

Exogenously applied silicate and abscisic acid ameliorates the growth of salinity stressed wheat (*Triticum aestivum* L) seedlings through Na⁺ exclusion

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Abstract

Hydroponic experiments were conducted to investigate the possible role of abscisic acid (ABA) and silicate (Si) on inducing salinity tolerance in wheat seedlings. Caryopses of two wheat genotypes Kharchia-65 (salt tolerant) and Punjab-85 (salt sensitive) were pre-treated with ABA (10 mM) for 24 h. Ten-day-old seedlings were exposed to 100 mM NaCl solution containing either 0 mM or 3 mM of sodium silicate for 16 days. We employed a fluorescent tracer trisodium salt of 8-hydroxy-1, 3, 6-pyrenetrisulphonic acid (PTS) for estimating Na⁺ transport pathway to shoot. Exogenously applied Si as alone or with ABA significantly improved seedling growth by inhibiting Na⁺ transport and the Na⁺/K⁺ ratio in both wheat genotypes. The genotype Punjab-85 maintained higher apoplastic Na⁺ concentration compared to Kharchia-65 under NaCl treatment. Moreover, Si and ABA application improved leaf chlorophyll contents and consequently net assimilation rate of NaCl- stressed wheat seedlings through up-regulation of antioxidative enzyme activities. The results suggested that application of Si alone or in combination with ABA can significantly limit Na⁺ bypass flow in both salt sensitive and tolerant wheat genotypes; however, ABA alone was effective in sensitive genotype only (Punjab-85). It is inferred from the results that Si had a more prominent role than ABA on plants in increasing biomass accumulation, proline contents and antioxidant enzyme activities, and inhibiting Na⁺ accumulation and bypass flow. Nevertheless, ABA assisted Si in the amelioration of salt stressed in much better way than Si alone.

Keywords: *Triticum aestivum*, ABA, Silicate, Salt tolerance, Bypass flow.

Abbreviations: ABA_Abscisic acid, Si_Silicate, SOD_Superoxide dismutase, POD_Peroxidase, CAT_Catalase, Chl_Chlorophyll, NAR_Net assimilation rate.

Introduction

Wheat (*Triticum aestivum* L.) is an important cereal crop used as staple food in many parts of the world. Numerous abiotic stresses such as salinity, drought and heat are the major constraints to wheat productivity in the arid and semi-arid regions. Of these, salinity is the major abiotic stress that is regarded highly deleterious to growth and productivity of wheat crop. It disturbs intercellular ionic homeostasis through osmotic stress and increased concentration of non-essential ions Na⁺ and Cl⁻ in the soil solutions (Hasegawa et al., 2000; Rodriguez-Navarro and Rubio, 2006). Improvement in salt tolerance is a complex phenomenon, where conventional breeding techniques have limited success in the past (Flowers, 2004). In order to tolerate high salinity level, plants not only have to cope with Na⁺ toxicity but also need to maintain high cellular K⁺ concentration (Amtman et al., 2006; Zhang et al., 2010). Excessive cellular Na⁺ and Cl⁻ concentrations in plants impair electron transport system, and accelerate reactive oxygen species (ROS) production (Foyer et al., 1994). Elevated ROS levels inside the cells are highly injurious to membranes and other cellular components such as chloroplast, protein, nucleic acids and lipids (Lin and Kao, 2000). To combat the ROS- incited intercellular damage, the

plants have developed antioxidant defense systems that regulate ROS levels (Najeeb et al., 2009, 2011). Abscisic acid (ABA) is an important endogenous plant hormone that regulates diverse functions of plant growth and development under drought and salinity (Beaudoin et al., 2000). In a saline environment, ABA treatment has been suggested to improve salinity tolerance by increasing growth of sorghum, rice and wheat crops (Amzallag et al., 1990; Gurmani et al., 2011; 2007). The ABA mediated growth improvement via modulation of ion transport in the plant roots, stomata and storage tissue (Roberts and Snowman, 2000; Halbrog et al., 2002). Seed ABA pretreatment has been reported to limit cellular Na⁺ concentration and increase osmoregulation via promoting proline and sugar accumulation in salt stressed rice leaves (Gurmani et al., 2011). Silicon (Si) is the second most abundant element in the soil that has beneficial impact on growth and development of crops such as wheat (Tuna et al., 2008), rice (Gong et al., 2006), barley (Liang et al., 1996) and maize (Parveen and Ashraf, 2010) grown in salt affected areas. It improves plant growth through reducing cellular inflow of Na⁺ ions, while maintaining chlorophyll content and photosynthetic activity under stressful environment

