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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.)

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Abstract

Soil salinity is an increasing problem for agricultural production worldwide. Plant growth regulator (PGR)-treated seeds have the potential to produce plants with a greater salt tolerance as salt tolerance in established plants is greater than in germinating seeds. This study aimed at evaluating the effect of seed pre-soaking with abscisic acid (ABA), 6-benzylaminopurine (BA) and chlormequat chloride (CCC) on the amelioration of salt stress in rice cv. IR-6 in hydroponic culture and in saline field conditions. The study placed particular emphasis on osmoregulation and accumulation/partitioning of ions and organic osmotic matters. We found that the addition of PGRs as seed treatment agents or chemicals to rice cv. IR-6 has a significant role in reducing salinity stress. ABA was the most effective PGR in reducing Na^+ and Cl^- concentrations and Na^+/K^+ ratio, increasing K^+ and Ca^{2+} concentrations, proline accumulation, soluble sugar content and grain yield. BA had the largest positive effect on soluble protein content. The three PGRs all had a significant positive effect on the grain yield of rice. Compared to control, ABA, BA and CCC treatments increased grain yield by 21%, 17% and 12%, respectively. Our results suggest that ABA was more effective than BA and CCC to ameliorate the toxic effects of salt stress in rice. It is economic to use these PGRs in the production system. Since they were used only for seed treatment and at very low concentration (10^{-5} or 10^{-6} M) their possible negative impact on human health can be avoided.

Keywords: abscisic acid; 6-benzylaminopurine; chlormequat chloride; ions; organic matters; rice (*Oryza sativa* L); salt stress; yield.
Abbreviations: ABA- abscisic acid; BA- 6-benzylaminopurine; CCC- chlormequat chloride; DW- dry weight; KT- kinetin; PGRs- plant growth regulators; RCBD- randomized complete block design; SEs- standard errors.

Introduction

Soil salinity has a significant negative impact on global agricultural productivity, and is a particularly acute issue in both irrigated and non-irrigated areas of the world (Zhang et al., 2010; Benderradji et al., 2011; Uddin et al., 2011). Establishment of ion homeostasis is an essential requirement for plants to survive under salt stress conditions. The transport of ions and their homeostasis are issues of special significance (Pardo et al., 2006). Potassium (K^+) and sodium (Na^+) that transport within plants have been studied extensively because of their significance in both plant nutrition and salt tolerance (Amtmann et al., 2004; Rodriguez-Navarro and Rubio, 2006; Rus et al., 2004). Plant salt tolerance requires not only adaptation to Na^+ toxicity but also the acquisition of abundant K^+ whose uptake by the plant cell is affected by high external Na^+ concentrations (Zhang et al., 2010). Rice (*Oryza sativa* L.) is moderately sensitive to soil salinity. A long-term effect of growing rice in saline soils is that excess salts accumulate in leaves (Yeo et al., 1990). Physiological studies suggest that a range of characteristics that include low shoot Na^+ concentrations, compartmentation of salt in older rather than younger leaves, tolerance of leaves to salt, and plant vigor, increase the ability of the plant to cope with salinity (Ren et al., 2005; Yeo et al., 1990). It is thought that the depressive effect of salinity on germination could be related to a decline in endogenous levels of plant hormones (Debez et al., 2001). It has been reported that PGRs, such as ABA, BA, KT and CCC, could regulate plant adaptation to salt stresses. Abscisic acid regulates various aspects of plant growth and development, including seed maturation and dormancy, as well as adaptation to abiotic environmental stresses (Beaudion et al., 2000). Gurmani et al. (2007) found that wheat (*Triticum aestivum*) treated with both ABA and BA lead to the significant decrease in Na^+ content and plant height, but increase in K^+ content in flag leaf, the number of grains per panicle, and grain yield. The induction of salt tolerance by kinetin in spring wheat can be attributed, in part, to its beneficial effects on ion homesostasis under saline conditions (Iqbal and Ashraf, 2005). Furthermore, Gadallah (1999) found that kinetin application reduced the harmful effects of combined saline-aerobic treatment through a reduction in the accumulation of inorganic ions Na^+, Ca^{2+} and Cl^-. BA pretreatment alone can help overcome the negative effect of salt stress on germination percentage, radicle elongation and fresh weight in barley (*Hordeum vulgare*) (Cavusoglu and Kabar, 2008). Spraying maize (*Zea mays* L.) leaves with BA caused a significant increase in the total number of microhairs per leaf (Ramadan and Flowers, 2004). The application of BA partially alleviated the salt stress symptoms in two roselle (*Hibiscus sabdariffa* L.) cultivars under salt stress via equilibration of cytosolutes including anthocyanins (Latef et al., 2009). In wheat, the grain yield per plant was increased by the application of CCC in the presence of salinity and the dry matter accumulation in the shoot system at earing stage (Gabr et al., 1977a). CCC consistently increased the yield of cotton plants, especially in the presence of salinity when using CCC as a seed-soaking medium alone (Gabr et al., 1977b). Since salt tolerance in established plants is greater than in germinating seeds, Naqvi et al. (1970) suggested investigating whether PGR-treated seeds could produce plants with a greater salt tolerance. The present study aimed at evaluating the effect of pre-soaking rice seeds with ABA, BA and CCC on the amelioration of salt tolerance of rice in hydroponics as well as in saline field conditions. Particular emphasis is placed on osmoregulation and accumulation/partitioning of ions and organic matters.

