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# EFFECT OF GROWTH REGULATORS ON GROWTH, YIELD AND IONS ACCUMULATION OF RICE (ORYZA SATIVA L.) UNDER SALT STRESS

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#### Abstract

A pot experiment was conducted in glass-house to assess the role of Abscisic acid (ABA), Benzyleadenine (BA) and Cycocel (CCC) on growth, yield, ion accumulation and proline production in three rice cultivars viz, Super Basmati, Shaheen Basmati (fine cultivar) and IR-6 (coarse cultivar) differing in yield. Seeds of each cultivar were soaked prior to sowing with ABA and BA each at 10<sup>-5</sup> M and CCC 10<sup>-6</sup> M for 24h. Salinity of 5dS/m was developed by adding NaCl salt in five equal splits daily to fifteen days old plants of all the cultivars. Plants were harvested two weeks after salt treatment. Shoot and root dry weight decreased at salinity stress as compared to control, however ABA, BA and CCC treatment caused a substantial increase in shoots and root dry weight over that of salt alone. Salt treatment increased the level of Na<sup>+</sup> and Cl<sup>-</sup> but decreased K<sup>+</sup> content in flag leaves as well as in roots of three rice cultivars. ABA and CCC treated plants showed significant decrease in Na<sup>+</sup> content but increased K<sup>+</sup> content in flag leaves of all the cultivars at salt stress. ABA was more effective to increase Ca<sup>2+</sup> content in flag leaf as well as in roots of all the cultivars as compared with BA and CCC. The levels of ions (Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup> and Cl<sup>-</sup>) were relatively higher in roots than in flag leaves, however higher accumulation of  $K^+$  and  $Ca^{2+}$ content with lower accumulation of Na<sup>+</sup> and Cl<sup>-</sup> in IR-6. The ranking of growth regulators for their effects on grain yield and 1000-grain weights were ABA>BA>CCC. Higher grain yield and 1000grain weight was recorded by IR-6. ABA and CCC treatment further augmented the stimulatory effect of salts on proline accumulation. Higher proline accumulation was observed in IR-6 as compared to Shaheen Basmati and Super Basmati. Rice cv. IR-6 performed better. The relatively low accumulation of Na<sup>+</sup> and Cl<sup>-</sup> and less translocation to flag leaf of IR-6 concomitant with high  $K^{+}$  accumulation, better proline content and greater leaf area under salt stress make this variety salt tolerant. These traits are augmented by ABA more effectively than BA. Keywords: Wheat, Abscisic acid, Benzyladenine, Cycocel, NaCl stress.

### Introduction

Salinization of agricultural soils represents one of the largest environmental challenges worlds wide. Over 6 % of the world's land and 20% of the world's irrigated land are currently affected by salinity (Rhoades *et al.*, 1992; Munns, 2005). In such soils, NaCl concentrations typically exceed 40 mM, and much higher values are frequently found (Munns, 2005), creating toxic growth conditions for most plants, including all major crop species. It has long been known that NaCl toxicity is largely attributed to the effect of Na<sup>+</sup>, and only rarely those of Cl<sup>-</sup> (Tester & Davenport, 2003), and that Na<sup>+</sup> toxicity is linked strongly to the plant's ability to sustain the acquisition and in plant

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distribution of  $K^+$  (Rains & Epstein, 1967; Zhu *et al.*, 1998; Kader & Lindberg, 2005). Generally salt stress caused both osmotic stress and ionic stress. Under salt stress, osmotic stress is created by an excess of salt in the soil, and ionic stress is caused by the over accumulation of salt in the cells. These stresses individually affect the physiological status (Lefevre *et al.*, 2001; Ueda *et al.*, 2003).

Phytohormones play an essential role in regulating plant growth and development. Cytokinins (CKs) have been implicated to control many developmental processes and environmental responses of plants, including leaf senescence, apical dominance, chloroplast development and regulation of cell division (Hutchison & Kieber, 2002). Seed priming with optimal concentration of the CKs has been shown to be the beneficial to germination, growth and yield of some crop species grown under saline conditions (Kaur *et al.*, 2002). CKs are involved in various processes in the growth and development of plants (Takei *et al.*, 2002). These effects of CKs are due to interactions with other plant hormones and environmental signals (Hare *et al.*, 1997).