(Liang, 1998). In wheat, it induces salinity tolerance via reducing plasma membrane permeability to Na^+ , and increasing shoot K^+ and Ca^{2+} concentrations (Tuna et al., 2008). In addition, its exogenous application up-regulates antioxidative enzyme activities i.e. superoxide dismutase (SOD), peroxidase (POD), guaiacol peroxidase (GPX), ascorbate peroxidase (APX) and glutathione reductase (GR), enhances photochemical efficiency and chlorophyll content that play a role in ameliorating adverse effect of salinity (Al-aghaby et al., 2004; Zhu et al., 2004). From the last several years, plant physiologists have been facing the challenge to investigate the pathways for water and ions movement into plant roots and shoots (Hose et al., 2001). For this purpose a number of tracers were experimented. Trisodium-8-hydroxy-1,3,6-pyrenetrisulphonic acid (PTS), is one of the water-soluble fluorescent non-hazardous chemicals that does not cross plant cell membranes or stick to cell walls (Yeo et al., 1987; Vesik et al., 2000). Generally bypass flow is a minute proportion 1–5% of the transpirational volume flow (Yeo et al., 1987), although it plays an essential role in ion transport at high transpiration rates (Garcia et al., 1997; Flowers and Flowers, 2005). Various reports been suggested to determine the bypass flow through PTS in plants such as *Zea mays*, *Vicia faba* (Peterson et al., 1981), *Gossypium hirsutum* (Skinner and Radin, 1994), *Allium cepa* (Cholewa and Peterson, 2001), *Triticum aestivum* and *Oryza sativa* (Garcia et al., 1997; Yadav et al., 1996; Gong et al., 2006). The role of Si and ABA in inducing salinity tolerance in crop plants has already been established (Din, 1997; Gong et al., 2006); however, the tolerance mechanisms are poorly understood. The present study evaluates the role of ABA alone as well as with Si on sodium transport, bypass flow, photosynthetic attributes and antioxidative enzyme activities in salt stressed wheat seedlings.

Results

In the absence of NaCl, ABA seed pre-treatment non-significantly increased the shoot dry weight of both genotypes; however, in the presence of 100 mM NaCl, it significantly improved the shoot dry biomass (37%) of Punjab-85 only (Table 1). While, silicate application alone or with ABA significantly improved the shoot dry weights of both genotypes under non-saline or saline condition. Under saline conditions, Si induced increased in shoot dry weight of Punjab-85 was 41% greater than control (non-treated plants) and 3.2% than ABA treatment. Kharchia-65 maintained relatively higher plant dry biomass compared to Punjab-85 under all the treatments (Table 1). Application of Si and ABA+ Si significantly increased the root dry biomass of Punjab-85 under all treatments. Noteworthy, ABA addition to Si contributed no further stimulation or reduction in root dry biomass of wheat seedlings. ABA appears to function independently of Si application, regardless of its relatively lower impact on wheat dry biomass improvement compared to Si under saline conditions. Salinity adversely affected the plant height of both wheat genotypes; the reduction in height was 13% of control in Kharchia-65, whereas, salinity induced approximately 2-fold greater height reduction in Punjab-85. ABA+Si treatment significantly improved the plant height of both wheat genotypes. Salinity induced root length diminution was 24% and 38% in Kharchia-65 and Punjab-85 respectively. Si treatment effectively ameliorated salt induced effect on root growth of both the genotypes, while only the Punjab -85 was responsive to ABA treatment. Amendments by ABA and Si treatments as alone or in combination significantly reduced the shoot Na^+ concentration in both

genotypes, although the magnitude of reduction was greater in Punjab-85. While, Si assisted ABA to further suppress Na^+ accumulation (Table 3). The PTS concentration significantly declined in response to Si and ABA + Si treatments in both genotypes; with ABA + Si treatment as the most efficient. Shoot K^+ concentration rose following any treatment of ABA or Si as alone or combined; while Si was relatively more effective than ABA for both the genotypes. Shoot Na^+/K^+ ratio of salt stressed wheat seedlings dropped in response to ABA, Si and ABA + Si treatments (Table 4). There was a significant suppression in transpiration rate of both the genotype in response to ABA treatment as compared to control (0 or 100 mM NaCl), where, this reduction was relatively higher in Punjab-85 compared to Kharchia-65 (Table 5). The Si alone or in combination with ABA showed no significant effect on transpiration rate. The NaCl treatment caused 2-fold reduction in net assimilation rate (NAR) of both wheat genotypes as compared to non-saline plants. The application of Si as alone significantly increased NAR over control. Salt treatment significantly suppressed leaf chlorophyll contents of both genotypes; however, application of Si with or without ABA significantly recovered chlorophyll contents of stressed plants. The NaCl treatment stimulated proline accumulation that was further augmented by ABA. In contrast, Si inhibited proline contents of NaCl stressed both wheat genotypes (Table 6). The ABA treatment had no significant effect on SOD activity of wheat plants, however, Si with or without ABA significantly enhanced SOD activity. The POD activity was significantly high under ABA + Si treatment, whereas, all other treatments remained ineffective. The same treatment was also effective in both the genotype for enhancing catalase activity (Fig. 1, 2 and 3).