Results

Effects of the three PGRs on ion accumulation in rice under salt stress in hydroponic experiment

Na^+ concentration in the shoot of rice increased significantly with the increase of external NaCl levels. Na^+ contents in the shoot and root were lower in PGR-treated plants compared to control under salt stress. Only ABA treated plants showed significant Na^+ reduction at both 50 and 75 mM salinity levels in both shoot and root (Table 2). Salinity stress caused a significant decrease whereas PGR treatments prevented the decrease in K^+ contents of shoot and roots. The ranking of effectiveness of PGRs to increase K^+ contents was ABA > BA > CCC at all the salinity levels in rice shoot and root. The increasing levels of salinity also increased Cl^- contents in the shoot and root of rice. However, the three PGR treatments reduced Cl^- content compared to untreated control. ABA and CCC treatments significantly decreased Cl^- contents in shoot of rice, although in the root only ABA significantly affected Cl^- content. Maximum Cl^- concentrations were recorded in the shoot of control plants under 75 mM NaCl stress. Salinity stress of 50 and 75 mM NaCl caused a significant reduction in Ca^{2+} contents in both shoot and root of rice. The three PGR treatments increased the Ca^{2+} content. ABA and BA significantly increased Ca^{2+} contents in shoot and root at 50 mM NaCl stress, while at 75 mM only ABA had significant effects, compared to the control. Salt stress significantly increased Na^+/K^+ and Na^+/Ca^{2+} ratios in the shoot and root of rice. However, the three PGR treatments all significantly decreased Na^+/K^+ and Na^+/Ca^{2+} ratios in both shoot and root at the two salinity levels compared to their corresponding control. The three PGRs reduced the two ratios in the order of ABA > BA > CCC (Table 2 and 3).

Effects of the three PGRs on organic osmotic matter accumulation in rice under salt stress in hydroponic experiment

ABA and CCC treatments significantly increased shoot proline content under 50 and 75 mM NaCl stress, while the increase due to BA was non significant. The highest proline content was recorded for the ABA treatment (Fig. 1). Shoot soluble sugar content increased significantly at 50 and 75 mM NaCl stress, however, it was higher in the three PGRs treated plants compared to their corresponding control. The three PGRs all increased shoot soluble sugar content significantly at 50 and 75 mM NaCl stress in the order of ABA > BA > CCC (Fig. 2). Shoot soluble protein content significantly diminished under 50 and 75 mM NaCl stress. However, the three PGR treatments increased shoot soluble protein content. The increase in shoot proline content due to BA and CCC was significant at 50 mM NaCl stress, at 75 mM NaCl stress only BA had a significant effect (Fig. 3).

Effects of the three PGRs on grain yield and yield attributes of rice under saline field conditions

The three PGRs all had a significant positive effect on the grain yield of rice. ABA proved to be the most effective in enhancing grain yield under salinity stress among three PGRs tested (Table 4). Compared to control, ABA, BA and CCC treatments increased grain yield by 21%, 17% and 12%, respectively. The 1000-grain weight and number of panicles per plant also significantly increased with the three PGRs, and the magnitude of the increase was higher in ABA followed by BA and CCC (Table 4). Similarly, panicle length was significantly increased by the three PGRs. The rank of the three PGRs to increase panicle length was BA > ABA > CCC (Table 4).

Effects of the three PGRs on ion accumulation in rice under saline field conditions

The three PGRs all lowered Na^+ accumulation, compared to control, However, only ABA and CCC significantly reduced Na^+ content in the flag leaf of rice (Table 5). K^+ concentration was increased by the three PGRs under saline field conditions and ABA and BA had a significant effect on increasing the K^+ concentration in rice (Table 5). ABA had a significant negative effect in the Cl^- content of rice (Table 5). The Ca^{2+} content in the flag leaf of rice significantly increased when ABA was used (Table 5).

Effect of the three PGRs on organic osmotic matter accumulation of rice under saline field conditions

All the three PGRs increased the proline content of rice under saline field conditions compared to control. The rank was ABA > CCC > BA. The increase in proline content due to ABA and CCC was significant but the increase due to BA was not significant (Table 6). Soluble sugar content was increased significantly by the three PGRs; with ABA causing the largest increase in soluble sugar content in the flag leaf of rice (Table 6). Soluble protein content was also increased by the three PGRs, but only the effect of BA was significant (Table 6).

Discussion

Under salt stress, one of the mechanisms of plant salt tolerance is the selective uptake and accumulation of inorganic ions, mainly Na^+, K^+ and Cl^- (Alian et al., 2000). Iqbal and Ashraf (2006) reported that BA and kinetin were effective in reducing Na^+ levels in wheat plants under saline conditions. Rodriguez (2000) reported that Na^+ affects the uptake of K^+ due to chemical similarities between the two ions. K^+ transport system involving good selectivity of K^+ over Na^+ can also be considered as an important salt tolerant determinant. Earlier studies reported that ABA reduced transpiration by closing stomata and thus leads to reduction of ion uptake in plants (Flowers and Yeo, 1995). In the present study, seed pretreatment with ABA was more

Table 1. Temperatures and rainfall during the experimental period. Experiments were conducted at the Arid Zone Research Institute (31^{o} 53$^{/}$ N, 70^{o} 51$^{/}$E), D. I. Khan, Pakistan from June to September, 2005

Month	Temperature ^{o}C			Rainfall (mm)	Times of Rainfall
	Maximum	Minimum	Mean		
June	49	32	41	0	0
July	44	30	37	142.5	6
August	42	28	35	96.0	4
September	40	24	32	62.0	2

