and root (**b**) of *P. distans* plants in the presence or absence of 1.5 mmol L^{-1} Si under salinity. Error bars represent the standard error. Different small letters on histograms represent statistically significant differences at $P \leq 0.05$

mechanisms at the root surfaces evidenced by greater H^+–ATPase activity and rhizosphere acidification (Figs. 5 and 6). Decreased Na^+ content due to Si supplementation has also been reported in halophytic grass *Spartina densiflora* under salinity [24]. Alternatively, greater shoot H^+-ATPase activities associated with more efficient Na^+ excretion mechanism in this organ may contribute to reduced Na^+ content and the consequent alleviation of salt toxicity (Fig. 4) as reported earlier in barley [10].

Salinity increased electrolyte leakage from *P. distans* leaves (Fig. 3). The increased electrolyte leakage in glycophytes such as wheat [11] and rice [39] in response to salinity has been interpreted as perturbation in membrane functioning. Si treated plants displayed reduction of electrolytes leakage from leaves (Fig. 3) which is a good measure of membrane functioning as reported in salt tolerant kentucky bluegrass supplied with Si [49]. Optimal membrane functioning has also been reported following Si application of Si accumulating and non-accumulating glycophytes under salinity [10, 11].

Salinity increased the phenolic content of roots and decreased it in shoots (Table 2). The increase in phenolics in roots under salinity might be related to alterations of plant secondary metabolism following oxidative stress [12, 43, 53] Salt treatment had no significant effect on the lignin content of *P. distans*. In a halophytic species *Atriplex prostrate*, salinity reduces lignification in the third and fourth internodes under salinity [54], while this treatment in both Si and non Si accumulating glycophytes increases the lignin contents [12]. However, salinity levels at which lignin biosynthesis starts might vary amongst species and tissues and it is possible that at higher salt concentrations *P. distans* plants display greater lignin content. Si application increased the lignin contents of both roots and shoots in *P. distans* plants under salinity (Table 2). Impacts of Si on plants lignin contents appear to be tissue and / or species specific. Thus, Si application reduced the lignin contents of canola plants under salinity [12]. In rice, Si induces lignin deposition in roots [16] but appears to inhibit lignin accumulation in shoots [55]. Our results for the increased lignin content following Si application are consistent with the results of Fleck et al. [12] in rice roots. The increased shoot lignin content in +Si *P. distans* plants under salinity might have some relevance to its tolerance mechanisms which beside exclusion at the root surfaces has also employed salt excretion mechanism in shoots as reported in other halophytes (40).

Salinity increased the cellulose contents of *P. distans* plants and the added Si strengthened this response (Table 2). Cell walls in grasses contain cellulose microfibrills interlinked with glucuronoarabinoxylans and phenolic network [56] which may combine with Si [57]. Increase of cellulose and lignin by Si under salinity may prevent plants lodging and increases light acquisition which further improves plant growth under stressful conditions.

5 Conclusion

The information on the beneficial aspects of Si nutrition on halophytes is scarce. The present study showed that Si application in a Si accumulating plant i.e. *P. distans* under salinity could reduce water deficit as well as salt toxicity through increase of osmoregulatory organic solutes and maintaining less tissue Na^+, respectively. Furthermore, improvement of membrane performance following Si application as evidenced by lower electrolyte leakage and higher rhizosphere acidification possibly through greater H^+–ATPase activity could help plant for further Na^+ secretion and exclusion from sensitive tissues. The increase of lignin and cellulose content in Si–fed plants grown under saline conditions might improve salt excretion and /or exclusion mechanisms through development of secondary cell walls in both roots and shoots.

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References

1. Munns R, Tester M (2008) Mechanisms of salinity tolerance. Ann Rev Plant Biol 59:651–81
2. Ashraf M, Harris PJC (2004) Potential biochemical indicators of salt tolerance in plants. Plant Sci 166:3–16
3. Parida AK, Das AB (2005) Salt tolerance and salinity effect on plants. Ecotox Environ Safe 60(3):324–349
4. Tester M, Davenport R (2003) Na^+ tolerance na^+ transport in higher plants. Ann Bot 5:503–527
5. Flowers TJ, Colmer TD (2015) Plant salt tolerance: adaptations in halophytes. Ann Bot 115:327–331
6. Duarte B, Sleimi N, Caçador I (2014) Biophysical and biochemical constraints imposed by salt stress: learning from halophytes. Front Plant Sci 5:746–755
7. Sommer M, Kaczorek D, Kuzyakov Y, Breuer J (2006) Silicon pools and fluxes in soils and landscapes– a review. J Plant Nutr Soil Sci 169:310–329
8. Epstein E (1994) The anomaly of silicon in plant biology. Proc Natl Acad Sci 91(1):11–1
9. Gong HJ, Randall DP, Flowers TJ (2006) Silicon deposition in the root reduces sodium uptake in rice (Oryza sativa L.) seedlings by reducing bypass flow. Plant Cell Environ 29:1970–1979
10. Liang YC, Zhang WH, Chen Q, Liu Y, Ding RX (2006) Effect of exogenous silicon (Si) on h^+–ATPase activity, phospholipids and fluidity of plasma membrane in leaves of salt–stressed barley (Hordeum vulgare L.) Environ Exp Bot 57:212–219
11. Tuna AL, Kaya C, Higgs D, Murillo–Amador B, Aydemir S, Girgin AR (2008) Silicon improves salinity tolerance in wheat plants. Environ. Exp Bot 62:10–16

