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Wheat seed priming in relation to salt tolerance: growth, yield and levels of free salicylic acid and polyamines

Muhammad Iqbal* & Muhammad Ashraf

*Department of Botany, University of Agriculture, Faisalabad 38040, Pakistan (*e-mail: iqbaluaf@yahoo.com)*

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The exogenous application of cytokinins through seed pre-treatment is reported to be involved in plant tolerance to salt stress. It was hypothesized that cytokinins could regulate the salinity-induced changes in hormonal balance for adequate physiological adaptation to stressful environments in plants. Thus, the present studies aimed to assess the effects of pre-sowing seed treatment with varying levels (100, 150 and 200 mg l^{-1}) of cytokinins, namely kinetin (Kin) and benzylaminopurine (BAP), on growth, yield, and leaves' endogenous levels of salicylic acid and polyamines (putrescine, spermidine and spermine) in two spring wheat (*Triticum aestivum*) cultivars, MH-97 (salt intolerant) and Inqlab-91 (salt tolerant). The primed and non-primed seeds were sown in a field in which NaCl salinity of 15 dS m^{-1} was developed. The experiments were conducted during the years 2002 and 2003. Of the different priming agents, Kin was very effective, particularly in improving growth and grain yield in both cultivars. Priming with mild concentration (100 mg l^{-1}) of Kin in the salt intolerant and moderate concentrations (150 mg l^{-1}) of BAP and Kin in the tolerant cultivar increased leaf free salicylic acid under saline conditions. BAP priming increased leaf free polyamines (Spermidine and Spermine) in both cultivars under salt stress. Plants of the salt tolerant cultivar raised from non-primed seeds accumulated more putrescine in the leaves as compared with plants raised from primed seeds. In conclusion, seed priming with moderate concentration of Kin showed a consistent beneficial effect on growth and grain yield in both cultivars under salt stress. The mechanism by which Kin-priming induced salt tolerance in wheat plants remains unclear.

Key words: growth, hormonal balance, kinetin, physiology, salt tolerance, seed priming, *Triticum aestivum*

Introduction

Among different strategies to cope with salinity issues, seed priming (pre-sowing seed treatment) is an easy, low cost, and low risk technique and this approach has recently been used to overcome the salinity problem in agricultural lands. Seed priming with optimal concentrations of

cytokinins has been shown to be beneficial to germination, growth, and yield of some crop species grown under saline conditions (Angrish *et al*. 2001, Kaur *et al*. 2002). The beneficial effects of seed priming with phytohormones on growth and grain yield are probably due to their involvement in the increased rate of translocation of photosynthates from leaves to grains (Ray & Choudhuri 1981, Kiseleva & Kaminskaya 2002).

Cytokinins (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, Rashotte *et al*. 2005). The quantitative and qualitative responses of plants to different CKs may also differ considerably. Cytokinins decline in plant tissues in response to salinity (Zhang & Zhang 1994, Wang *et al*. 2001). Therefore, the possible involvement of exogenously applied CKs in response to adverse environmental conditions has been suggested by Hare *et al*. (1997) and Brault and Maldiney (1999).

Stress tolerance of plants is also correlated with their capacity to enhance the synthesis of polyamines (PAs) upon encountering the stress (Kasukabe *et al*. 2004). The molecular genetic analyses have shown that altered PA levels have profound effects on plant growth and development (Watson & Malmberg 1998). Which of the three PAs (putrescine, Put; spermidine, Spd; or spermine, Spm) plays central roles in stress responses of plants may depend on the plant species and the types of stress (Kasukabe *et al*. 2004, Iqbal & Ashraf 2005). Similarly, salicylic acid (SA), an endogenous growth regulator of phenolic nature also plays an important role in the plant response to adverse environmental conditions, such as salt stress (Borsani *et al*. 2001, Tari *et al*. 2002). Recently, it was shown that pre-sowing seed treatment with SA did not influence endogenous cytokinin contents (Sakhabutdinova *et al*. 2003). However, to our knowledge, it has not been reported whether pre-sowing seed treatment with cytokinins may alter the endogenous levels of SA and/or PAs in salt stressed wheat plants. In addition, although priming-induced salt tolerance has been reported in some crops, knowledge about physiological or biochemical basis of priming-induced beneficial effects under stressful environments is scarce. Plant hormones could be the possible factors responsible for priming-induced salt tolerance. We hypothesized that cytokinins could regulate the salinity-induced changes in hormonal balance and thus induce adequate physiological adaptation in plants.

The objective of our studies was to determine whether pre-sowing seed treatment with cytokinins could alter the endogenous SA and PAs (Put, Spd, and Spm) concentrations in wheat plants under salt stress. In addition, it was assessed whether and how the changes in SA and PAs could enhance growth and yield processes in salt stressed plants. Another aim of the present studies was to determine to what extent CKs as priming agents could induce salt tolerance in genetically diverse wheat plants.