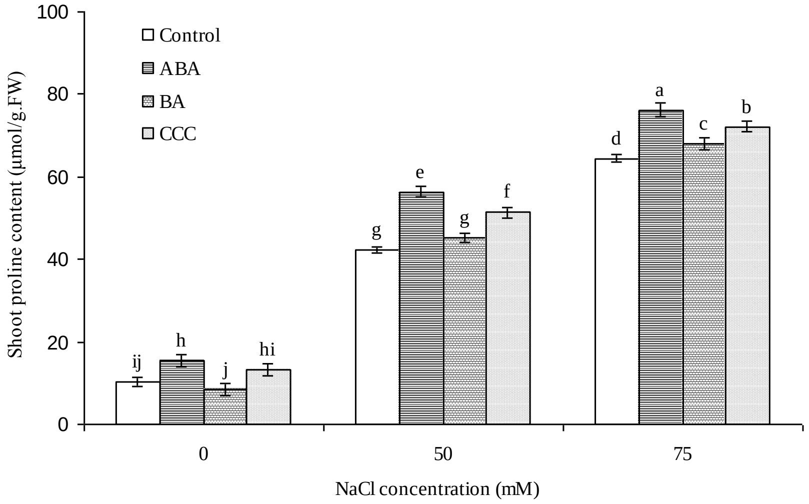

Fig 1. Effect of ABA, BA and CCC on shoot proline content of rice at 0, 50 and 75 mM NaCl stress. Values are means ± SE (*n* = 5) and bars indicate SE. Columns with different letters indicate significant difference at *P* < 0.05 (one-way ANOVA and Duncan's multiple range tests)

effective in reducing Na$^+$ and Cl$^-$ uptake from the culture media. Furthermore, both Na$^+$ and Cl$^-$ transport from root to shoot was also reduced by ABA under salt stress. The result of Na$^+$ content changes in flag leaf by the three PGRs in field experiment was consistent with that in shoot in hydroponic experiment. ABA and BA significantly increased K$^+$ content in the flag leaf in the field experiment. In the hydroponic study, only ABA was effective in increasing the K$^+$ content in shoot of rice under 50 and 75 mM NaCl stress. Gadallah (1999) found that kinetin application reduced the harmful effects of combined saline-aerobic treatment through a reduction in the accumulation of inorganic ions (Na$^+$, Ca^{2+} and Cl$^-$). Calcium has been reported to exhibit positive role in providing salt tolerance (Lauchli and Schubert, 1989). The Ca^{2+} as a secondary messenger initiates the stress signal transduction leading to salt adaptation. Externally applied calcium reduces the effect of NaCl presumably by facilitating higher K$^+$/Na$^+$ selectivity (Liu and Zhu, 1998). Our study showed that both ABA and BA significantly increased Ca^{2+} content at 50 and 75 mM NaCl stress in the hydroponic experiment, while under the field condition only ABA had a significant effect in increasing Ca^{2+} content. Generally, plant species that have low amounts of proline when grown in well-watered and non-saline soil may increase the content of this amino acid upon imposition of drought or salt stresses (Teixeira and Pereira, 2007). In most plants, salinity stress causes changes in gene expression, leading to an increased synthesis of osmoprotectant and osmoregulators (Shao et al., 2006; Ibraheem et al., 2011). Proline and glycinebetaine are thought to function as osmoprotectants for proteins (Bohnert and Jenson, 1996). Accumulation of proline and glycinebetaine provides an environment compatible with the macromolecular structure and function and helps plants to adapt to negative consequences of salinity (Jaleel et al., 2007). In this present study ABA and CCC increased proline accumulation significantly in the shoot of rice in hydroponic condition as well as in the flag leaf in the field experiment. Our results indicated that soluble sugar content was also increased under salt stress; interestingly, ABA, BA and CCC all increased soluble sugar content in hydroponic experiment as well as in field study. Accumulation of soluble sugar in response to PGRs under salt stress is expected to be helpful for plants to cope with salt stress. Therefore, it is generally agreed that salinity and water stress induce soluble sugar accumulation (Prado et al., 2000). Earlier studies indicated that ABA was implicated in the regulation of soluble sugar transport and metabolism (Kashem et al., 1998). It was found that both ABA and gibberellins (GA) help to regulate soluble sugar concentration (Bethke et al., 1997). Ashraf and Waheeda (1993) reported that leaf soluble proteins were decreased due to salt stress in all lines of wheat irrespective of their salt tolerance. In current study, soluble protein contents were also decreased by salinity stress, however, BA increased soluble protein content significantly in both the experiments. Iqbal and Ashraf (2006) reported that pre-treatment with mild and moderate concentrations of kinetin increased grain yield in both the salt-intolerant and salt-tolerant cultivar of wheat. Aldesuquy and Ibrahim (2001) suggested that the improvement in wheat grain yield due to the increased rate of translocation of photosynthate from leaves to grains was caused by pre-treatment with PGRs. Grain yield is highly dependent upon the number of panicles and bearing tillers per plant (Nerson, 1980) and salt stress leads to reduce tillering and or tiller abortion (Nicolas et al.,

Table 2. Effects of ABA, BA and CCC seed soaking on Na^+ and K^+ content (mmol/g.DW) and Na^+/K^+ ratios in shoot and root of rice at 0, 50 and 75mM NaCl stress. Values are means ± SEs (*n* = 5). Different letters within each index indicate significant differences at $P < 0.05$ (one-way ANOVA and Duncan's multiple range tests)

