

Exogenous abscisic acid (ABA) and silicon (Si) promote salinity tolerance by reducing sodium (Na^+) transport and bypass flow in rice (*Oryza sativa indica*)

Ali Raza Gurmani^{1*}, Asghari Bano², Najeeb Ullah³, Hakim Khan¹, Muhammad Jahangir¹ and Timothy J. Flowers⁴

¹Department of Agriculture, University of Haripur, Khyber Pakhtunkhwa, Pakistan

²Department of Plant Sciences, Quaid-i-Azam University, Islamabad Pakistan

³Department of Plant and Food Sciences, Faculty of Agriculture and Environment, The University of Sydney, NSW 2006, Australia

⁴School of Life Sciences, University of Sussex, Falmer, Brighton BN1 9QG, United Kingdom

*Corresponding author: argurmani@uoh.edu.pk

Abstract

Excessive sodium (Na^+) and chloride (Cl^-) concentrations in rice shoots reduce seedlings growth. In a hydroponic study, we investigated the role of abscisic acid (ABA) seed pre-treatment on growth of salt-stressed rice, in the presence and/or absence of silicate (Si). Caryopses of rice IR4630 (salt tolerant) and IR15324 (salt sensitive) were soaked in an ABA solution (10^{-5} M) for 24 h, and the plants were then allowed to grow in a medium without or with silicate (Si, 0 or 3 mM). Twenty-one-day-old plants were salinised with NaCl (50 mM). We found the application ABA+Si the most effective treatment for improving plant growth under saline conditions. Si treatment alone or with ABA significantly increased the plant total dry biomass under saline or non-saline conditions, whereas, the effect of ABA seed pre-treatment was significant only on total dry biomass of salt sensitive genotype IR15324. Under saline conditions, ABA, Si and ABA+Si application ameliorated plant growth via suppression of Na^+ accumulation in shoots and lowering down Na^+/K^+ ratios in both the rice genotypes. In addition, treatment with Si alone or with ABA significantly reduced Na^+ concentrations in the leaf blades and sheaths, increased net assimilation rate and stomatal conductance of salt affected rice seedlings. We concluded that silicon can be applied as a nutrient for rice under saline or non-saline conditions. Furthermore, seed soaking with ABA has a potential for enhancing salt tolerance, particularly in the sensitive rice genotypes.

Keywords: *Oryza sativa* L., ABA, Silicate, salt tolerance, bypass flow.

Abbreviations: ABA – Abscisic acid, Si – Silicate, PTS -trisodium salt of 8-hydroxy-1, 3, 6-pyrenetrisulphonic acid, NAR – Net Assimilation Rate, WUE – Water Use Efficiency, SDW – shoot dry weight, RDW – Root Dry Weight.

Introduction

Salinity is one of the major factors limiting crop growth and productivity in irrigated areas of the world (Zhang et al., 2010). Plant species exhibit great variability in salinity tolerance, with some having the capacity to grow in sea water, whereas most crop plants are intolerant of just one fifth of such a concentration. Since tolerance to salt in plants is a complex trait, conventional breeding techniques have had limited success in improving this trait in crops (Flowers, 2004). Salt tolerance in plants not only depends on controlling Na^+ uptake and localisation but also on the acquisition of K^+ , which is commonly suppressed by high external Na^+ concentrations (Amtman et al., 2006; Zhang et al., 2010). Because of the importance of K^+ and Na^+ homeostasis in plant nutrition, K^+ and Na^+ transporters have been extensively investigated (Rodriguez and Rubio, 2006; Maathuis, 2007). However, limited attention had been paid to the factors influencing net ionic transport into shoots and the impact of transpiration. Abscisic acid (ABA) regulates various aspects of plant growth and development, such as seed maturation, dormancy and adaptation to abiotic stresses (Beaudoin et al., 2000; Sreenivasulu et al., 2012). A positive role of ABA on plant growth and induction of salt tolerance through improving ionic relations in plant tissues (Holbrook et al., 2002) has been suggested in different crops (Din and

Flowers, 2002; Travaglia et al., 2010; Gurmani et al., 2011). ABA induces salt and drought tolerance through regulating stomatal behaviour, leaf initiation and leaf expansion (Halbrook et al., 2002; Sharp and LeNoble, 2002). Bohra et al., (1995) reported a strong association between ABA application and inhibition of Na^+ accumulation in rice tissues. Silicon (Si) is an essential nutrient for the majority of plant species (Epstein, 1999; Ma and Yamaji 2008). Increases in its uptake and accumulation have been linked with improved resistance to biotic (Rodrigue et al., 2003) and abiotic stresses (Hodson and Sangster, 2002, Tahir et al., 2010). For example, deposition of Si in the exodermis and endodermis reduces Na^+ uptake through apoplastic transport, and consequently improves salt tolerance in rice (Gong et al., 2006; Faiyue et al., 2010b). In the experiments we report here, we have investigated treatments that might enhance the salt tolerance of rice, since this crop is so sensitive to salt and its tolerance has been hard to change (see Singh and Flowers 2011). From preliminary studies (Din, 1997), we noticed a remarkable enhancement of salt tolerance in rice by short term ABA treatment. Here we have investigated how seed pre-treatment of rice with ABA could improve its subsequent salt tolerance by investigating physiological processes that affect Na^+ uptake and accumulation. Since a significant

Table 1. Effect of silicate and abscisic acid on dry biomass (mg) of rice in the absence and presence of NaCl.