12. Hashemi A, Abdolzadeh A, Sadeghipour HR (2010) Beneficial effects of silicon nutrition in alleviating salinity stress in hydroponically grown canola, Brassica napus L. plants. J Soil Sci Plant Nutr 56:244–253
13. Sattar A, Cheema MA, Ali H, Sher A, Ijaz M, Hussain M, Hassan W, Abbas T (2016) Silicon mediates the changes in water relations, photosynthetic pigments, enzymatic antioxidants activity and nutrient uptake in maize seedling under salt stress. Grassland Sci 62:262–269
14. Yeo AR, Flowers S, Rao G, Welfare K, Senanayake N, Flowers TJ (1999) Silicon reduces sodium up take in rice (Oryza sativa L.) in saline conditions and this is accounted for by a reduction in the transpirational bypass flow. Plant Cell Environ 22:559–565
15. Romero–Aranda MR, Jurado O, Cuartero J (2006) Silicon alleviates the deleterious salt effect on tomato plant growth by improving plant water status. J Plant Physiol 163:847–855
16. Fleck T, Nye A, Repenning TC, Stahl F, Zahn M, Schenk KM (2011) Silicon enhances suberization and lignification in roots of rice (Oryza sativa). J Exp Bot 62:2001–2011
17. Coskun D, Britto DT, Huynh WQ, Kronzucker HJ (2016) The role of silicon in higher plants under salinity and drought stress. Front Plant Sci 7:1072
18. Yin L, Wang S, Tanaka K, Fujihara S, Itai A, Den X (2016) Silicon mediated changes in polyamines participate in silicon-induced salt tolerance in Sorghum bicolor L. Plant Cell Environ 39:245–258
19. Markovich O, Steiner E, Kouril S, Tarkowski P, Aharoni A, Elbaum R (2017) Silicon promotes cytokinin biosynthesis and delays senescence in Arabidopsis and Sorghum. Plant Cell Environ 40:1189–1196
20. Tarasoffa CS, Mallory–Smitha CA, Ballb DA (2007) Comparative plant responses of Puccinellia distans and Puccinellia nuttalliana to sodic versus normal soil types. J Arid Environ 70:403–417
21. Bandani M, Abdolzadeh A (2007) Effects of silicon nutrition on salinity tolerance of Puccinellia distans (jacq.) parl. J Agr Sci Nat Resour 14(3):111–119
22. Langlosi E, Bonis A, Bouzille JB (2003) Sediment and plant dynamics in saltmarshes pioneer xone: Puccinellia as a key species. Estuar Coast Shelf *S* 56:239–249
23. Alshammary SF, Qian YL, Wallner SJ (2004) Growth response of four turfgrass species to salinity. Agr Water Manag 66:97–111
24. Mateos-Naranjo E, Andrades-Moreno L, Davy AJ (2013) Silicon alleviates deleterious effects of high salinity on the halophytic grass Spartina densiflora. Plant Physiol Biochem 63:115–121
25. Hoagland DR, Arnon DI (1950) The water culture method for growing plant without soil. California Agri. Exp. Sta. Cir. No. 347. University of California Berkley Press, CA, p 347
26. Abdolzadeh A, Raghimi M, Mehraban P, Ghlipour M, Mierzaali E (2012) The Potential of Puccinellia distans for cultivation in uncommon salty waters. J Water Soil 26(2):484–493
27. Kiani CZ, Abdolzadeh A, Sadeghipour HR (2014) Silicon nutrition potentiates the antioxidant metabolism of rice plants under iron toxicity. Acta Physiol Plant 36:493–502
28. Kim YH, Khan AL, Waqas M, Shim JK, Kim DH, Lee KY, Lee IJ (2014) Silicon application to rice root zone influenced the phytohormonal and antioxidant responses under salinity stress. J Plant Growth Regul 33(2):137–149
29. Elliot CL, Snyder GH (1999) Autoclave–indused digestion for the colorimetric determination of silicon: Silicon. Annu Rev Plant Phys 50:641–644
30. McCready RM, Guggolz J, Silviera V, Owens HS (1950) Determination of Starch and amylose in vegetables. Anal Chem 22(9):1156–1158
31. Bradford M (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254
32. Lavid N, Schwrtz A, Yarden O, Tel-Or E (2001) The involvement of polyphenols and peroxidase activities in heavy–metal accumulation by epidermal glands of waterlily (Nymphaeaceae). Planta 212:323–331
33. Yemm EW, Cocking EC, Ricketts RE (1955) The determination of amino-acids with ninhydrin. Analyst 80:209
34. Kochert A (1987) Carbohydrate determination by phenol–sulfuric acid method. In: Hellebust JA, Craige JS (eds) Handbook of physiology and biochemical methods. Cambridge University Press, London, pp 95–97