Abscisic acid (ABA) regulates various aspects of plant growth and development, including seed maturation and dormancy, as well as adaptation to abiotic environmental stresses (Beaudoin *et al.*, 2000). Exogenous ABA application decreased Na<sup>+</sup> accumulation and increased K<sup>+</sup>:Na<sup>+</sup> ratio in sorghum and rice shoot and also in leaves (Amzallag *et al.*, 1990). ABA reduces transpiration by closing stomata and thus leads to reduce ion uptake in plant (Yeo *et al.*, 1985). In view of this information, Ashraf & Foolad (2005; 2007) proposed that plant growth regulators, compatible solutes, antioxidants or inorganic salt can be exogenously applied as a foliar spray, or through seed priming to induce stress tolerance. For example, exogenous application of salicylic acid can induce salt tolerance and water stress tolerance in wheat (Waseem *et al.*, 2006; Arfan *et al.*, 2007). Similarly, Raza *et al.* (2006; 2007) found that foliar application of glycinebetaine improved the growth of wheat by improving photosynthesis, K<sup>+</sup>/Na<sup>+</sup> ratio, and antioxidant capacity. Present study was conducted to assess the effects of pre-soaking seed treatment with different plant growth regulator on growth and ion accumulation of rice plants under salt stress.

#### **Materials and Methods**

The seeds of three cultivars of rice i.e. Super Basmati (salt sensitive) and Shaheen Basmati (moderately salt tolerant) and one coarse rice cultivar IR-6 (salt tolerant) were obtained from rice research program, Crop Sciences Institute, National Agriculture Research Centre, Islamabad, Pakistan. Aqueous solutions of Abscisic acid (ABA) Benzyladenine (BA) in the concentration of  $10^{-5}$  M and Cycocel (CCC) at  $10^{-6}$  M were used for seed soaking. Healthy rice seeds were surface sterilized with 95% ethanol for two to three minute followed by three washing with sterilized deionized water. Seeds were soaked separately in 250 ml of the ABA, BA and CCC solutions as well as distilled water, for 24 h at room temperature in black painted flasks, aerated with aeration pump (Blue Sky BS-410). Soaked seeds were rinsed three times with deionized water prior to sowing.

Seeds were sown for nursery raising in plastic trays containing washed silica sand. After 10days of germination, plants were transferred to plastic pots of (30x40 cm) containing 10 kg soil collected from the field of NARC (15 cm from the upper soil layer), soil was dried, ground, sieved (<2 mm). Salinity 5, 7 and 9 dS/m was developed by using NaCl salt on soil weight basis as prescribed by Richards (1954). The NaCl Solution was applied to fifteen days old plants of all the cultivars with five equal splits in five days.

## 1416

The experiment was conducted in a glasshouse during the mid of June 2004 and harvested in Sep 2004. Experiment was repeated in 2005 during the same growing season. Recommended fertilizer was applied to the pots @ 120, 90, 60, 5 and 1 kg ha<sup>-1</sup> N,  $P_2O_5$  and  $K_2O$ , Zn and B on soil weight basis in the form of urea, di-ammonium phosphate, sulphate of potash, zinc sulphate and borax. All the P, K, zinc and boron were applied at the time of sowing while N was applied in three equal splits.

At vegetative stage 6 plants from each treatment (two plants per replicate) were uprooted and washed with distilled water. After being dried with filter paper, the roots were removed. Samples were dried in an oven at  $65^{\circ}$ C and the dry weight of shoots and roots were recorded. At flag leaf stage, 6 plants per treatment were up rooted their flag leaf area was calculated by leaf area meter (Li- Cor, model LI-3000A).

The proline contents were determined from the fresh flag leaf by the method of Bates *et al.*, (1973). Flag leaves and their roots were dried in oven. Oven dried leaves and roots were ground finely so as to pass through a 2 mm sieve. Dried samples were digested in digestion mixture (Nitric acid-perchloric acid) according to the method of Chapman & Pratt (1961). The ions Na<sup>+</sup> and K<sup>+</sup> in flag leaves and in roots were determined using a flame photometer (Sherwood model 410, Japan) Ca<sup>2+</sup> was determined with Atomic Absorption (Shimadzu 6200AA Japan). Chloride was determined by the method of Chapman and Pratt (1961). The Agronomic data were recorded for grain yield per pot, plant height and 1000-grain weight. Data was statistically analyzed by Mini tab soft ware.

#### **Results and Discussion**

All the three cultivars of rice (10 d old seedlings) were exposed to NaCl salinity @ 5, 7 and 9 dS/m but seedlings of all the cultivars died with the increase in salt concentrations above 5dS/m except a few seedlings of cv. IR-6 which survived. Hence the 5 dS/m was selected for further experimentation.

Salt stress caused a significant decrease in dry weight of shoots and roots in all the rice cultivars. Rice cv. Super Basmati was more affected by salt stress as compared to cv. Shaheen Basmati and cv. IR-6. ABA, BA and CCC increased significantly shoot and root dry weight as compared to untreated plants experiencing salt stress (ANOVA not shown), but the magnitude of increase was higher in ABA under salt stress. Maximum shoot and root dry weight was recorded in cv. IR-6 with ABA treatment as compared to cv. Shaheen Basmati and cv. Super Basmati under salt stress (Table-1). Interaction between salinity and growth regulators were significant for shoot dry weight in cv. Super Basmati and cv. IR-6, while in root dry weight only cv. Shaheen Basmati had significant effect.