	0 mM NaCl						50 mM NaCl					
	SDW		RDW		Total dry weight		SDW		RDW		Total dry weight	
	IR4630	IR15324	IR4630	IR15324	IR4630	IR15324	IR4630	IR15324	IR4630	IR15324	IR4630	IR15324
Control	235±9 ^d	204±10 ^e	60±3.0 ^c	40±2.3 ^d	296±12 ^{bc}	244±9 ^d	170±7 ^{bc}	112±7 ^e	45±30 ^b	28±3.0 ^{cd}	215±9 ^b	140±7 ^d
ABA	250±11 ^{cd}	235±10 ^d	62±2.2 ^{bc}	46±2.0 ^d	312±9 ^b	281±10 ^c	185±9 ^b	134±6 ^{de}	50±3.0 ^{ab}	33±2.0 ^c	235±11 ^b	167±7 ^c
Si	280±12 ^{ab}	266±12 ^{bc}	68±2.1 ^{ab}	30±1.3 ^e	348±12 ^a	296±11 ^{bc}	224±8 ^a	148±6 ^{cd}	51±2 ^{ab}	23±1.4 ^d	275±8 ^a	171±8 ^c
ABA+Si	294±11 ^a	275±11 ^{abc}	70±3.0 ^a	39±1.4 ^d	364±12 ^a	314±10 ^b	235±10 ^a	153±8 ^{cd}	52±4.0 ^a	25±2.0 ^d	287±11 ^a	178±11 ^c

ABA was supplied during imbibition for 24 h; silicate was applied throughout the culture period, while NaCl (50 mM) was applied to 21-d old plants and harvested after 96h. Within column, values followed by the same letter (s) are not significantly different at $P < 0.05$. SDW, shoot dry weight; RDW, root dry weight.

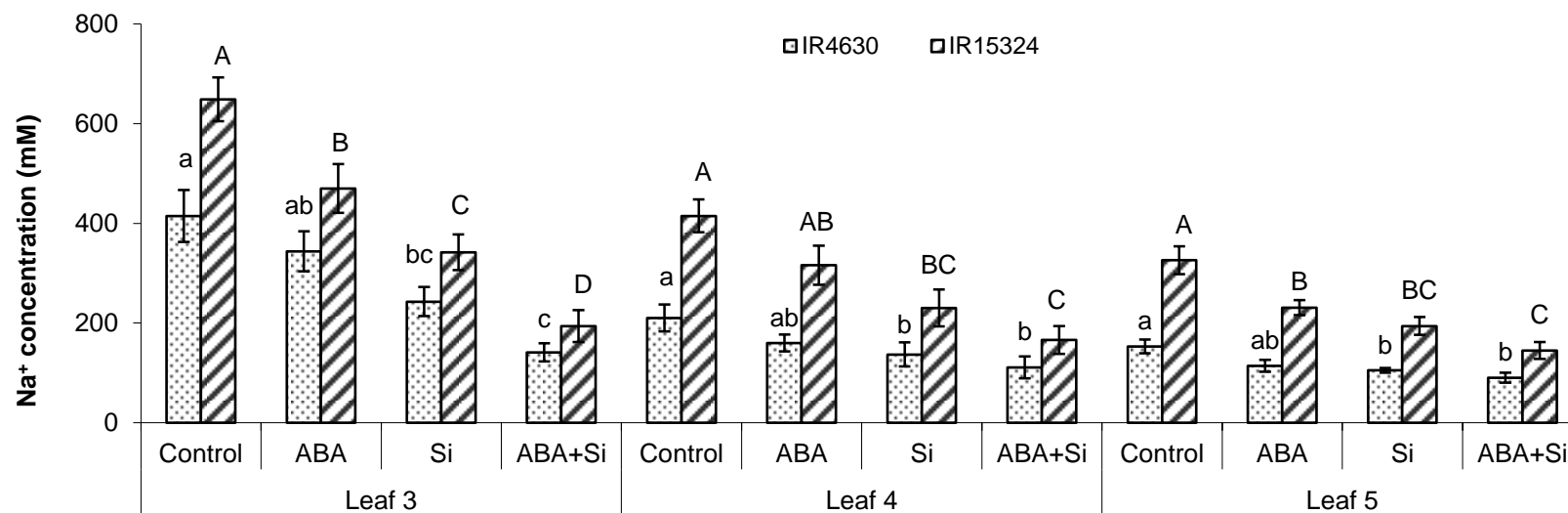


Fig 1. Effect of silicate with and without abscisic acid on Na⁺ concentrations in 3rd, 4th and 5th leaf blades of rice genotypes IR4630 (stippled bars) and IR15324 (cross-hatched bars). ABA (10⁻⁵ M) was supplied during seed treatment for 24 h; silicate was applied throughout the culture solution. Twenty one day old rice seedlings were treated with NaCl (50 mM) for 96 h. LSD (0.05) was applied on individual genotypes in leaf 3rd, 4th and 5th. Bars overtopped by the same letter were not significantly different at $P < 0.05$ in individual genotype. ABA, abscisic acid; Si, silicate.

fraction of Na⁺ uptake in rice growing under saline conditions takes place through bypass flow (Yeo et al., 1999; Faiyue et al., 2012), we included Si application with seed pre-treatment with ABA to evaluate their possible interactions via Na⁺ transport, bypass flow and Na⁺ distribution in the 3rd, 4th and 5th leaf blades and sheaths of two rice genotypes differing in salt tolerance.