35. Bates LS, Waldern RP, Teare ID (1973) Rapid determination of free proline for water stress studies. Plant Soil 39:205–207
36. Kokubo A, Kuraishi S, Sakurai N (1989) Culm strength of barley. Plant Physiol 91:876–882
37. Correa–Aragunde N, Lombardo C, Lamattina L (2008) Nitric oxide: an active nitrogen molecule that modulates cellulose synthesis in tomato roots. New Phytol 179:386–396
38. Zimmer M (1999) Combined methods for the determination of lignin and cellulose in leaf litter. Sci Soil 4:20–32
39. Lutts S, Kinet JM, Bouharmont J (1996) Nacl–induced senescence in leaves of rice (Oryza sativa L.) cultivars differing in salinity resistance. Ann Bot 78:389–398
40. Hossain MB, Matsuyama N, Kawasaki M (2016) Hydathode morphology and role of guttation in excreting sodium at different concentrations of sodium chloride in eddo. Plant Prod Sci 19:528–539
41. Romheld V (1984) Different strategies for iron acquisition in higher plants. Physiol Plant 70:231–234
42. Ames BN (1966) Assay of inorganic phosphate, total phosphate and phosphatases. Method Enzymol 8:115–118
43. Z1 Gerivani, Vashaee E, Sadeghipour HR, Aghdasi M, Shobbar ZS, Azimmohseni M (2016) Short versus long term effects of cyanide on sugar metabolism and transport in dormant walnut kernels. Plant Sci 252:193–204
44. Janicka–Russak M, Kabaa K (2012) Abscisic acid and hydrogen peroxide induce modification of plasma membrane h^+–ATPase from Cucumis sativus L. roots under heat shock. J Plant Physiol 169:1607–1614
45. Gulzar S, Ajmal Khan M, Ungar IA, Liu X (2005) Influence of salinity on growth and osmotic relations of Sporobolus ioclados. Pakistan J Bot 37:119–129
46. Guo Q, Wang P, Ma Q, Zhang JL, Bao AK, Wang SM (2012) Selective transport capacity for K^+ over Na^+ is linked to the expression levels of ptSOS1 in halophyte Puccinellia tenuiflora. Funct Plant Biol 39(12):1047–1057
47. Acosta-Motos JR, Ortuño MF, Bernal-Vicente A, Diaz-Vivancos P, Sanchez-Blanco MJ, Hernandez JA (2017) Plant responses to salt stress: Adaptive mechanisms. Agron 7:1–38
48. Farshidi M, Abdolzadeh A, Sadeghipour HR (2012) Silicon nutrition alleviates physiological disorders imposed by salinity in hydroponically grown canola (Brassica napus L.) plants. Acta Physiol Plant 34:1779–1788
49. Chai Q (2010) Silicon effects on Poa pratensis responses to salinity. Hort Sci 45(12):1876–1881
50. Hosseini SA, Maillard A, Hajirezaei MR, Ali N, Schwarzenberg A, Jamois F, Yvin JC (2017) Induction of barley silicon transporter HvLsi1 and HvLsi2, increased silicon concentration in the shoot and regulated starch and ABA homeostasis under osmotic stress and concomitant potassium deficiency. Front Plant Sci 8:1–15
51. Rios JJ, Martínez-ballesta MC, Ruiz JM, Blasco B, Carvajal M (2017) Silicon-mediated improvement in plant salinity tolerance: The role of aquaporins. Front Plant Sci 8:1–10
52. Slama I, Abdelly C, Bouchereau A, Flowers T, Savoure A (2015) Diversity, distribution and roles of osmoprotective compounds

accumulated in halophytes under abiotic stress. Ann Bot 115:433–447
53. Petridisa A, Theriosa I, Samourisb G, Tananakic C (2012) Salinity–induced changes in phenolic compounds in leaves and roots of four olive cultivars (Olea europaea L.) and their relationship to antioxidant activity. Environ Exp Bot 79:37–43
54. Wang LW, Showalter AM, Ungar IA (1997) Effect of salinity on growth, ion content and cell wall chemistry in Atriplex prostrate (Chenopodiaceae). Am J Bot 84(9):1247–1255
55. Suzuki S, Ma JF, Yamamoto N, Hattori T, Sakamoto M, Umezawa T (2012) Silicon deficiency promotes lignin accumulation in rice. Plant Biotechnol 29:391–394
56. Cosgrove DJ (2005) Growth of the plant cell wall. Nat Rev Mol Cell Biol 6:850–861
57. Zhang C, Wang L, Zhang W, Zhang F (2013) Do lignification and silicification of the cell wall precede silicon deposition in the silica cell of the rice (Oryza sativa L.) leaf epidermis? Plant Soil 372:137–149