Shoot												
	Na^+				K^+				Na^+/K^+			
NaCl (mM)	0mM	50mM	75mM	Mean	0mM	50mM	75mM	Mean	0mM	50mM	75mM	Mean
Control	0.060 ± 0.005 g	1.22 ± 0.08 cd	1.84 ± 0.02 a	1.04	0.93 ± 0.14 cd	0.63 ± 0.07 ef	0.61 ± 0.03 f	0.72	0.06 ± 0.02 g	1.97 ± 0.21 cd	3.07 ± 0.27 a	1.70
ABA	0.036 ± 0.004 g	0.78 ± 0.08 f	1.27 ± 0.09 c	0.70	1.17 ± 0.08 ab	0.93 ± 0.06 cd	0.82 ± 0.10 cde	0.96	0.03 ± 0.01 g	0.85 ± 0.08 f	1.60 ± 0.24 de	0.83
BA	0.057 ± 0.006 g	1.09 ± 0.00 de	1.62 ±0.06 b	0.92	1.30 ± 0.14 a	0.86 ± 0.08 cde	0.70 ± 0.05 def	0.94	0.04 ± 0.01 g	1.28 ± 0.15 ef	2.34 ± 0.25 bc	1.22
CCC	0.053 ± 0.010 g	0.98 ± 0.09 e	1.60 ± 0.10 b	0.88	1.05 ± 0.11 bc	0.76 ± 0.17 def	0.65 ± 0.08 ef	0.78	0.05 ± 0.02 g	1.32 ± 0.23 ef	2.53 ± 0.39 b	1.31

Root												
	Na^+				K^+				Na^+/K^+			
NaCl (mM)	0mM	50mM	75mM	Mean	0mM	50mM	75mM	Mean	0mM	50mM	75mM	Mean
Control	0.040 ± 0.004 f	0.89 ± 0.10 bc	1.20 ± 0.09 a	0.73	0.77 ± 0.03 b	0.45 ± 0.02 f	0.37 ± 0.02 f	0.53	0.045 ± 0.005 e	1.90 ± 0.12 bc	3.61 ± 0.41 a	1.88
ABA	0.036 ± 0.010 f	0.57 ± 0.08 e	0.82 ± 0.07 bcd	0.48	0.93 ± 0.02 a	0.72 ± 0.05 bc	0.56 ± 0.03 de	0.74	0.038 ± 0.006 e	0.79 ± 0.05 d	1.43 ± 0.05 cd	0.76
BA	0.041 ± 0.004 f	0.72 ± 0.17 cde	1.01± 0.06 ab	0.59	0.95 ± 0.02 a	0.65 ± 0.03 cd	0.46 ± 0.04 ef	0.68	0.044 ± 0.005 e	1.12 ± 0.23 d	2.23 ± 0.29 b	1.13
CCC	0.037 ± 0.010 f	0.65 ± 0.12 de	0.96 ± 0.10 b	0.55	0.90 ± 0.05 a	0.61 ± 0.06 cd	0.43 ± 0.05 f	0.65	0.041 ± 0.010 e	1.13 ± 0.27 d	2.33 ± 0.36 b	1.13

Table 3. Effects of ABA, BA and CCC seed soaking on Cl^- and Ca^{2+} concentrations (mmol/g.DW) and Na^+/Ca^{2+} ratios in shoot and root of rice at 0, 50 and 75 mM NaCl stress. Values are means ± SE (*n* = 5). Different letters within each index indicate significant differences at $P < 0.05$ (one-way ANOVA and Duncan's multiple range tests)

Shoot												
	Cl^-				Ca^{2+}				Na^+/Ca^{2+}			
NaCl (mM)	0mM	50mM	75mM	Mean	0mM	50mM	75mM	Mean	0mM	50mM	75mM	Mean
Control	0.080 ± 0.020 f	0.76 ± 0.04 c	1.11 ± 0.04 a	0.65	0.035 ± 0.010 bc	0.023 ± 0.013 d	0.020 ± 0.010 d	0.026	1.98 ± 0.48 g	56.6 ± 10.0 c	95.5 ± 5.0 a	51.4
ABA	0.067 ± 0.010 f	0.60 ± 0.04 e	0.85 ± 0.04 b	0.54	0.068 ± 0.005 a	0.050 ± 0.005 b	0.036 ± 0.005 bc	0.051	0.61 ± 0.09 g	15.5 ± 0.5 f	36.0 ± 1.0 d	17.3
BA	0.090 ± 0.010 f	0.73 ± 0.02 cd	1.08 ± 0.03 a	0.61	0.052 ± 0.010 b	0.047 ± 0.003 b	0.029 ± 0.004cd	0.043	1.23 ± 0.33 g	23.5 ± 1.0 ef	57.6 ± 3.0 bc	27.4
CCC	0.073 ± 0.010 f	0.65 ± 0.05 de	0.93 ± 0.02 b	0.51	0.047 ± 0.005 b	0.032 ± 0.004 cd	0.024 ± 0.002 d	0.035	1.20 ± 0.29 g	32.2 ± 5.6 de	69.30 ± 4.9 b	34.2