Results

Plant dry biomass

The more tolerant genotype IR4630 maintained significantly higher fresh and dry biomass compared to the more sensitive genotype IR15324 under all the treatments. In the absence of salinity, Si application significantly increased total dry biomass of both rice genotypes, even though there was a reduction in root dry biomass of IR15324 (Table 1). In NaCl-treated rice seedlings, Si application had a similar positive effect on plant dry biomass. Interestingly, ABA pre-treatment only significantly increased the dry biomass (shoot and total) of IR15324. The combined application of ABA and Si increased shoot dry biomass and the total dry biomass of both genotypes compared to control plants (Table 1).

Bypass flow and sodium potassium transport

We investigated the accumulation of Na⁺ and the apoplastic tracer (PTS) in shoots of plants treated with NaCl (for 96 h) in response to application of ABA and Si, alone or in combination. The salt tolerant genotype IR4630 contained lower Na⁺ concentrations in its tissues as compared to IR15324 under all treatments (Table 2). Application of ABA or Si significantly reduced shoot Na⁺ concentration of both genotypes, IR4630 and IR15324. However, the combined treatment of ABA+Si caused a greater reduction in shoot Na⁺ accumulation than either treatment with Si or ABA alone. After 96 h of NaCl treatment, the percentage reduction in Na⁺ concentration in IR4630 tissues due to ABA, Si and ABA+Si was 18%, 35% and 49%, respectively, while it was 43%, 24% and 57%, respectively in IR15324 compared to their respective controls. PTS accumulation under Si or Si+ABA treatment was significantly lower than in control plants of both genotypes, although no significant effect was observed in plants of either genotype when treated with ABA alone (Table 2). Application of ABA+Si caused maximal suppression in shoot PTS concentrations (65 and 55%) followed by Si alone (50 and 45%) and ABA (12 and 24%) in IR4630 and IR15324, respectively (Table 2). There was a significant increase in rice shoot potassium (K⁺) concentrations under Si or Si+ABA treatment in both genotypes when compared to control plants grown without such treatments; ABA pre-treatment alone only significantly increased K⁺ concentrations in the salt sensitive genotype IR15324 (Table 3). As a consequence of changes in monovalent cation concentrations, Na⁺/K⁺ ratios fell significantly on application of Si and ABA, alone or in combination; again Si was the major contributor to this reduction (Table 3).

Distribution of sodium in rice leaves

The Na⁺ concentrations in the sheaths and blades of the 3rd, 4th and 5th leaves were higher in IR15324 compared to IR4630 under all treatments (Fig 1 and 2). Although different treatments (viz. ABA, Si and Si+ABA) suppressed Na⁺ accumulation in rice leaves, the Na⁺ distribution in different

leaves followed a similar pattern. In the leaf blades of both genotypes, the 3rd leaf contained the highest Na⁺ concentrations followed by the 4th and 5th leaf (Fig 1). The reduction from 3rd to 4th leaf for Na⁺ concentrations was statistically significant but non-significant from 4th to 5th leaf in all the treatments except for ABA+Si, in which there was no significant change in Na⁺ distribution between all three leaves.

The Na⁺ concentrations in the leaf sheaths were almost double those in the leaf blades, but, again, the concentrations in the sheaths of the younger leaves were generally lower than those in the older leaves and were lower in IR4630 than in IR15324 (Fig 2). In both genotypes, the Na⁺ concentrations were lowered (compared to control plants) by treatment with ABA and Si in all three sheaths.

Photosynthetic parameters

In the presence of NaCl, silicate alone as well as in combination with ABA significantly increased the net assimilation rate (NAR) of leaf 4 over that of the control plants in both genotypes (Table 4). The salt tolerant genotype IR4630 maintained a significantly higher NAR than IR15324 under different treatments. Application of Si or ABA+Si significantly increased leaf NAR compared to control plants. The increase in leaf NAR due to ABA+Si application was higher than with either Si or ABA alone. There was also a significant increase in stomatal conductance in both genotypes with Si application, while combined application of Si and ABA caused a significant increase in conductance only in the salt sensitive genotype IR15324 (Table 4). The highest stomatal conductance was recorded with Si-treated plants of the salt tolerant genotype IR4630, while minimum stomatal conductance was observed in control plants of genotype IR15324. Compared to control plants, there was a significant increase in transpiration rate of both rice genotypes growing in the presence of NaCl when treated with Si alone (Table 5). No significant change in transpiration rate was recorded under ABA alone or ABA+Si treatment. There was a significant increase in water use efficiency (WUE) in ABA+Si treated plants of both genotypes. However, ABA pre-treatment alone only significantly increased the WUE of the salt sensitive genotype, IR15324 (Table 5). No significant change in WUE of any rice genotype was observed when treated with silicate alone.