Iqbal & Ashraf (2005) reported that, plant growth regulators have been successfully applied to ameliorate the toxic effects of salinity on germination and plant growth. Presowing treatment with optimal concentration of CKs has been shown to be beneficial to germination, growth and the yield of some crop species grown under saline conditions (Kaur *et al.*, 2002; Khan *et al.*, 2002).

Salinity stress decreased significantly the height of rice cultivars under salt stress (ANOVA not shown), but the effect of plant growth regulators was non significant. The ranking of plant height of rice cultivars exposed to salt stress were cv. Shaheen Basmati>cv. Super Basmati>cv. IR-6. ABA, BA and CCC had no marked effect on plant height of any variety under salt stress (Table-1). Zidan *et al.* (1990) reported that 100 mM NaCl in the growth medium caused reduction in the length of epidermal cell and in the rate of apparent cell productions.

<b>I</b>			ot dry weight		i) unu e uo/m			
	Super I	Basmati	Shaheen	Basmati	IR	-6		
Treatment	So	<b>S</b> 1	So	<b>S</b> 1	So	<b>S</b> 1		
Control	1.05 ab	0.50 b	1.19 a	0.60 a	1.03 bcd	0.70 d		
ABA	1.39 ab	0.77 ab	1.68 a	0.84 a	1.35 abc	0.94 cd		
BA	1.61 a	0.69 ab	1.92 a	0.78 a	1.60 a	0.85 cd		
CCC	1.52 ab	0.67 ab	1.75 a	0.74 a	1.52 ab	0.80 d		
	LSD (0.0	(5) = 1.04	LSD(0.05) = 1.337 $LSD(0.01)$		) = 0.539			
	Root dry weight (g)							
Treatment	So	S1	So	<b>S</b> 1	So	<b>S</b> 1		
Control	0.36 a	0.14 a	0.41 ab	0.19 b	0.46 a	0.24 a		
ABA	0.38 a	0.20 a	0.42 ab	0.30 ab	0.45 a	0.34 a		
BA	0.47 a	0.19 a	0.55 a	0.27 ab	0.63 a	0.31 a		
CCC	0.42 a	0.17 a	0.50 ab	0.25 ab	0.64 a	0.30 a		
	LSD(0.05	5) = 0.359	LSD(0.01) = 0.338		LSD(0.05) = 0.566			
Plant height (cm)								
Treatment	So	<b>S</b> 1	So	<b>S</b> 1	So	<b>S</b> 1		
Control	105.14 a	100.31 a	118.20 a	115.20 a	100.45 a	95.25 a		
ABA	103.28 a	98.31 a	117.00 a	112.20 a	98.23 a	93.27 a		
BA	107.33 a	99.28 a	120.00 a	113.20 a	104.38 a	94.34 a		
CCC	104.29 a	100.35 a	117.20 a	114.20 a	99.38 a	94.33 a		
LSD(0.05)	14	.10	11.	.75	15.49			
$S_0 = 0.34 \text{ dS/m}$	$r_{1} = 5 dS/m$							

Table.1. Effect of ABA, BA and CCC seed soaking on the shoot dry weight, root dry weight and plant height of three cultivars of rice at 0.34 dS/m (control) and 5 dS/m NaCl.

Salt stress significantly increased the Na<sup>+</sup> content of flag leaves in all the cultivars. The maximum increase was recorded in cv. Super Basmati over control. The Na<sup>+</sup> content in flag leaf was less in ABA, BA and CCC treated plants under salt stress as compared to that of untreated salt stressed plant, but the magnitude of decrease was higher in ABA treatment than that of BA and CCC under salt stress. The growth regulators treated plants of cv. IR-6 was the least accumulator of Na<sup>+</sup> content under salt stress (Table-2).

Salt stress caused a reduction in K<sup>+</sup> content of flag leaves of all the cultivars. Highest K<sup>+</sup> content was observed in cv. IR-6 followed by cv. Shaheen Basmati and cv. Super Basmati. Plant growth regulators treated plants increased  $K^+$  content in the ranking ABA>BA>CCC under salt stress. Lowest K<sup>+</sup> content were recorded with untreated plants of cv. Super Basmati under salt stress (Table 2).

NaCl stress significantly increased the Cl<sup>-</sup> content in flag leaves of all the cultivars. Rice cv. Super Basmati accumulated the highest Cl<sup>-</sup> content in flag leaf. ABA, BA and CCC treatment also increased the Cl<sup>-</sup> content in flag leaves, but the magnitude of increase was lower in growth regulators treatments as compared to untreated salt stressed plants, ABA treated showed least increase. Minimum accumulation of Cl<sup>-</sup> was observed in cv. IR-6 with ABA treatment. Interaction between salinity level and growth regulators were non significant for Cl<sup>-</sup> level in flag leaf of all the cultivars (Table 2).