Root												
	Cl^-				Ca^{2+}				Na^+/Ca^{2+}			
NaCl (mM)	0mM	50mM	75mM	Mean	0mM	50mM	75mM	Mean	0mM	50mM	75mM	Mean
Control	0.065 ± 0.012 f	0.59 ± 0.04 cd	0.84 ± 0.04 a	0.50	0.023 ± 0.002 c	0.016 ± 0.001 c	0.015 ± 0.001 c	0.017	2.10 ± 0.34 f	55.0 ± 7.5 b	90.0 ± 6.5 a	49.0
ABA	0.055 ± 0.010 f	0.46 ± 0.06 e	0.69 ± 0.07 bc	0.40	0.036 ± 0.001 a	0.034 ± 0.002 b	0.026 ± 0.002 b	0.033	0.90 ± 0.14 f	18.0 ± 3.4 e	33.0 ± 4.7 cd	17.3
BA	0.073 ± 0.010 f	0.56 ± 0.06 de	0.82 ± 0.06 a	0.48	0.040 ± 0.002 a	0.029 ± 0.004 b	0.023 ± 0.002 c	0.029	1.20 ± 0.06 f	25.0 ± 4.7 de	44.0 ±5.4 bc	23.4
CCC	0.061 ± 0.010 f	0.53 ± 0.04 de	0.77 ± 0.05 ab	0.45	0.030 ± 0.008 b	0.024 ± 0.002 c	0.021 ± 0.001 c	0.026	1.20 ± 0.26 f	27.0 ± 3.0 de	45.0 ± 5.3 bc	24.4

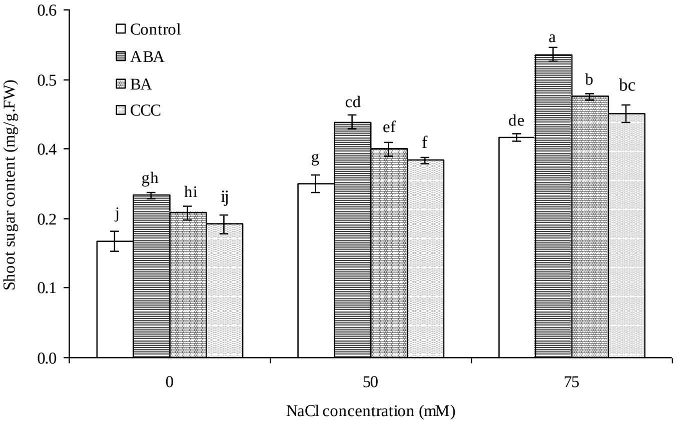

Fig 2. Effect of ABA, BA and CCC on shoot soluble sugar content of rice at 0, 50 and 75 mM NaCl stress. Values are means ± SE (n = 5) and bars indicate SE. Columns with different letters indicate significant difference at $P < 0.05$ (one-way ANOVA and Duncan's multiple range tests)

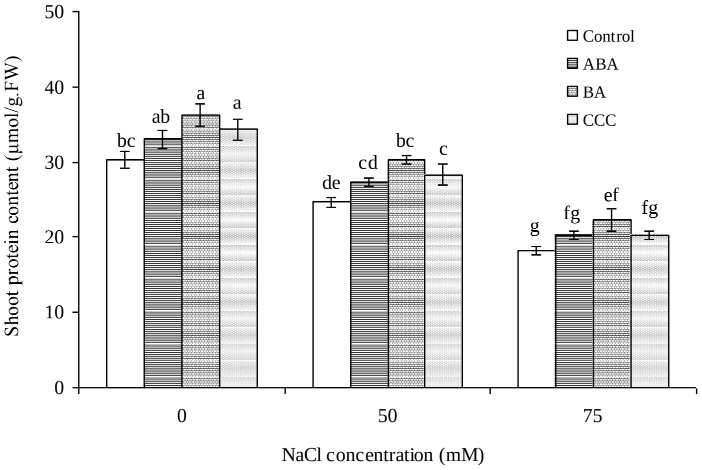

Fig 3. Effect of ABA, BA and CCC on shoot soluble protein content of rice at 0, 50 and 75 mM NaCl stress. Values are means ± SE (n = 5) and bars indicate SE. Columns with different letters indicate significant difference at $P < 0.05$ (one-way ANOVA and Duncan's multiple range tests)

1993). ABA also improved the transport of photoassimilates from the leaves and stem to the developing grains, that is, it effectively increased the sink strength of the grains (Travaglia et al., 2010). Our results indicated that the three PGRs alleviate the inhibitory effects of salt stress by increasing grain yield, 1000-grain weight, panicle length and number of panicles per plant.

Materials and methods

Hydroponic experimental procedure and plant growth conditions

Seeds of rice cv. IR-6 were obtained from Rice Research Institute, National Agricultural Research Center, Pakistan, by which the variety was bred. The variety has high yield. It is 115 cm tall and matures within 120 d (Mushtaq and Akram, 2005). Healthy rice seeds were surface sterilized with 70% ethanol for two minutes followed by washing three times with sterilized deionized water. A total of 500 seeds for each PGR treatment were soaked with ABA and BA at the concentration of 10^{-5} M and CCC at 10^{-6} M and aerated for 24 hours at 25°C. After soaking, seeds were sown in plastic trays containing washed silica sand filled with Yoshida nutrient solution (Yoshida et al., 1976). After 10 d, 300 healthy and uniform plants from each PGR treatment were selected and transplanted into black painted well-aerated plastic boxes of 6 litre capacity filled with the same nutrient solution. After 5 d adaptation, these 300 plants were randomly divided into three groups and transferred into a Yoshida nutrient solution supplemented with 0, 50 and 75 mM NaCl, respectively, for 25 d. Solutions were renewed every 3 d and pots were randomly arranged. Plants (40 d old) were harvested for the estimation of the contents of ions, proline, sugar and protein and biomass. Experiments were conducted in the glasshouse during August and September 2006. During this period the day/night temperature was 30 ± 8 / 13 ± 5°C and the photoperiod was 12 ± 2 / 12 ± 2 h. Photon flux density was 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and relative humidity was 65%.