Discussion

Short term (96 h) exposure to NaCl negatively influenced the growth and development of rice seedlings causing 28% and 45% reduction in shoot dry mass of IR4630 and in IR15324 respectively compared to non-saline plants. Although the two genotypes exhibited a similar trend in biomass reduction, the genotype IR4630 exhibited better growth performance compared to IR15324. The genotype IR15324 achieved improved salinity tolerance through limiting Na⁺ uptake and bypass flow, and maintaining higher net assimilation rate (NAR), stomatal conductance and WUE. Our results are consistent with the findings of Koyama et al., (2001) and Roshandel (2007), who found the genotype IR4630 relatively more salt tolerant compared to IR15324. Silicate addition to the growth medium improved the total dry biomass of both rice genotypes in the absence or presence of salt, other than for the root dry weight of IR15324, which decreased on Si application. The positive effects of Si on plant growth in the absence of salt reflect its role as an essential nutrient (Epstein, 1999). A role for Si in the presence of salt has also

Table 2. Effect of silicate and abscisic acid on Na⁺ concentrations and PTS (an indicator of bypass flow) in shoot of rice growing under saline conditions (50 mM NaCl).

Genotypes	Na ⁺ concentration in shoot (μmol g ⁻¹)				PTS concentration in shoot (μmol g ⁻¹)			
	Control	ABA	Si	ABA+Si	Control	ABA	Si	ABA+Si
IR4630	600±75 ^{bc}	493±50 ^{cd}	390± 23 ^{de}	304±21 ^e	26±4 ^b	23±5 ^{bc}	13±1.2 ^e	9±1.9 ^d
IR15324	890±85 ^a	672±32 ^b	504± 30 ^{cd}	378±27 ^{de}	33±4 ^a	25±3 ^{ab}	18±1.35 ^d	15±1 ^e

ABA was supplied during imbibition for 24 h; silicate was applied throughout the culture period, while NaCl (50 mM) and PTS (2mM) was applied to 21-d old plants for 96h. Values within rows followed by the same letter (s) are not significantly different at P<0.05: PTS, trisodium salt of 8-hydroxy-1, 3, 6-pyrenetrisulphonic acid

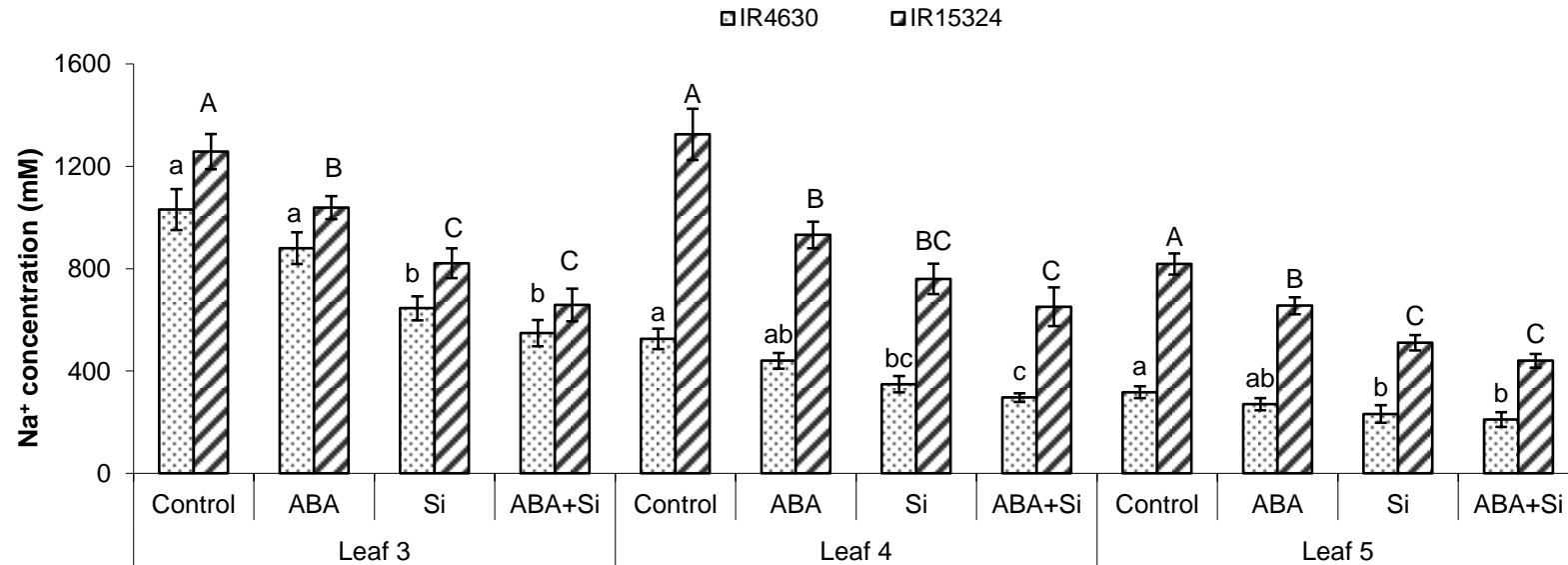


Fig 2. Effect of silicate with and without abscisic acid on Na⁺ concentrations in 3rd, 4th and 5th leaf sheaths of rice genotypes IR4630 (stippled bars) and IR15324 (cross-hatched bars). ABA (10⁻⁵ M) was supplied during seed treatment for 24 h; silicate was applied throughout the culture solution. Twenty one day old rice seedlings were treated with NaCl (50 mM) for 96 h. LSD (0.05) was applied on individual genotypes in leaf 3rd, 4th and 5th. Bars overtopped by the same letter were not significantly different at P<0.05 in individual genotype. ABA, abscisic acid; Si, silicate.