Discussion

The maintenance of higher shoot biomass in Kharchia-65 was associated with lower Na^+ accumulation, higher K^+ concentration and K^+/Na^+ ratio in plant tissues. Improvement in salt tolerance by addition of silicon has been reported in barley (Liang et al., 1996 and 1998), rice (Yeo et al., 1999; Gong et al., 2006), wheat (Ahmed et al., 1992), maize (Parveen and Ashraf, 2010) and tomato (Al-Aghaby et al., 2004). The data indicated that the increase in root and shoot biomass was maximum in ABA + Si > Si treatment suggesting that ABA synergistically work with Si for improving biomass of salt stressed wheat plants. Significant effect of ABA on root and shoot dry weight under stressed condition may be attributed by ABA induced signal transduction in response to salt stress. Shoot growth recovery from salinity, under the application of ABA and ABA + Si reported here was correlated with shoot Na^+ exclusion from both wheat genotypes (Table 1 & 3). The better ability of Kharchia-65 to restrict Na^+ uptake and maintain K^+ level than Punjab-85 was associated with maintenance of higher NAR and chlorophyll. The overriding effect of Si on the PTS accumulation in Si and ABA + Si treatment is noteworthy. The greater suppression of Na^+/K^+ ratio in Kharchia-65 compared to Punjab-85 indicated higher salt tolerant potential of Kharchia-65. These results confirmed the finding of Din, (1997), who suggested Kharchia-65 as salt tolerant genotype. Presence of higher Na^+ concentrations in the tissues of salt sensitive genotype (Punjab-85) manifested Na^+ ion toxicity to various metabolic processes leading to growth reduction. Din and Flowers, (2002) reported ABA seed pre-treatment induced reduction in Na^+ and increased in K^+ uptake in a salt sensitive wheat genotype. From the analysis of xylem

Table 1. Abscisic acid and/or silicate induced changes dry biomass of wheat genotypes Kharchia-65 and Punjab-85 in the absence and presence of NaCl (100 mM) treatment.

Treatment	0 mM NaCl				100 mM NaCl			
	Shoot dry weight (mg)		Root dry weight (mg)		Shoot dry weight (mg)		Root dry weight (mg)	
	Kharchia-65	Punjab-85	Kharchia-65	Punjab-85	Kharchia-65	Punjab-85	Kharchia-65	Punjab-85
Control	208±24 ^{cd}	156±7 ^e	60±7 ^{bc}	46±4 ^d	153±6 ^{de}	92±8.5 ^e	32±3 ^{bcd}	23±2 ^{ef}
ABA	239±16 ^{bc}	193±11 ^{de}	54±5 ^{cd}	55±3 ^{cd}	171±10 ^{cd}	126±7 ^{bc}	23±4 ^{ef}	26±2 ^{def}
Si	270±1 ^{ab}	213±10 ^{cd}	71±3 ^a	59±3 ^{bc}	183±8 ^{ab}	130±7 ^{bc}	41±2 ^a	32±2 ^{bcd}
ABA+Si	294±15 ^a	242±9 ^{bc}	69±3 ^{ab}	63±5 ^{abc}	192±8 ^a	147±7 ^{abc}	38±2 ^{ab}	35±3 ^{abc}

SDW , shoot dry weight; RDW, root dry weight; ABA was supplied as seed soaking for 24 h; silicate was applied throughout the culture solution while NaCl 100 mM was applied at 10 d old plants. Values followed by the same letter (s) or not significantly different at P<0.05. ABA, abscisic acid; Si, silicate.

Table 2. Effect of abscisic acid and/or silicate on root and shoot length wheat genotypes Kharchia-65 and Punjab-85 in the absence and presence of NaCl (100 mM).