	0		Na <sup>+</sup> (mg/g)		/	
	Super	Basmati		Basmati	IF	R-6
Treatment	So	<b>S</b> 1	So	<b>S</b> 1	So	S1
Control	4.63 d	31.53 a	4.26 b	24.49 a	4.43 c	27.16 a
ABA	3.75 d	27.88 с	3.69 b	24.04 a	3.69 c	21.48 b
BA	4.06 d	29.48 b	4.29 b	26.39 a	4.07 c	23.32 b
CCC	3.94 d	28.38 bc	3.91 b	25.05 a	3.97 c	22.26 b
LSD(0.01)	LSD (0.0	(15) = 1.32	LSD (0.0	(11) = 6.50	LSD (0.0	(11) = 2.63
			$\mathbf{K}^{+}(\mathbf{mg/g})$			
Treatment	So	S1	So	<b>S</b> 1	So	S1
Control	62.09 a	28.93 c	64.57 a	32.05 c	67.24 a	33.63 d
ABA	63.05 a	37.33 b	65.31 a	40.29 b	69.03 a	47.21 b
BA	64.93 a	34.91 bc	64.90 a	39.32 b	70.79 a	43.21 bc
CCC	62.71 a	34.45 bc	62.74 a	36.35 bc	67.42 a	41.65 c
LSD(0.01)	6.	.98	7.04		5.	15
			Cl <sup>-</sup> (mg/g)			
Treatment	So	S1	So	S1	So	S1
			2.97 b	25.88 a		25.13 a
Control	<u>3.03 c</u>	28.17 a			2.92 c	
ABA	2.71 c	25.34 b	2.77 b	23.73 a	2.60 c	22.37 b
BA	2.91 c	26.70 ab	2.90 b	25.30 a	2.94 c	23.62 ab
CCC	2.76 c	25.61 ab	2.82 b	25.00 a	2.92 c	22.66 ab
$\frac{\text{LSD}(0.01)}{\text{S}_{2} = 0.34 \text{ dS/m}}$		.62	2.	50	2.	58

 Table. 2. Effect of ABA, BA and CCC seed soaking on the Na<sup>+</sup>, K<sup>+</sup> and Cl<sup>-</sup> concentrations (mg/g DW) on flag leaf of three cultivars of rice at 0.34 dS/m (control) and 5 dS/m NaCl.

 $S_0 = 0.34 \text{ dS/m}; S_1 = 5 \text{ dS/m}$ 

Salinity stress caused a significant reduction in  $Ca^{2+}$  content in flag leaf of all the cultivars. Maximum decrease in  $Ca^{2+}$  content was recorded in cv. Super Basmati as compared to cv. Shaheen Basmati and cv. IR-6 under salt stress. ABA, BA and CCC treatment significantly increased the  $Ca^{2+}$  content in flag leaf of all the cultivars; however cv. IR-6 was more responsive to ABA treatment (Table-4).  $Ca^{2+}$  act as second messenger and involved in stomatal opening acting in consent with ABA (Sanders *et al.* 2002).

Under salt stress one of the mechanisms of salt tolerance is accompanied by selectivity in uptake and accumulation of inorganic ions, mainly Na<sup>+</sup>, K<sup>+</sup> and Cl<sup>-</sup> (Alian *et al.*, 2000). Munns (1993) reported that depression in plant growth may be caused by sodium and chloride toxicity. Increase in growth with contemporary ABA treatment has been reported in saline conditions and was mainly attributed to reduction in Na<sup>+</sup> with elevated ABA levels (Amzallag *et al.*, 1990). Ashraf & O'Leary, (1996) reported that uptake and accumulation of toxic ions such as Na<sup>+</sup> and Cl<sup>-</sup> in crop species including wheat are enhanced under saline condition.

The concentration of Na<sup>+</sup> content was higher in roots than that of flag leaves of all the cultivars. Lowest Na<sup>+</sup> accumulation was recorded with cv. IR-6 in ABA treatment as compared to cv. Shaheen Basmati and cv. Super Basmati under salt stress. Growth regulators decreased Na<sup>+</sup> content of roots in all the cultivars in the ranking ABA>BA>CCC (Table 3).