been suggested for rice (Gong et al., 2006), as well as for barley (Liang et al., 1996, 1998), wheat (Tunae et al., 2008) and tomato (Al-Aghabary et al., 2004). In salt-treated rice seedlings, the improved seedling growth on Si application was associated with 35% and 44% reduction in Na⁺ concentration in the shoots of IR4630 and IR15324, respectively. Since a significant proportion of Na⁺ that reaches the shoots does so via bypass flow it is likely that Si reduces Na⁺ transport through influencing bypass flow (Gong et al., 2006). Si does not enter through the sites of lateral root emergence (Faiyue et al., 2010a), but through the lateral roots themselves, where the exodermis is undeveloped (Faiyue et al., 2010b). In the presence or absence of salt, ABA pre-treatment was more effective in improving dry biomass of the salt-sensitive genotype IR15324 than the more tolerant IR4630. However, ABA caused a significant reduction of Na⁺ accumulation in the shoots of both genotypes, suggesting that its mode of action was not simply through enhancing growth. Application of ABA+Si caused a greater reduction in Na⁺ concentrations in the tissues of both rice genotypes than treatment with ABA or Si alone, although the interaction was only statistically significant in IR15324. Na⁺ accumulation was reduced by whatever its pathway; ABA may have switched the transcription of genes affecting membrane bound transporters reducing future symplastic transport or altered the development of lateral roots, thus affecting bypass flow and Na⁺ accumulation. Si deposition in the lateral roots would decrease bypass flow. There was no clear effect of ABA on the distribution of ions between blades and sheaths of leaves. Further work would be required to evaluate whether a small effect of ABA on bypass flow significantly contributed to reduce Na⁺ accumulation or ABA treatment switched on other mechanism that enhanced K⁺/Na⁺ selectivity in both rice genotypes. It is remarkable that a short (24 h) ABA treatment during seed imbibition can affect subsequent plant performance, some 6 days later, when the ABA concentrations in plants did not differ (Din, 1997). We speculate that ABA improved plant performance by regulating developmental pathways (Pitman, 1974). Although ABA is generally considered as a plant growth inhibitor, increased growth with ABA treatment (foliar/root treatment) has been reported in salt-affected rice that was largely attributed by reduction in Na⁺ uptake (Bohra et al., 1995). The build-up of salt in rice leaves impairs photosynthetic gas exchange (Yeo and Flowers 1985). Therefore, it is predictable that any reduction in Na⁺ transport by ABA+Si might enhance photosynthesis. Application of Si alone and with ABA improved net assimilation rate and stomatal conductance of both NaCl-treated rice genotypes; conversely, ABA alone had no effect on these parameters. Our results are consistent with the findings of Yeo et al., (1991) who also reported that Si application increased stomatal conductance and net assimilation rate of three different genotypes of salt-stressed rice. Plant water relations are very important to proper photosynthetic functioning and consequently for plant growth. Application of Si+ABA improved plant water relation by increasing water use efficiency, although the individual effects of ABA and Si were not sufficient to increase water use efficiency in either genotype. An increase in transpiration rate and water use efficiency by the addition of Si has been reported in maize under saline conditions (Parveen and Ashraf, 2010). From a practical point of view, ABA seed pre-treatment has a potential for enhancing salt tolerance in sensitive rice genotypes under saline conditions. Effects of seed priming on subsequent plant performance under saline conditions have been already reported; for example, pre-treatment with kinetin alleviated wheat from the

adverse effect of salt by suppressing Na⁺ uptake (Iqbal and Ashraf, 2005). The highly significant effects of ABA pre-treatment on the sensitive rice genotype suggest that resistance could be controlled by the way in which ion transport pathways are switched during development.