Treatment	0 mM NaCl				100 mM NaCl			
	Plant Height (cm)		Root Length (cm)		Plant Height (cm)		Root Length (cm)	
	Kharchia-65	Punjab-85	Kharchia-65	Punjab-85	Kharchia-65	Punjab-85	Kharchia-65	Punjab-85
Control	37±0.98 ^c	39±1.0 ^c	33±2 ^{ab}	24±1.2 ^c	32±0.69 ^c	29±0.66 ^d	25±0.76 ^{bc}	15±0.8 ^d
ABA	38±0.40 ^c	38±0.86 ^c	32±3 ^b	26±2.1 ^c	32±0.92 ^c	30±0.61 ^{cd}	23±1.4 ^{bc}	18±0.73 ^c
Si	40±1.0 ^{bc}	40±1.0 ^{bc}	36±1.6 ^a	28±1.2 ^{bc}	33±0.62 ^{bc}	32±0.63 ^c	30±0.67 ^a	20±0.81 ^c
ABA+Si	44±2.0 ^a	42±0.98 ^{ab}	34±2 ^{ab}	30±1.0 ^{bc}	36±0.51 ^a	34±0.77 ^{ab}	28±0.52 ^{ab}	22±0.55 ^{bc}

ABA was supplied as seed soaking for 24 h; silicate was applied throughout the culture solution while NaCl 100 mM was applied at 10 d old plants. Values followed by the same letter (s) or not significantly different at P<0.05. ABA, abscisic acid; Si, silicate.

Table 3. Abscisic acid and/or silicate induced changes in shoot sodium and potassium concentration of NaCl (100 mM) stressed wheat genotypes Kharchia-65 and Punjab-85.

Treatment	Na ⁺ concentration (μmol g ⁻¹)		K ⁺ concentration (μmol g ⁻¹)	
	Kharchia-65	Punjab-85	Kharchia-65	Punjab-85
Control	1360±99 ^b	1640±91 ^a	215±32 ^b	328±30 ^a
ABA	1132±82 ^{bcd}	1270±80 ^{bc}	160±27 ^{bc}	211±34 ^b
Si	1060±90 ^{cd}	1160±78 ^{bcd}	114±18 ^c	139±20 ^c
ABA+Si	967±76 ^d	1040±58 ^{cd}	92±10 ^c	120±8 ^c

ABA was supplied as seed soaking for 24 h; silicate was applied throughout the culture solution while NaCl 100 mM was applied at 10 d old plants. Values followed by the same letter (s) or not significantly different at P<0.05. ABA, abscisic acid; Si, silicate;

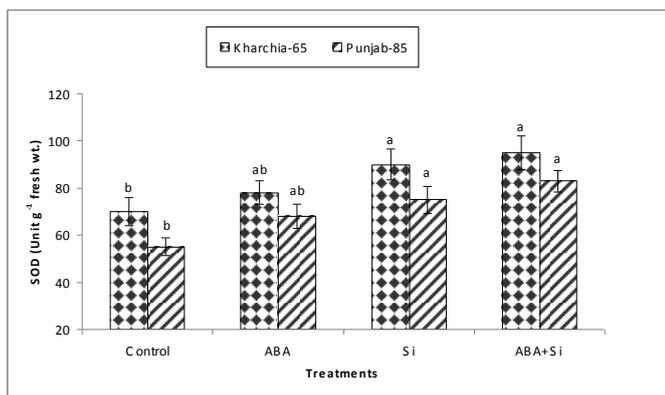


Fig 1. Effect of silicate and abscisic acid on superoxide dismutase (SOD) in shoot of wheat genotypes Kharchia-65 and Punjab-85at 100 mM NaCl. Values followed by the same letter (s) or not significantly different at P<0.05.

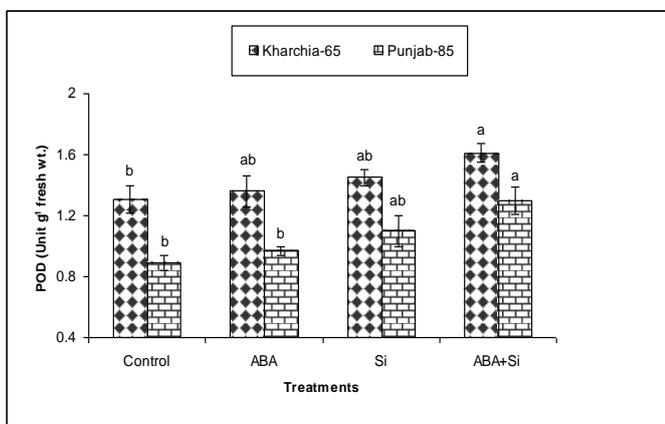


Fig 2. Effect of silicate and abscisic acid on peroxidase (POD) in shoot of wheat genotypes Kharchia-65 and Punjab-85at 100 mM NaCl. Values followed by the same letter (s) or not significantly different at P<0.05.