			Na <sup>+</sup> (mg/g)			
	Super 1	Basmati		Basmati	IR-6	
Treatment	So	<b>S</b> 1	So	<b>S</b> 1	So	<b>S</b> 1
Control	5.40 de	36.92 a	4.78 c	34.40 a	4.40 c	30.35 ab
ABA	4.05 e	30.22 c	4.28 c	28.53 b	3.84 c	24.49 b
BA	6.44 d	33.33 b	5.27 c	30.86 b	4.61 c	27.31 b
CCC	4.76 de	30.49 c	4.73 c	29.42 b	4.27 c	25.38 b
LSD(0.01)	1.	89	2.	65	2.	86
			$\mathbf{K}^{+}(\mathbf{mg/g})$			
Treatment	So	<b>S</b> 1	So	<b>S</b> 1	So	<b>S</b> 1
Control	44.42 c	21.40 f	48.22 b	24.30 c	51.45 c	28.29 f
ABA	47.39 ab	26.36 d	51.29 a	29.26 c	54.37 ab	34.35 d
BA	49.43 a	23.39 ef	49.85 ab	27.71 cd	55.46 a	31.45 c
CCC	46.23 bc	24.36 de	51.11 a	26.27 d	53.32 b	30.06 ef
LSD(0.01)	2.	53	1.91		1.86	
			Cl <sup>-</sup> (mg/g)			
Treatment	So	S1	So	<b>S</b> 1	So	<b>S</b> 1
Control	2.70 d	25.26 a	2.42 d	23.10 a	2.14 d	22.15 a
ABA	2.22 d	21.36 c	2.10 d	19.42 c	1.82 d	18.34 c
BA	2.45 d	23.22 b	2.22 d	21.31 b	2.20 d	20.24 b
CCC	2.32 d	23.36 bc	2.15 d	20.38 bc	2.00 d	19.61 bc
LSD(0.01)	1.	53	1.56		1.48	
$S_0 = 0.34 \text{ dS/m}$	$s_1 = 5 \text{ dS/m}$					

Table 3. Root Na<sup>+</sup>, K<sup>+</sup> and Cl<sup>-</sup> concentrations (mg/g DW) of three cultivars of rice at 0.34 dS/m (control) and 5 dS/m NaCl when the seeds were soaked with solutions of ABA, BA and CCC.

Salt stress decreased K<sup>+</sup> content in root of all the cultivars. Relatively higher K<sup>+</sup> content were recorded in roots of rice than in flag leaves in salt stress with respect to K<sup>+</sup> content. The ranking of varieties to increase  $K^+$  content in roots under salt stress was cv. IR-6>cv. Shaheen Basmati>cv. Super Basmati. ABA, BA and CCC increased significantly K<sup>+</sup> content in roots as compared to untreated salt stressed plants; however magnitude of increase was higher in ABA. Maximum  $K^+$  content was recorded with cv. IR-6 with ABA treatment (Table 3).

Although salt stress caused an increase in root CI<sup>-</sup> content in all the three cultivars. Highest Cl<sup>-</sup> content was recorded with cv. Suepr Basmati as compared to cv. Shaheen Basmati and cv. IR-6. ABA, BA and CCC also increased the Cl<sup>-</sup> content in all the cultivars but the extent of increase was less, however ABA was more effective than BA and CCC. Lowest Cl<sup>-</sup> content was recorded in cv. IR-6 with ABA treatment (Table-3).

Salinity stress significantly decreased the  $Ca^{2+}$  content in roots of rice cultivars; however the concentration of Ca<sup>2+</sup> content was relatively higher in roots than that of flag leaves. Growth regulators increased Ca<sup>2+</sup>content in all the cultivars as compared to untreated salt stressed plants. Maximum Ca2+ content were recorded with cv. IR-6 in ABA treatment (Table 4).

		with solution	ns of ABA, BA	A and CCC.			
		Flag	leaf-Ca <sup>2+</sup> (m	ng/g)			
	Super 1	Basmati	Shaheen	Basmati	IF	R-6	
Treatment	So	<b>S</b> 1	So	<b>S</b> 1	So	<b>S</b> 1	
Control	0.82 a	0.66 a	0.84 a	0.73 a	0.92 b	0.82 b	
ABA	1.70 a	1.00 a	2.21 a	1.80 a	2.62 a	2.22 ab	
BA	2.00 a	0.94 a	2.18 a	1.41 a	2.82 a	1.81 ab	
CCC	1.82 a	0.89 a	2.02 a	1.21 a	2.51 a	1.82 ab	
LSD(0.05)	1.425		1.74		1.5	547	
		Ro	ot-Ca <sup>2+</sup> (mg	/g)			
				-			
Treatment	So	<b>S</b> 1	So	<b>S</b> 1	So	<b>S</b> 1	
Control	1.13 bc	0.87 c	1.60 ab	1.20 b	2.10 bc	1.52 c	
ABA	2.89 ab	2.62 abc	2.98 ab	2.80 ab	3.22 ab	3.00 ab	
BA	3.09 a	2.01 abc	3.61 a	2.20 ab	3.99 a	2.61 abc	
CCC	2.90 ab	1.81 abc	2.90 ab	1.99 ab	3.01 ab	2.40 bc	
LSD(0.05)	1.9	935	2.09		1.	1.47	
		Leaf proli	ne (µ moles	s g <sup>-1</sup> f. wt.)			
Treatment	So	S1	So	<b>S</b> 1	So	<b>S</b> 1	
Control	9.31 b	50.20 a	12.26 b	55.34 a	14.22 c	60.13 a	
ABA	15.32 b	62.14 a	18.21 b	69.22 a	21.14 c	78.12 b	
BA	10.19 b	50.25 a	12.23 b	58.17 a	16.17 c	62.15 ab	
CCC	13.25 b	58.14 a	15.19 b	65.54 a	18.21 c	74.21 ab	
LSD(0.01)	24	.09	16	.47	17	.13	

Table 4. Flag leaf, root  $Ca^{2+}$  (mg/g DW) and leaf proline ( $\mu$  moles g<sup>-1</sup> f. wt.) concentrations of three cultivars of rice at 0.34 dS/m (Control) and 5 dS/m NaCl, when the seeds were soaked with solutions of ABA. BA and CCC.