Materials and methods

Plant materials

Caryopses of rice (*Oryza sativa* L.) were obtained from the International Rice Research Institute (IRRI, Philippines). During a preliminary study six rice genotypes (Amber-355, IR26, IR28, IR4630, IR2153 and IR15324) were screened for salinity tolerance by exposing to 50 mM NaCl in a culture solution (Yoshida et al., 1976). Consequently, genotypes IR4630 and IR15324 were selected as salt tolerant and salt sensitive respectively. The salt tolerance of these two genotypes was also explored by Roshandel (2007), who found that after exposure to 50 mM NaCl, leaves of IR15324 contained almost double the amount of the Na⁺ than those of IR4630. Caryopses of both genotypes were soaked for 24 h in an aerated solution of ABA (10⁻⁵ M) and/or distilled water (control) in black-painted flasks. The ABA was dissolved in absolute ethanol (final concentration, 0.00044 % v/v). After the treatment, caryopses were washed three times with distilled water to remove any external ABA and ethanol, and were sown on nylon mesh supported on Perspex grids floated on culture solution (Yoshida et al., 1976). Seven-day-old seedlings were transplanted into boxes (3 dm³), painted black containing Yoshida culture solution (with or without Si). The additional silicon was applied as sodium silicate (BDH, 25.5-28.5% SiO₂) and the solution pH was adjusted to 4.5 with 0.01 N H₂SO₄. The treatments included: control (distilled-water-treated seeds), ABA-treated seeds, Si-treated plants and ABA+Si-treated plants. The growth conditions were a 12 h photoperiod with a minimum of 400-500 μmol m⁻² s⁻¹ photosynthetically active radiation (HQ1 metal halide, Newry and Eyre, Brighton, UK) 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. The 21-day-old rice seedlings were salinised by adding 50 mM NaCl. After 4 days salinisation, the plants were harvested, and dry biomasses were recorded after drying plant samples in an oven at 65°C for 72 h.

Determination of ions and Bypass flow

Plant tissues were extracted with distilled water for 2 h at 90 °C in a water bath, and the extract used for determining Na⁺ and K⁺ concentrations by atomic absorption spectroscopy (Unicam 919, Cambridge, UK). Bypass flow was determined using the fluorescent membrane-impermeant dye, the trisodium salt of 8-hydroxy-1, 3, 6-pyrenetrisulphonic acid (PTS, 0.2 mM) [former nomenclature 3-hydroxy-5, 8, 10-pyrenetrisulphonic acid and supplied as Pyranin (Bayer UK)] by the method described by Yeo et al., (1987) and Yadav et al., (1996). Seedlings were grown in boxes that could be drained completely, and the solution was replaced with minimum root disturbance. The shoots were extracted in 10 mL of distilled water for 2 h at 90 °C, and the fluorescence measured at 510 nm using an excitation wavelength of 403 nm (Perkin Elmer, LS3).

Gas exchange

Measurements of carbon dioxide and water exchange between the fourth leaf and the surrounding air were made in

Table 3. Effect of silicate and abscisic acid on K⁺ concentrations and Na⁺/K⁺ ratio in shoots of rice growing under saline conditions (50 mM NaCl).

Genotypes	K ⁺ concentration in shoot (μmol g ⁻¹)				Na ⁺ /K ⁺			
	Control	ABA	Si	ABA+Si	Control	ABA	Si	ABA+Si
IR4630	745±58 ^{cd}	812±50 ^{abc}	860±24 ^{ab}	902±34 ^a	0.87±0.11 ^{bc}	0.65±0.1 ^{cd}	0.45±.03 ^{de}	0.34±0.03 ^e
IR15324	648±41 ^d	756±44 ^c	780±31 ^{bc}	831±40 ^{abc}	1.4±0.12 ^a	0.93±0.07 ^b	0.66±.05 ^{cd}	0.5±0.05 ^{de}

ABA was supplied during imbibition for 24 h; silicate was applied throughout the culture period, while NaCl (50 mM) was applied to 21-d old plants for 96h. Within rows, values followed by the same letter (s) are not significantly different at P<0.05.

Table 4. Effect of silicate with and without abscisic acid on photosynthetic gas exchange (NAR; net assimilation rate, Gs; stomatal conductance) in the 4th leaf of rice growing under saline conditions (50 mM NaCl).

Genotypes	NAR (μmol m ⁻² S ⁻¹)				Gs (mmol m ⁻² S ⁻¹)			
	Control	ABA	Si	ABA+Si	Control	ABA	Si	ABA+Si
IR4630	12±0.76 ^b	12.5±0.71 ^b	16.5±0.85 ^a	17±0.59 ^a	380±15 ^{cd}	359±33 ^{de}	463±21 ^a	421±26 ^b
IR15324	8.44±1.0 ^c	9.5±0.68 ^c	12.5±0.82 ^b	13.5±0.86 ^b	330±17 ^{ef}	295±19 ^f	405±15 ^{bc}	380±17 ^{cd}

ABA was supplied during imbibition for 24 h; silicate was applied throughout the culture period, while NaCl (50 mM) was applied to 21-d old plants for 96h. Measurements (each of five minutes) were taken with five replications between 14:00 and 17:00 h with equal intervals. Within rows, values followed by the same letter (s) or not significantly different at P<0.05.