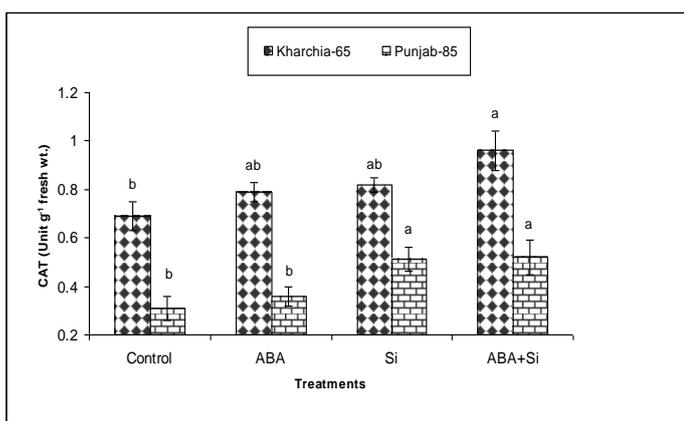


Fig 3. Effect of silicate and abscisic acid on catalase activity (CAT) in shoot of wheat genotypes Kharchia-65 and Punjab-85at 100 mM NaCl. Values followed by the same letter (s) or not significantly different at P<0.05.

priming has influence on ion transport. It is remarkable that a short term (24 h) ABA seed treatment can affect subsequent performance, some 6 days later, when ABA concentrations in the plants did not differ (Din, 1997). We speculated that ABA influenced plant growth by regulating developmental pathways. Positive role of seed priming and subsequent effects on plant performance under saline conditions has already been documented i.e. seed treatment with kinetin alleviated the adverse effect of salt by inhibiting Na⁺ uptake and increasing wheat growth (Iqbal and Ashraf, 2005). Seed ABA pre-treatment reduced bypass flow only in salt sensitive genotype; however, Si application as alone or with ABA significantly reduced PTS concentration in both genotypes. Our results are in agreement with earlier findings that Si reduces bypass flow in rice seedlings (Gong et al., 2006; Faiyue et al., 2010). Exogenously applied Si through culture solution decreased Na⁺ uptake in rice by limiting bypass flow (Yeo et al., 1999). As the wheat plants have much lower bypass flow than rice (Garcia et al., 1997), the Si application proved more effective for wheat. Si application inhibited Na⁺ uptake and bypass flow via its deposition in the exodermis of seminal roots (Gong et al., 2006). We found that ABA seed pre-treatment reduced transpiration rate of salt stressed wheat, which helped to minimize salinity induced dehydration, and consequently induced salinity tolerance. The observed lack of any significant effect of Si on transpiration rate of Kharchia-65 was not regulated by ABA application. Whereas, in Punjab-85 ABA + Si treated plants exhibited decrease transpiration rate over the control plants. As uptake of solutes from root to shoot is associated with transpirational volume flow (Yeo et al., 1987, 1999), decreased transpiration inhibited solute transport to aboveground parts. The results of present experiment confirmed findings of Yeo et al., 1999 and Gong et al., (2006), who inferred that Si application had positive effect on the transpiration rate of rice seedling. Application Si alone or with ABA reduced Na⁺ transport and increased transpiration rate, which represent that Si had direct influence on Na⁺ uptake from the root. Ahmed et al., (1992) reported that Si had multifaceted effect on Na⁺ transport mechanisms through which it limits Na⁺ movement in salt affected plants. Increase in NAR via Si with or without ABA might be correlated with wheat growth response under saline conditions. These treatments improved wheat physiological and metabolic functions contributing towards growth recovery. The Si was less effective than ABA in augmenting proline production under salt stress, however, its effect was more pronounced on up-regulation of SOD activities. During the present experiment ABA seed treatment increased proline accumulation in wheat genotypes. Since proline accumulation is an indication of adoptive response in plants to salt stress (Teixeira and Pereira, 2007), it confirmed that ABA regulated proline metabolism, and consequently induced salt tolerance (Savoure et al., 1997; Gurmani et al., 2011). Due to strong relationship between an efficient antioxidant system and plant salinity tolerance (Olmos et al., 1994), monitoring of antioxidative enzyme level in the cell could be a good indicator of plant tolerance to salt stress. In present investigations, SOD, POD and CAT activities in leaves of both the wheat genotypes were higher with the application of Si+ABA under saline conditions (Fig. 1, 2 and 3). Our results explained that Si raised SOD activity in leaves of both wheat genotypes that detoxified superoxide radicals by H₂O₂ generation (Olmos *et al.*,

sap collected from the stumps, they found that ABA pre-treatment lowered the sodium concentration and increased the selectivity in favour of K⁺, demonstrating that ABA

1994). Up-regulation of antioxidative enzyme activities under different amendments manifested the positive role of Si and ABA for inducing salinity tolerance in wheat. Exogenously applied Si has been found to improve cellular membrane stability and consequently salt tolerance in different plant species (Zhu et al., 2004; Lee et al., 2010). Similarly, ABA-pretreatment was found to activate a comprehensive defense mechanism enabling plants to defy salt stress (Li et al., 2010). ABA appears to assist Si in the amelioration of salt induced inhibition in plant growth, Na⁺ accumulation, PTS concentration, K⁺ accumulation and proline contents.