 $S_0 = 0.34 \text{ dS/m}; S_1 = 5 \text{ dS/m}$ 

Externally supplied Ca<sup>2+</sup> reduces the toxic effects of Na Cl , presumably by facilitating higher K<sup>+</sup>/Na<sup>+</sup> selectivity (Cramer *et al.*, 1987). Gadallah (1999) reported that Kinetin application reduced the harmful effects of salt treatment through a reduction in the accumulation of inorganic ions (Na<sup>+</sup>, Ca<sup>2+</sup>and Cl<sup>-</sup>). Marshner (1995) reported that plant species and even different cultivars within the species differ greatly in their response to salinity. The higher uptake of beneficial mineral elements, such as K<sup>+</sup>, is an important mechanism of salt tolerance in most crop species (Greenway & Munns 1980).

Salt stress caused a significant increase in proline content of rice in all the cultivars. ABA and CCC proved to be more effective in increasing proline content but highly significant increase was due to ABA in flag leaf of rice under salt stress. Rice cv. IR-6 accumulated maximum proline content as compared to cv. Shaheen Basmati and cv. Super Basmati.

Flower & Yeo (1989) reported that accumulation of proline and some other organic solutes associated with stress might serve as a compatible solute in order to maintain the osmotic balance between the cytoplasm and vacuole. The salt tolerant rice variety cv. IR-6 accumulated higher proline than that cv. Shaheen Basmti and cv. Super Basmati which are relatively salt sensitive.

			g leaf area (c			
	Super I	Basmati		Basmati	IF	R-6
Treatment	So	S1	So	S1	So	<b>S</b> 1
Control	40 ab	29 cd	43 ab	31 cd	39 ab	28 cd
ABA	38 b	26 d	41 b	30 d	37 b	27 d
BA	43 a	32 c	46 a	35 c	42 a	31 c
CCC	42 ab	30 cd	44 ab	33 cd	41 a	30 cd
LSD(0.05)	4.	4.15		.12	3.	62
		100	0-grain weigh	t (g)		
Treatment	So	<b>S</b> 1	So	S1	So	S1
Control	18.19 a	16.28 a	18.21 a	17.21 a	19.24 a	18.21 a
ABA	20.25 a	19.15a	21.21a	20.17 a	22.25 a	22.32 a
BA	21.16 a	17.58 a	22.42 a	19.15 a	23.18 a	20.14 a
CCC	20.20 a	16.32 a	20.16 a	18.19 a	22.2 a	20.29 a
LSD(0.05)	9.	11	9.40		9.53	
		Gr	ain yield (Kg/	ha)		
	Super B	Basmati	Shaheen Basmati		IR-6	
Treatment	So	<b>S</b> 1	So	S1	So	S1
Control	13.63 b	4.40 c	14.73 bc	6.50 e	15.52 bc	7.60 e
ABA	15.70 a	5.63 c	17.03 a	8.60 d	18.33 a	10.27 d
BA	13.51 b	5.27 c	14.38 c	8.22 de	15.03 c	9.50 d
CCC	15.20 ab	5.02 c	16.37 ab	7.93 de	17.15 ab	9.22 de
	LSD(0.0	)5) 1.72	LSD(0.0	01) 1.73	LSD(0.0	01)1.76
S = 0.24  dS/m	r c = 5 dc/m	,	,	,		,

Table. 5. Effect of ABA, BA and CCC seed soaking on the Na<sup>+</sup>, K<sup>+</sup> and Cl<sup>-</sup> concentrations (mg/g DW) on flag leaf area, 1000 grain weight and grain yield of three cultivars of rice at 0.34 dS/m (control) and 5dS/m NaCl.

 $S_0 = 0.34 \text{ dS/m}; S_1 = 5 \text{ dS/m}$ 

Flag leaf transport assimilates to spike and developing grain. Flag leaf area was decreased significantly by salinity stress; however seed pre-treatment with BA proved to be effective to overcome the inhibitory effect of salt on flag leaf area, while ABA and CCC had no marked effect on the salt induced decrease in flag leaf area in all the cultivars (ANOVA not shown). Maximum flag leaf area was recorded in cv. Shaheen Basmati with BA seed treatment, while minimum flag leaf area was in cv. Super Basmati with ABA seed treatment (Table-5). Which may record account for ABA induced inhibition of cell division and cell expansion.