Table 5. Effect of silicate with and without abscisic acid on transpiration rate and water use efficiency (WUE) of rice growing under 50 mM NaCl

Genotypes	Transpiration (g day ⁻¹)				Water Use Efficiency (g DW kg ⁻¹ water)			
	Control	ABA	Si	ABA+Si	Control	ABA	Si	ABA+Si
IR 4630	4.0±0.17 ^{ef}	3.5±0.15 ^f	5.5±0.2 ^{bc}	4.5±0.18 ^{de}	1.4±0.14 ^{bc}	1.6±0.07 ^{ab}	1.3±0.06 ^c	1.8±0.12 ^a
IR 15324	6.0±0.29 ^b	4.9±0.17 ^{cd}	7.0±0.43 ^a	6.2±0.39 ^b	0.94±0.06 ^d	1.4±0.1 ^{bc}	1.0±0.07 ^d	1.3±0.11 ^c

ABA was supplied as seed soaking for 24 h; silicate was applied throughout the culture solution while NaCl 50 mM was applied at 21 d old plants. Whole-plant transpiration was measured gravimetrically and corrected for water loss by evaporation during treated and chase periods. WUE was determined as shoot dry mass after 25 d per unit of water transpired for 25 d. Within rows, values followed by the same letter (s) or not significantly different at P<0.05.

a temperature controlled green house under natural daylight supplemented with high pressure sodium illumination (SON/T, Camplex Plant Care LTD) by infrared gas analysis in an open system at ambient partial pressures of carbon dioxide and water vapour (CIRAS-1, PP system, UK). At the time of measurement, the leaf temperature was 22-28 °C, and the light flux density was 600-800 μ mol m⁻² S⁻¹. Measurements (each of five minutes) were taken from five replicates between 14:00 and 17:00 h with equal intervals. Net assimilation and stomatal conductance were calculated by on-board software. Whole-plant transpiration was measured gravimetrically, and corrected for water loss by evaporation during treatment with 50 mM NaCl (Yeo et al., 1999; Gong et al., 2006). Water use efficiency was determined as shoot dry mass after 25 days per unit of water transpired for 25 days (Flowers et al., 1988; Yadav et al., 1996).

Statistical analysis

Data was statistically analyzed by using Minitab software. The experimental design was a 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

ABA increased plant growth and reduced Na⁺ uptake in the sensitive rice genotype, IR15324. In combination with silicate, ABA elevated the salinity tolerance of both genotypes, and reduced Na⁺ transport and bypass flow, increasing stomatal conductance, net assimilation rate and shoot biomass under salt stress. It suggested that ABA plays a regulatory role in inducing salt tolerance in rice by influencing Na⁺ uptake and transport.

Acknowledgements

This work was supported by Higher Education Commission, Government of Pakistan, which is acknowledge with gratitude. The authors are also thankful to School of Life Sciences, University of Sussex, UK for laboratory and greenhouse facilities.

References

Al-aghaby K, Zhu Z, Shi Q (2004) Influence of silicon supply on chlorophyll content, chlorophyll fluorescence and antioxidant enzyme activities in tomato plants under salt stress. *J Plant Nutr* 27: 2101-2115.

Amtmann A, Armengaud P, Volkov V (2006) Potassium nutrition and salt stress. In: Blatt MR (ed) *Membrane transport in plants*. Blackwell, Oxford, UK, 15: 293-339.

Beaudoin N, Serizet C, Gosti F, Giraudat J (2000) Interactions between abscisic acid and ethylene signaling cascades. *Plant Cell* 12: 1103-1116.

Bohra JS, Dorffling H, Dorffling K, (1995) Salinity tolerance of rice (*Oryza sativa* L.) with reference to endogenous Abscisic acid. *J Agron Crop Sci* 174: 79-86.

Din J (1997) Effect of hormonal pre-treatments on the response of rice (*Oryza sativa* L.) and wheat (*Triticum aestivum* L.) to salinity. Dissertation, University of Sussex, UK.

Din J, Flowers TJ (2002) Effect of ABA seed pre-treatment on the response of wheat (*Triticum aestivum* L.) to salinity, with special reference to plant growth, ion relations and protein patterns. In: Ahmad R, Malik KA (Ed) *Prospects for saline agriculture*. Kluwer Academic Publishers, Netherlands, pp. 145-153.

Epstein E (1999) Silicon, Annual review of plant physiology and plant molecular biology. *Plant Biol* 50: 641-664.

Faiyue B, Vijayalakshmi C, Nawaz S, Nagato Y, Taketa S, Ichii M, Al-azzawi M, Flowers TJ (2010 a) Studies on sodium bypass flow in lateral rootless mutants *Irt1* and *Irt2*, and crown rootless mutant *cr11* of rice (*Oryza sativa* L.). *Plant Cell Environ* 33: 687-701.

Faiyue B, Al-azzawi M, Flowers TJ (2010 b) The role of lateral roots in bypass flow in rice (*Oryza sativa* L.). *Plant Cell Environ* 33: 702-716.

Faiyue B, Al-Azzawi M, Flowers TJ (2012) A new screening technique for salinity resistance in rice (*Oryza sativa* L.) seedlings using bypass flow. *Plant Cell Environ* 35: 1099-1108

Flowers T J (2004) Improving crop salt tolerance. *J Exp Bot* 55: 307-319.

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.

Gurmani AR, Bano A, Khan SU, Din J, Zhang JL (2011) Alleviation of salt stress by seed treatment with abscisic acid (ABA), 6-benzylaminopurine (BA) and chlormequat chloride (CCC) optimizes ion and organic matter accumulation and increases yield of rice (*Oryza sativa* L.). *Aust J Crop Sci* 5(10): 1278-1285.