Material and methods

Plant material

Seeds of two wheat (*Triticum aestivum* L.) genotypes Kharchia-65 (salt tolerant) and Punjab-85 (salt sensitive) were obtained from the National Agricultural Research Centre, Islamabad, Pakistan. The salt tolerance of these two genotypes were explored by Din, (1997), who found that after exposure to NaCl, leaves of Kharchia-65 contained higher plant biomass and less Na⁺ concentrations, while Punjab-85 accumulated almost double Na⁺ concentrations and less plant biomass.

Treatment with ABA, Si and plant growth

A total of 600 seeds were soaked for 24 h in an aerated solution of abscisic acid (ABA; 10 mM) or distilled water (control) in black-painted flasks. The residual ABA was removed from seeds by washing the soaked seeds three times with distilled water. Thereafter, seed of each genotype were first sown in 4 plastic trays containing vermiculite irrigated with Hoagland's solution (Hoagland and Arnon, 1950). Four treatments; control, ABA, Si and ABA+Si were made. After 7 days, 500 healthy and uniform plants were selected and transplanted into their respective black-painted boxes (3.0 dm³) containing 3.0 L Hoagland's solution. Silicon was applied as sodium silicate (BDH, 25.5-28.5% SiO₂) solution to produce an estimated concentration of 3 mM; during the addition of sodium silicate, the pH could rise to 9.5, which was adjusted to 6 with 0.01 N H₂SO₄. Silicon was applied along with Hoagland's solution from the first day of sowing. Ten-day-old seedlings (500 plants of both genotypes) were randomly divided into two groups and transferred into a Hoagland's solution supplemented with 0 and 100 mM NaCl, respectively, for 16 days. Salinity was imposed as increments of 25 mM NaCl per day along with Hoagland's nutrients solution until the desired concentration of 100 mM was obtained, whereas; the non-saline plants were supplemented with Hoagland's nutrients solution only. Plants (26 d old) were harvested for determining Na⁺ and K⁺ concentrations, bypass flow, gas exchange, chlorophyll, proline, antioxidant enzyme activities and biomass. Experiment was conducted in the glasshouse at School of Life Sciences, University of Sussex, UK. The growing conditions were 12 h photoperiod of minimum of 400-500 μmol m⁻² s⁻¹ photosynthetically active radiation (HQ1 metal halide, Newry & Eyre, Brighton, UK) maintained at 27 °C and 1.0-1.5 K Pa saturation vapour pressure deficit (SVPD). The temperature during the dark period was 25 °C and SVPD 0.6 K Pa.

Bypass flow and Ions

Bypass flow was determined using a fluorescent, membrane-impermeant dye, the trisodium salt of 8-hydroxy-1, 3, 6-pyrenetrisulphonic acid (PTS) [former nomenclature 3-hydroxy-5, 8, 10-pyrenetrisulphonic acid and supplied as pyranin (Bayer UK)] with the method described by Yeo et al., (1987) and Yadav et al., (1996). Seedling were grown in boxes from which the solution could be easily drained, and replaced with the minimum disturbance to the root system. Plants of both genotypes (10d old) were treated either with 100 mM NaCl + PTS (30 mg dm⁻³) or unsalinised nutrient solution. After 16d, shoots were extracted with 10 mL of distilled water for 2 h at 90 °C and the fluorescence was measured at 510 nm using an excitation wavelength of 403 nm (Perkin Elmer, LS3). Sodium and potassium were extracted with distilled water for 2 h at 90 °C and the extract was analyzed by atomic absorption spectroscopy (Unicam 919, Cambridge, UK).

Gas exchange

The net assimilation rate was measured on the 3rd leaf using a portable gas analyzer system (IRGA (LCA-4)). Measurements were taken from five replicates between 14:00 and 16:00 h with equal intervals (Ben-Asher et al., 2006). Whole-plant transpiration was measured gravimetrically and corrected for water loss by evaporation (Gong et al., 2006) during treatment with 100 mM NaCl.