Field experimental procedure and site description

Experiments were conducted at the Arid Zone Research Institute (31° 53′ N, 70° 51′E), Pakistan from June to September, 2005. Temperatures and rainfall during the experimental period are shown in Table 1. Seeds of rice cv. IR-6 were soaked with ABA and BA at a concentration of 10^{-5}M and CCC at 10^{-6}M for 24h at 25°C. Seeds were sown in non-saline soil for seedling raising and transplanted (35d old seedlings) into saline soil. The experimental design was a randomized complete block design (RCBD) with four treatments (control, ABA, BA and CCC) and five replications with 100 plants in each replication. Plants were spaced at 10cm × 20cm. Before layout of the experiment in the field, composite soil samples were taken. Laboratory analysis of the soil indicated that the soil was salty clay, with low organic matter (0.72%). The pH was 8.4 and EC 7.34 dS m^{-1}, the ion contents were 18 me L^{-1} Na^+, 3.4 me L^{-1} K^+, 2.3 me L^{-1} Ca^{2+}, 2.3 me $L^{-1}$$Mg^{2+}$, 10 me L^{-1} Cl^-, 1.7 me L^{-1} CO_3^- and 0.68 me L^{-1} HCO_3^-. Soil particle size distribution was determined by Hydrometer method (Moodie et al., 1959). The ion contents were determined by the method of Flowers and Hajibagheri (2001). Organic matter was determined by the method of Li et al. (2009). Fertilizers were applied at a rate of 120, 90, 60, 5 and 1 kg ha^{-1} of N, P_2O_5, K_2O, Zn and B in the form of urea, diammonium phosphate, sulphate of potash, zinc sulphate and borax, respectively.

Measurements of biomass, ions and yield

For the hydroponic experiments, shoots and roots of plants were separated after harvest. For each index estimation, four plants were pooled in each of the five replications (n = 5). Fresh weights were determined immediately and samples oven dried at 70 °C for 3 d to obtain dry weights. Dried samples were digested in Nitric acid-perchloric acid digestion mixture. The ion contents of Na^+ and K^+ were determined using a flame photometer (Sherwood model 410, Japan), Ca^{2+} was determined with Atomic Absorption Spectrophotometer (Shimadzu 6200AA, Japan) and Cl^- was determined by the method of Murcia et al. (1995). Shoot soluble protein content was estimated by the method of Lowery et al. (1951), using bovine serum albumin as standard. Shoot proline contents were determined using ninhydrin acid reagent according to

Table 4. Effect of ABA, BA and CCC on grain yield and yield attributes of rice under saline field conditions. Values are means ± SE (*n* = 5). Different letters within each index indicate significant differences at P < 0.05 (one-way ANOVA and Duncan's multiple range tests)

Treatment	Grain yield (Kg/ha)	Number of panicles/plant	1000-Grain weight (g)	Panicle length (cm)
Control	4833 ± 124 c	16.25 ± 0.40 c	20.75 ± 0.34 c	18.65 ± 0.37 c
ABA	5827 ± 88 a	22.25 ± 0.45 b	23.32 ± 0.15 a	22.40 ± 0.12 b
BA	5659 ± 91 ab	20.57 ± 0.31 a	22.47 ± 0.10 ab	23.80 ± 0.21 a
CCC	5413 ± 85 b	19.75 ± 0.26 b	21.34 ± 0.18 b	22.00 ± 0.16 b

Table 5. Effect of ABA, BA and CCC on ion accumulation in flag leaf of rice under saline field conditions. Values are means ± SE (*n* = 5). Different letters within each index indicate significant differences at P < 0.05 (one-way ANOVA and Duncan's multiple range tests)

Treatment	Na^+ (mmol/g.DW)	K^+ (mmol/g.DW)	Cl^- (mmol/g.DW)	Ca^{2+} (mmol/g.DW)
Control	1.56 ± 0.05 a	0.79 ± 0.13 c	0.86 ± 0.05 a	0.025 ± 0.002 b
ABA	0.80 ± 0.10 c	1.30 ± 0.10 a	0.69 ± 0.02 b	0.045 ± 0.005 a
BA	1.26 ±0.12 ab	1.16 ± 0.12 ab	0.83 ± 0.04 a	0.037 ± 0.003 ab
CCC	1.05 ± 0.11 bc	0.92 ± 0.11 bc	0.76 ± 0.04 ab	0.027 ± 0.004 b

Table 6. Effect of ABA, BA and CCC on some organic osmotic matter contents in flag leaf of rice under saline field condition. Values are means ± SE (*n* = 5). Different letters within each index indicate significant differences at P < 0.05 (one-way ANOVA and Duncan's multiple range tests)

Treatment	Proline content (μmol/g.FW)	Soluble sugar content (mg/g.FW)	Soluble protein content (μmol/g.FW)
Control	67.27 ± 2.10 b	0.380 ± 0.013 c	24.27 ± 0.91 b
ABA	88.24 ± 4.60 a	0.540 ± 0.010 a	27.33 ± 1.04 ab
BA	72.39 ± 4.30 b	0.480 ± 0.010 b	29.27 ± 0.98 a
CCC	80.27 ± 2.10 ab	0.400 ± 0.008 b	26.30 ± 1.14 ab

Bates et al. (1973) and shoot soluble sugar content was determined by the method of Hedge and Hofreiter (1962). For the field experiments, grain yield (kg/ha) and 1000-grain weight were recorded according to the RCBD (*n* = 5). Four flag leaves from four individual plants in the same treatment were collected as one sample unit to determine ion, proline, soluble protein and soluble sugar contents. Four panicles from four individual plants were collected as one sample unit to measure number of panicles per plant and panicle length.