Leaf area reduction under saline conditions was recorded by Neuman & Smith, (1997). 1000-grain weight of all the cultivars was significantly (P < 0.01) reduced under salt stress (ANOVA not shown). All the growth regulators increased 1000-grain weight over control. The interaction between growth regulator and salinity level was non significant in all the three cultivars (Table 5).

Salinity stress caused a significant reduction in the grain yield of all the three cultivars (P<0.01). All the growth regulators increased grain yield over untreated salt stressed but such that there was no significant difference with that of control (unstressed) hormone treated plants. Rice cv. Super Basmati had higher grain yield with ABA seed treatment as compared to cv. Shaheen Basmati and cv. IR-6. Grain yield of rice cv. Shaheen Basmati was also increased with growth regulators but the magnitude of increase was less than that of cv. IR-6. Minimum grain yield was recorded with salt sensitive rice cv. Super Basmati (Table 5).

Significant relationship between flag leaf characteristics, grain filling and ultimate grain yield by growth regulators has been reported (Stahli *et al.*, 1995). Our results are in agreement with those of earlier studies (Farida *et al.*, 2003).

It is inferred from the present investigation that cv. IR-6 performed better and had high yield than that of cv. Shaheen Basmati and cv. Super Basmati. The mechanism of salt tolerance in cv. IR-6 relates to the selectivity of ions (less accumulation of toxic ions, Na<sup>+</sup> and Cl<sup>-</sup> and lower rate of transport to flag leaf in contrast greater accumulation of K<sup>+</sup> and Ca<sup>2+</sup> content) and better osmotic adjustment by increase in proline content and greater leaf area under salt stress. These effects were further enhanced by seed priming with ABA and BA. ABA being more effective than BA but both of them were equally effective to increase yield.

#### References

- Alian, A., A. Altman and B. Heuer. 2000. Genotypic difference in salinity and water stress tolerance of fresh market tomato cultivars. *Plant. Sci.*, 152: 59-65.
- Amzallag, G.N., H.R. Lerner, and A. Poljakoff-Mayber. 1990. Exogenous ABA as a modulator of the response of sorghum to high salinity. J. Exp. Bot., 41: 1529-1534.
- Arfan, M, H.R. Athar, and M. Ashraf. 2007. Does exogenous application of salicylic acid through the rooting medium modulate growth and photosynthetic capacity in two differently adapted spring wheat cultivars under salt stress? J. Plant Physiol. 6 (4): 685-694.
- Ashraf, M. and J.W.O. Leary. 1996. Responses of some newly developed salt-tolerant genotypes of spring wheat to salt stress:II. Water relation and photosynthetic capacity. *Acta. Bot. Neerl.*, 45: 29-39.
- Ashraf, M. and M.R. Foolad. 2005. Pre-sowing seed treatment-a shotgun approach to improve germination, plant growth, and crop yield under saline and non-saline conditions. *Adv. Agron.*, 88: 223-271.
- Ashraf, M. and M.R. Foolad. 2007. Roles of glycinebetaine and proline in improving plant abiotic stress resistance. *Env. Exp. Bot.*, 59(2): 206-216.
- Bates, L.S., R.P Waldren and L.D Teare. 1973. Rapid determination of free proline for water stress studies. *Plant Soil*, 39: 205-207
- Beaudoin, N., C. Serizet, F. Gosti, and J. Giraudat, 2000. Interactions between abscisic acid and ethylene signiling cascades. *Plant Cell.*, 12: 1103-1116.
- Chapman, H.D. and P.F. Pratt. 1961. Methods of analysis for soil, plant and water: Division of Agriculture Science. *University of California: Riverside. CA*: 1188 pp.
- Cramer, G. R. J. Lyreh, A. Lauchli, and E. Epstein. 1987. Influence of Na<sup>+,</sup> K<sup>+</sup> and Ca<sup>2+</sup> in roots of salt stressed cotton seedlings. *Plant Physiol.*, 83: 510-516.
- Farida, M.S., A.R. Sakhabutdinova, M.V. Bezrukova, R.A. Fatkhutdinova, D.R. Fatkhutdinova. 2003. Changes in the hormonal status of wheat seedlings induced by salicylic acid and salinity. *Plant. Sci.*, 164, 317-322.
- Flower, T.J., and A.R. Yeo. 1989. Effects of salinity on plant growth and crop yield. In: Cherry J. Environmental stress in Plants. Biochemical and Physiological mechanisms. *Springer. Verlan*, pp: 101-119.
- Gadallah, M.A.A. 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.
- Greenway, H, and R. Munns. 1980. Mechanism of salt tolerance in non-halophytes. *Annu. Rev. Plant Physiol.*, 31: 149-190.
- Hare, P.D., W.A. Cress, and J. Van Staden. 1997. The involvement of Cytokinin in plant responses to environmental stress. *Plant Growth Regul.*, 23: 79-103.
- Hutchison, C.E, and J.J. Kieber. 2002. Cytokinin signiling in Arabidopsis. Plant Cell, 14: 47-59.
- Iqbal, M, and M. Ashraf. 2005. Pre-sowing seed treatment with cytokinin and its effect on growth, photosynthetic rate, ionic levels and yield of two wheat cultivars differing in salt tolerance. J. Integ. Plant Biol., 47: 1315-1325.