Determination of chlorophyll, proline and antioxidant enzymes activities

Chlorophyll was extracted from fresh leaves with 80% ethanol by heating in water bath at 85 °C for 10 min. The extracts were subsequently cooled in dark room and optical density was measured at 666 nm as rapidly as possible, and with minimal exposure to light using a spectrophotometer (Unico-UV 2100 Japan). Chlorophyll contents were calculated according to Arnon, (1949). Proline content was determined by the method of Bates et al., (1973). Fresh leaves (16-days after salinisation) were homogenized in 10 mL of 3% aqueous sulfosalicylic acid and filtered through Whatman 42. The filtrate was mixed with 2 mL acidic - ninhydrin and 2 mL of glacial acetic acid and placed in a water bath for 1 h at 100 °C. After cooling, the reaction mixture was extracted with 4 mL toluene and absorbance was measured at 520 nm with a (Unico-UV 2100 Japan): spectrometer. The SOD activity was determined by measuring the ability of the enzyme to inhibit phytochemical reduction of tetrazoleum blue according to Giannopolitis and Ries, (1977). The reaction mixture (3 mL) contained: K-Na-phosphate buffer (60 mM, pH 7.8), methionine (13 mM), riboflavin (2 μM), P-tetrazoleum blue (63 μM), EDTA (0.1 mM) and 100 μL of enzyme extract. The reaction was run for 10 min under illumination with 15 W fluorescence lamps. The complete reaction medium incubated in darkness was used as dark control. The complete reaction medium without enzyme, in which maximum color developed in light, was used as light control. The reaction was stopped by switching off light and placing the sample into darkness. Optical density was measured at 650 nm.

Table 4. Abscisic acid and/or silicate induced changes in K⁺ concentrations and Na⁺/K⁺ ratio in the shoots of NaCl (100 mM) treated wheat genotypes Kharchia-65 and Punjab-85.

Treatment	K ⁺ concentration (μmol g ⁻¹)		Na ⁺ /K ⁺ (Ratio)	
	Kharchia-65	Punjab-85	Kharchia-65	Punjab-85
Control	960±60 ^b	739±73 ^c	1.4±0.17 ^b	2.2±0.20 ^a
ABA	1054±100 ^{ab}	891±81 ^{bc}	1.1±0.13 ^{cde}	1.4±0.19 ^b
Si	1172±82 ^a	926±67 ^{bc}	0.9±0.06 ^{de}	1.3±0.08 ^{bcd}
ABA+Si	1210±70 ^a	950±45 ^b	0.8±0.10 ^e	1.1±0.09 ^{cde}

ABA was supplied as seed soaking for 24 h; silicate was applied throughout the culture solution while NaCl 100 mM was applied at 10 d old plants. Values followed by the same letter (s) or not significantly different at P<0.05. ABA, abscisic acid; Si, silicate.

Table 5. Effect of silicate and abscisic acid on transpiration rate and net assimilation rate in the shoot of wheat genotypes Kharchia-65 and Punjab-85

Treatment	Transpiration ((g water d ⁻¹))		Net Assimilation Rate (μmol m ⁻² S ⁻¹)	
	Kharchia-65	Punjab-85	Kharchia-65	Punjab-85
Control (0 NaCl)	5.4±0.23 ^b	7.5±0.32 ^a	22.2±1.4 ^a	19.2±1.34 ^{ab}
Control (100 mM NaCl)	4.2±0.21 ^c	5.5±0.14 ^b	13.2±1.1 ^{cd}	9.4±0.7 ^e
ABA (100 mM NaCl)	3.5±0.26 ^{cd}	4.0±0.15 ^c	13.5±1.1 ^{cd}	10.7±0.6 ^{cd}
Si (100 mM NaCl)	5.0±0.16 ^b	6.8±0.14 ^{ab}	17.1±1.3 ^b	15.2±1.2 ^c
ABA+Si (100 mM NaCl)	4.5±0.37 ^{bc}	5.4±0.30 ^b	18±1.2 ^{ab}	16.4±1.5 ^{bc}

ABA was supplied as seed soaking for 24 h; silicate was applied throughout the culture solution while NaCl 100 mM was applied at 10 d old plants. Values followed by the same letter (s) or not significantly different at P<0.05. ABA, abscisic acid; Si, silicate.

Table 6. Effect of silicate and abscisic acid on chlorophyll concentrations and proline content in shoot of wheat genotypes Kharchia-65 and Punjab-85

Treatment	Chlorophyll concentration (mg g ⁻¹ d. wt.)		Proline content (mmol g ⁻¹ Fw)	
	Kharchia-65	Punjab-85	Kharchia-65	Punjab-85
Control (0 NaCl)	22±1.4 ^a	20±1.3 ^{ab}	2.5±0.42 ^{de}	1.2±0.16 ^f
Control (100 mM NaCl)	16±1.0 ^c	14±1.2 ^c	6.5±0.71 ^b	4.6±0.30 ^{bc}
ABA (100 mM NaCl)	17±1.3 ^b	15±1.1 ^c	8.5±0.65 ^a	7.0±0.40 ^{ab}
Si (100 mM NaCl)	21±1.5 ^a	17±1.0 ^b	5.5±0.44 ^{bc}	3.0±0.21 ^d
ABA+Si (100 mM NaCl)	20±1.4 ^{ab}	18±1.3 ^b	7.1±0.72 ^{ab}	5.8±0.72 ^b

ABA was supplied as seed soaking for 24 h; silicate was applied throughout the culture solution while NaCl 100 mM was applied at 10 d old plants. Values followed by the same letter (s) or not significantly different at P<0.05. ABA, abscisic acid; Si, silicate.