Holbrook NM, Shashidar VR, James RA, Munns R (2002) Stomatal control in tomato with ABA deficient roots: response of grafted plants to soil drying. *J Exp Bot* 53: 1503-1514.

Hodson MJ, Sangster AG (2002) Silicon and abiotic stress. In: Matoht, (Ed) *Second silicon in agriculture conference*. Press-Net Tokyo, Japan, pp 99-104.

Hogland DR, Arnon D (1950) The water culture method for growing plants without soil. Univ. of Calif. Agric Exp Station, Berkley, Circular, 347.

Iqbal M, Ashraf M (2005) Pre-sowing seed treatment with cytokinins and its effect on growth, photosynthetic rate, ionic levels and yield of two wheat cultivars differing in salt tolerance. *J Integr Plant Bio* 147: 1315-1325.

Koyama ML, Levesley A, Koebner RMD, Flowers TJ, Yeo AR (2001) Quantitative trait loci for component physiological traits determining salt tolerance in rice. *Plant Physiol* 125: 406-422.

Liang Y (1998) Effect of silicon on leaf ultrastructure, chlorophyll content and photosynthetic activity in barley under salt stress. *Pedosphere* 8: 289-296.

Liang YC, Shen QR, Shen ZG, Ma TS (1996) Effect of silicon on salinity tolerance of two barley cultivars. *J Plant Nutr* 19: 173-183.

Maathuis FJM (2007) Monovalent cation transporters; establishing a link between bioinformatics and physiology. *Plant Soil* 301: 1-15.

Ma J, Yamaji N (2008) Functions and transport of silicon in plants. *Cell Mol Life Sci* 65: 3049-3057.

Parveen N, AshrafM (2010) Role of silicon in mitigating the adverse effects of salt stress on growth and photosynthetic attributes of two maize (*Zea mays* L) cultivars grown hydroponically. *Pak J Bot* 42: 1675-1684.

- Pitman MG, Schaefer N, Wildes RA (1974) Membrane Transport in Plants. In: Zimmermann U, Dainty J (Ed) Springer, Berlin, Heidelberg and New York. pp 391-396.
- Rodrigues FA, Vale FXR, Korndorfer GH, Prabhu AS, Datnoff LE, Oliveira AMA, Zambolim L (2003) Influence of silicon on sheath blight of rice in Brazil. *Crop Prot* 22: 23-29.
- Rodriguez-Navarro A, Rubio F (2006) High-affinity potassium and sodium transport systems in plants. *J Exp Bot* 57: 1149-1160.
- Roshandel P (2007) Xylem sap analysis reveals new facts of salt tolerance in rice genotypes. *Braz J Plant Physiol* 19(3): 185-192.
- Sharp RE, LeNoble ME (2002) ABA, ethylene and the control of shoot and root growth under water stress. *J Exp Bot* 53: 33-37.
- Singh RK, Flowers TJ. 2011. Physiology and molecular biology of the effects of salinity on rice. In: Pessaraki M, ed. *Handbook of Plant and Crop Stress*. Boca Raton: CRC Press, 899-939.
- Sreenivasulu N, Harshavardhan VT, Govind G, Seiler C, Kohli A (2012) Contrapuntal role of ABA: Does it mediate stress tolerance or plant growth retardation under long-term drought stress? *Gene* 506 (12): 265-273
- Travaglia C, Reinoso H, Cohen A, Luna C, Tommasino E, Castillo C, Bottini R (2010) Exogenous ABA increases yield in field grown wheat with moderate water restriction. *J Plant Growth Regul* 29: 366-374.
- Tahir, M.A, Rahmatullah, Aziz T, Ashraf M (2010) Wheat genotypes differed significantly in their response to silicon nutrition under salinity stress. *J Plant Nutr* 33: 1658-1671
- Tuna A, 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.
- Yadav R, Flowers TJ, Yeo AR (1996) The involvement of the transpirational bypass flow in sodium uptake by high and low sodium-transporting lines of rice developed through intravarietal selection. *Plant Cell Environ* 19: 329-336.
- Yeo AR, Flowers SA, Rao G, Welfare K, Senanayake N, Flowers TJ (1999) Silicon reduces sodium uptake 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.
- Yeo AR, Caporn SJ, Flowers TJ (1985) The effect of salinity upon photosynthesis in rice (*Oryza sativa* L): gas exchange by individual leaves in relation to their salt content. *J Exp Bot* 36: 1240-1248.
- Yeo AR, Lee KS, Izzard P, Boursier PJ, Flowers TJ (1991) Short-term and long-term effect of salinity on leaf growth in rice (*Oryza sativa* L.). *J Exp Bot* 42: 881-889.
- Yeo AR, Yeo ME, Flower TJ (1987) The contribution of an apoplastic pathway to sodium uptake by rice roots in saline conditions. *J Exp Bot* 38: 1141-1153.
- Yoshida S, Forno DA, Cock JH, Gomez KA (1976) Laboratory manual for physiological studies of rice. The International Rice Research Institute, Los Banos, Philippines, pp 83.
- Zhang JL, Flowers TJ, Wang SM (2010) Mechanisms of sodium uptake by roots of higher plants. *Plant Soil* 326: 45-60.