Statistical analysis

Results of all the indexes are presented as means with standard errors (SEs) (*n* = 5). One-way ANOVA (analysis of variance) and Duncan's multiple range 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).

Conclusions

The addition of plant growth regulators as seed treatment to rice cv. IR-6 has a significant role in the partial alleviation of salinity stress. ABA was the most effective in reducing Na^+ and Cl^- concentrations and Na^+/K^+ ratio and at increasing K^+ and Ca^{2+} concentrations, proline accumulation, soluble sugar content and grain yield. ABA was the most effective at increasing soluble protein content. Grain yield in the field experiment was increased significantly in the ranking ABA > BA > CCC. Our results suggest that ABA was more effective than BA and CCC to ameliorate the toxic effects of salinity stress in rice. It is economic to use these PGRs in the production system. Since they were used only for seed treatment and at very low concentration (10^{-5} or 10^{-6} M) their possible negative impact on human health can be avoided. Consequently, using PGRs-treated seeds may reduce the negative effects of salinity in rice cultivation.

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References

Aldeuquy HS, Ibrahim AH (2001) Water relations, abscisic acid and yield of wheat plants in relation to the interactive effect of seawater and growth bio-regulators. J. Agron. Crop Sci. 187: 97-104

Alian A, Altman A, Heuer B (2000) Genotypic difference in salinity and water stress tolerance of fresh market tomato cultivars. Plant Sci. 152: 59-65

Amtmann A, Armengaud P, Volkov V (2004) Potassium nutrition and salt stress. In: Blatt MR (ed) Membrane Transport in Plants. Blackwell, Oxford. pp. 293-339

Ashraf M, Waheeda A (1993) Responses of some local/exotic accessions of lentil (*Lensculinaris medic*.) to salt stress. J. Agron. Crop Sci. 170: 103-112

Bates LS, Waldren RP, Teare ID (1973) Rapid determination of free proline for water-stress studies. Plant Soil 39: 205-207

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

Benderradji L, Brini F, Amar SB, Kellou K, Azaza J, Masmoudi K, Bouzerzour H, Hanin M (2011) Sodium transport in the seedlings of two bread wheat (*Triticum aestivum* L.) genotypes showing contrasting salt stress tolerance. Aust. J. Crop Sci. 5: 233-241

Bethke P, Schuurink R, Jones R (1997) Hormonal signaling in cereal aleurone. J. Exp. Bot. 48: 1337-1356

Bohnert HJ, Jenson RG (1996) Strategies for engineering water-stress tolerance in plants. Trends Biotechnol. 14: 89-97

Cavusoglu K, Kabar K (2008) Comparative effects of some plant growth regulators on the germination of barley seeds under saline conditions. Sci. Eng. J. Fırat Univ. 20: 43-55

Debez A, Chaibi W, Bouzid S (2001) Effect du NaCl et de regulatoeurs de croissance sur la germination d’ *Atriplex halimus* L. Cah. Agric.10: 135-138

Flowers TJ, Hajibagheri MA (2001) Salinity tolerance in *Hordeum vulgare*: ion concentrations in root cells of cultivars differing in salt tolerance. Plant Soil 231: 1-9

Flowers TJ, Yeo AR (1995) Breeding for salinity resistance in crop plants: where next. Aust. J. Plant Physiol. 22: 875-884

Gabr AI, Sharaky MM, El-Ashkar SA (1977a) The combined effect of soil salinity and CCC on dry matter accumulation and yield of wheat plants. Biol. Plant. 19: 101-106

Gabr AI, Sharaky MM, El-Ashkar SA (1977b) The effect of different combinations of soil salinity and CCC on dry matter accumulation and yield of cotton plants. Biol. Plant. 19: 391-393

Gadallah MAA (1999) Effect of kinetin on growth, grain yield and some mineral elements in wheat plants growing under excess salinity and oxygen deficiency. Plant Growth Regul. 27: 63-74

Gurmani AR, Bano A, Salim M (2007) Effect of abscisic acid and 6-benzyladenine on growth and ion accumulation of wheat under salinity stress. Pak. J. Bot. 39: 141-149

Hedge JE, Hofreiter BT (1962) Methods of estimating starch and carbohydrates. In: Whistler RL, BeMiller JN (ed) Carbohydrate Chemistry for Food Scientists. Academic Press, New York

Ibraheem O, Dealtry G, Roux S, Bradley G (2011) The effect of drought and salinity on the expressional levels of sucrose transporters in rice (*Oryza sativa* Nipponbare) cultivar plants. Plant Omics Journal 4: 68-74

Iqbal M, Ashraf M (2005) Presowing seed treatment with cytokinins and its effect on growth, photosynthetic rate, ionic levels and yield of two wheat cultivars differing in salt tolerance. Acta Bot. Sin. 47: 1315-1325