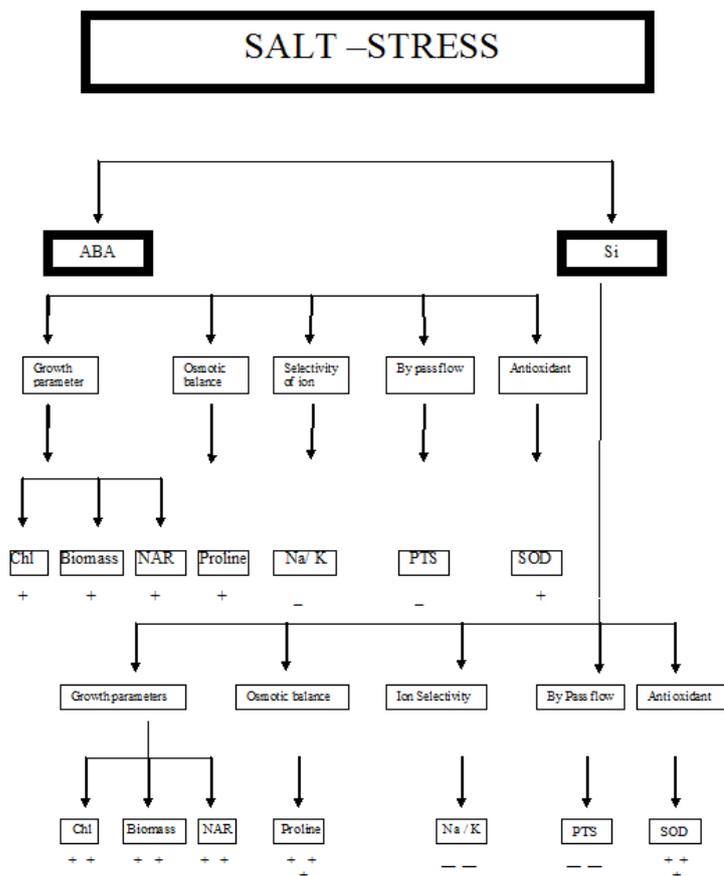


Fig 4. Putative mechanism of action of ABA and Si to alleviate salinity stress in plants. The number of + and - represent the magnitude of stimulation and inhibition of a particular parameter.

The unit of SOD activity was taken as the amount of enzyme able to inhibit tetrazolium blue reduction by 50%. SOD activity was expressed in arbitrary units per g of fresh weight. Peroxidase activity (POD) was assayed by the method described by Pundir et al., (1999). The assay mixture contained 1.8 mL 50 mM L⁻¹ sodium phosphate buffer, 0.1 mL phenol and 0.1 mL enzyme extract was incubated at 40 °C for 5 minutes. The H₂O₂ solution was added after incubation and absorbance was recorded at 520 nm. Catalase (CAT) activity was determined by the method of Aebi, (1984). The reaction mixture contained 50 mM L⁻¹ sodium phosphate buffer, 50 mM L⁻¹ H₂O₂ and 50 µL of enzyme extract in a 3 mL volume. The activity was determined by observing decline in absorbance at 240 nm as a result of H₂O₂ consumption and expressed as amount H₂O₂ decomposed per minutes per mg of protein.

Statistical analysis

Data was statistically analyzed using Minitab software. Design of the experiment was randomized complete block factorial design (RCBD) with two ways ANOVA (analysis of variance). LSD tests were performed to test the differences among treatments at P < 0.05 using Minitab software (Minitab 15.0, Minitab Inc., State College, PA, USA).

Conclusion

It is inferred from the present findings that Si was more effective than ABA in both genotypes. However, Si may be applied in combination with ABA for better tolerance to salt stress. Pre-sowing seed treatment with ABA could increase plant growth by reducing Na⁺ transport in sensitive wheat genotype. The ABA+Si treatment ameliorated the adverse effect of salinity by excluding Na⁺ transport and bypass flow, and improved plant growth attributes under salt stress (Fig. 4).

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