- Kader, M.A and S. Linberg. 2005. Uptake of Sodium in protoplasts of salt-sensitive and salt tolerant cultivars of rice, *Oryza sativa* L. determined by the florescent dye SBFI. *J. Expl. Bot.*, 56: 3149-3158.
- Kaur, J, O.S. Singh and N. Arora. 2002. Kinetin like role of TDZ (thidiazuron) in salinity amelioration in wheat(*Triticum aestivum*). J. Res. Punjab Agr. Univ., 39: 82-84.
- Khan, MA, B. Gul and DJ. Weber. 2002. Improving seed germination of Salicornia rubra (Chenopodiaceae) under saline conditions using germination-regulating chemicals. Western North Am. Nat., 62: 101-105.
- Lefevre, I, E. Gratia and S. Luttus. 2001. Discrimination between the ionic and osmotic components of salt stress in relation to free polyamine level in rice (*Oryza sativa*). *Plant Sci.*, 161: 943-952.
- Marshner, H. 1995. Saline Soils. In: Mineral nutrition of higher plants. *Academic Press, New York.*, pp: 657-680.
- Munns, R. 1993. Physiological processes limiting plant growth in saline soils: Some dogmas and hypothesis. *Plant Cell Environ.*, 16: 1107-1114.
- Munns, R. 2005. Gene and salt tolerance: bringing them together. New Phytol., 167: 645-663.
- Neuman, D., and B.A. Smith. 1997. The influence of leaf water status and ABA on growth and stomata of *Phaseolus* seedling with hypoxic root. *J. Exp. Bot.*, 42:1499.1506.
- Rains, D.W and E. Epstein. 1967. Sodium absorption by barley roots: Its mediation by mechanism 2 of alkali cation transport. *Plant. Physiology*. 42: 319-323.
- Raza, S. H., H. R., Athar, and M. Ashraf. 2006. Influence of exogenously applied glycinebetaine on the photosynthetic capacity of two differently adapted wheat cultivars under salt stress. *Pak. J. Bot.*, 38: 341-351.
- Raza, S.H., H.R. Athar, M. Ashraf, and A. Hameed. 2007. GB-induced modulation of antioxidant enzymes activities and ion accumulation in two wheat cultivars differing in salt tolerance. *Env. Exp. Bot.* doi:10.1016/j.envexpbot.2006.12.009.
- Rhoades, J.D, A. Kandiah, and A.M. Mashali. 1992. The use of saline water for crop production (FAO irrigation and drainage paper 48). FAO of the United Nations, *Rome*.
- Richards, L.A. 1954. Diagnosis and improvement of saline and alkali soils. USDA Agric. Hand book 60. Washington. D. C.
- Sanders, D., J. Pelloux, C. Bromlac, and J.F. Harper. 2002. Calcium at the crossroads of signaling. *Plant Cell*, 14(suppl.) 5401-5417.
- Stahli, D, D. Pasrission-Fabert, A. Blouet, and A. Guckert. 1995. Contribution of the wheat (*Triticum aestivum*) flag leaf to grain yield in response to plant growth regulator. *Plant. Growth. Reg.*, 16: 293-297.
- Takei K., T. Takahashi, T. Sugi yama, H. Sakakibara. 2002. Multiples routes communicating nitrogen availability from roots to shoots, a signal transduction pathway mediated by Cytokinin. J. Exp. Bot., 53: 971-977.
- Tester, M and R. Devenport. 2003. Na<sup>+</sup> tolerance and Na<sup>+</sup> transport in higher plants. *Annal. Bot.*, 91: 503-527.
- Ueda, A, M. Kanechi, Y. Uno, N. Inagaki. 2003. Photosynthetic limitations of a halophyte sea aster (Aster tripolium L.) under water stress and NaCl stress. J. Plant Res., 116: 65-70.
- Waseem, M., H. R. Athar, and M. Ashraf. 2006. Effect of salicylic acid applied through rooting medium on drought tolerance of wheat. *Pak. J. Bot.*, 38(4): 1127-1136.
- Yeo, A.R, S.J. Caporn. and T.J. Flowers. 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.
- Zhu, J.K., J.P. Liu and L.M. Xiong. 1998. Genetic analysis of salt tolerance in Arabidopsis: evidence for a critical role of potassium nutrition. *Plant Cell*, 10: 1181-1191.
- Zidan, I, H., Azaizeh and P.M. Neumann. 1990. Does salinity reduce growth in maize root epidermal cell by inhibiting their cell dividing capacity. *Plant Physiol.*, 93: 7-11.

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