Iqbal M, Ashraf M (2006) Wheat seed priming in relation to salt tolerance: growth, yield and levels of free salicylic acid and polyamines. Ann. Bot. Fennici 43: 250-259

Jaleel CA, Gopi R, Sankar B, Manivannan P, Kishorekumar A, Sridharan R, Panneerselvam R (2007) Studies on germination, seedling vigour, lipid peroxidation and proline metabolism in *Catharanthus roseus* seedlings under salt stress. South Afri. J. Bot. 73: 190-195

Kashem MA, Hori H, Itoh K, Hayakawa T, Todoroki Y, Hirai N, Ohigashi H, Mitsui T (1998) Effect of (+)-$8',8',8'$-trifluoroabscisic acid on ά-amalyse expression and sugar accumulation in rice cells. Planta 205: 319-326

Latef AAA, Shaddad MAK, Ismail AM, Alhmad MFA (2009) Benzyladenine can alleviate saline injury of two roselle (*Hibiscus sabdariffa*) cultivars via equilibration of cytosolutes including anthocyanins. Int. J. Agric. Biol. 11: 151-157

Lauchli A, Schubert S (1989) The role of calcium in the regulation of membrane and cellular growth processes under salt stress. In: Cherry JH(ed) Environmental Stress in Plans. Springer-Verlag, Berlin. pp. 131-138

Li FR, Zhao WZ, Liu JL, Huang ZG (2009) Degraded vegetation and wind erosion influence soil carbon, nitrogen and phosphorus accumulation in sandy grasslands. Plant Soil 317: 79-92

Liu J, Zhu JK (1998) A calcium sensor homolog required for plant salt tolerance. Sci. 280: 1943-1945

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the folin phenol reagent. J. Boil. Chem. 193: 265-275

Moodie D, Smith HW, Mc-Creary RA (1959) Laboratory Manual for Soil Fertility. Washington State College Press, Pullman, Washington. pp. 31-39

Murcia MA, Vera A, Ortiz R, García-Carmona F (1995) Measurement of ion levels of spinach grown in different fertilizer regimes using ion chromatography. Food Chem. 52: 161-166

Mushtaq A, Akram M (2005) Hand book on rice varieties of Pakistan. Rice Research Institute, Kala Shah Kaku, National Agricultural Research Institute, Islamabad. pp. 66

Naqvi SM, Ansari R, Ansari AQ (1970) Effect of (2-chloroethyl)-trimethylammoium chloride (CCC) on increasing salt tolerance in wheat. J. Exp. Bot. 21: 712-713

Nerson H (1980) Effect of population density and number of ears on wheat yield and its components. Field Crop Res. 3: 225-234

Nicolas ME, Munns R, Samarakoon AB, Gifford RM (1993) Elevated CO_2 improves the growth of wheat under salinity. Aust. J. Plant Physiol. 20: 349-360

Pardo JM, Cubero B, Leidi EO, Quintero FJ (2006) Alkali cation exchanger: roles in cellular homeostasis and stress tolerance. J. Exp. Bot. 57: 1181-1199

Prado FE, Boero C, Gallardo M, González JA (2000) Effect of NaCl on germination, growth and soluble sugar content in *Chenopodium quinoa* Willd. seeds. Bot. Bull. Acad. Sin. 41: 27-34

Ramadan T, Flowers TJ (2004) Effects of salinity and benzyl adenine on development and function of microhairs of *Zea mays* L. Planta 219: 639-648

Ren ZH, Gao JP, Li LG, Cai XL, Huang W, Chao DY, Zhu MZ, Wang ZY, Luan S, Lin HX (2005) A rice quantitative trait locus for salt tolerance encodes a sodium transporter. Nat. Genet. 37: 1141-1146

Rodriguez-Navarro A (2000) Potassium transport in fungi and plants. Biochem. Biophys. Acta. 1469: 1-30

Rodriguez-Navarro A, Rubio F (2006) High-affinity potassium and sodium transport systems in plants. J. Exp. Bot. 57: 1149-1160

Rus A, Lee B, Munoz-Mayor A, Sharkhuu A, Miura K, Zhu JK, Bressan RA, Hasegawa PM (2004) AtHKT1 facilities Na^+ homeostasis and K^+ nutrition in planta. Plant Physiol. 136: 2500-2511

Shao HB, Chen XY, Chu LY, Zhao XN, Wu G, Yuan YB, Zhao CX, Hu ZM (2006) Investigation on the relationship of proline with wheat anti-drought under soil water deficits. Colloid. Surface. B. 53: 113-119

Teixeira J, Pereira S (2007) High salinity and drought act on an organ-dependent manner on potato glutamine synthetase expression and accumulation. Environ. Exp. Bot. 60: 121-126

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

Uddin MK, Juraimi AS, Ismail MR, Hossain MA, Othman R, Rahim AA (2011) Effect of salinity stress on nutrient uptake and chlorophyll content of tropical turfgrass species. Aust. J. Crop Sci. 5: 620-629

Yeo AR, Yeo ME, Flowers SA, Flowers TJ (1990) Screening of rice (*Oryza sativa* L.) genotypes for physiological characters contributing to salinity resistance, and their relationship to overall performance. Theor. Appl. Genet. 79: 377-384

Yoshida S, Forno DA, Cock JH, Gomez KA (1976) Laboratory Manual for Physiological Studies of Rice, 3rd edn. International Rice Research Institute, Philippines

Zhang JL, Flowers TJ, Wang SM (2010) Mechanisms of sodium uptake by roots of higher plants. Plant Soil 326: 45-60