Material and methods

The seeds of two spring wheat cultivars, MH-97 (salt intolerant) and Inqlab-91 (salt tolerant) (Hollington 2000), were obtained from the Wheat Section, Ayub Agricultural Research Institute, Faisalabad, Pakistan. Solutions of 100, 150 and 200 mg l^{-1} of kinetin (Kin) and benzyl aminopurine (BAP) were used for seed priming. Distilled water (DW) was used for hydropriming. Healthy wheat seeds (17 g for each treatment) were primed separately in 100 ml of solutions of Kin and BAP as well as in DW for 12 h at room temperature in plastic cups (250 ml). After pre-soaking, the seeds were surface dried on filter paper and then allowed to air-dry for 12 h at room temperature. The air-dried seeds (primed seeds) as well as untreated seeds (non-primed seeds) were used for field experiments.

Field experiments were conducted during winter 2002–2003 at the Botanic Garden, University of Agriculture, Faisalabad (latitude 31°30´N, longitude 73°10´E, altitude 213 m), where the average photosynthetically active radiation (PAR) of the entire growth period was 1098 μmol m^{-2} s^{-1}, and maximum and minimum relative humidity 79% and 32%, respectively. The average maximum and minimum temperatures were 28 °C and 12 °C, respectively. Eight

blocks having lengths, widths and depths of 732, 137 and 46 cm, respectively, and lined with polythene sheets were filled with thoroughly mixed sandy loam soil (pH = 7.56; electrical conductivity of the saturation paste extract = 2.84 dS m^{-1}; saturation percentage = 25.5). In order to develop 15 dS m^{-1} NaCl salinity, the calculated amount of NaCl was dissolved in water required for complete saturation of the soil in four blocks (replicates). Thus, the soil was completely saturated with salt solution (150 mM) so as to homogenize soil salinity. The other four blocks served as control (2.84 dS m^{-1}). Each block was split into two plots. After three weeks, when the soil moisture content (12%–14%) was suitable for germination, the primed and non-primed seeds of each treatment of either wheat cultivar were sown randomly in rows in separate plots keeping row to row spacing at 15 cm. The electrical conductivity of the soil (EC_e) was checked periodically and maintained. In the second year, before sowing, the EC_e was again maintained by adding a calculated amount of NaCl in solution form to rectify the fluctuations in salinity within plots. The plants were irrigated carefully with tap water (EC_w = 0.65 dS m^{-1}) using plastic pipes during entire experiments so as to minimize dilution in salinity at irrigation points. The experiments were laid out in a split-split plot design.

At the boot stage, 12 plants (3 plants per replicate) from each treatment (control or salt) were uprooted and washed with DW. After drying with filter paper, roots were carefully removed. The samples were dried in an oven at 65 °C for two weeks and shoot dry weights recorded. At maturity, grain yield $plant^{-1}$ (g) was recorded.

Determination of salicylic acid

Salicylic acid was extracted and purified following the procedure of Enyedi *et al*. (1992) and Seskar *et al*. (1998) with some modifications. Fresh leaves already frozen and stored in 30 ml of 80% cold (–70 °C) aqueous methanol (MeOH) (4:1 v/v) supplemented with 20 mg l^{-1} butylated hydroxytoluene (BHT) were ground in a mortar, using aqueous 80% MeOH-BHT as an extracting solvent. To check recoveries during extraction and purification, 300 ng naphthaleneacetic acid (NAA) was added as an internal standard before homogenization. The homogenate was vortexed for 10 min and filtered with suction through Whatman No. 42 paper. The residues in the flask and on the filter paper were rinsed three times with 10-ml aliquots of MeOH-BHT and two times with 100% MeOH. The extracts were combined, mixed and used for SA purification. The extract was concentrated to an aqueous residue by rotary flask evaporation (RFE) at 40 °C.

Sublimation of SA was prevented by the addition of 0.2 M sodium hydroxide (Verberne *et al*. 2002). The aqueous fraction was transferred to 50-ml polypropylene centrifuge tubes. The flask, used for RFE, was rinsed with 10 ml of *n*-hexane. Partitioning with *n*-hexane (pH 8) and then with *n*-hexane (pH 3) was used for SA clean-up. The pH was then adjusted to 2.8 and the samples were centrifuged for 15 min at 13 000 *g* to remove any precipitate. The supernatant fractions were decanted into clean centrifuge tubes and partitioned thrice against 10-ml portions of cold diethylether-BHT. Each 10-ml portion of ether was partitioned, in turn, against a 10-ml portion of 1 mM HCl. The same 10 ml of 1 mM HCl was used for all three 10-ml portions of ether. All three ether fractions were combined and evaporated by RFE to dryness *in vacuo*. The residue was immediately dissolved into 500 μl of 80% ice-cold MeOH-BHT in a 1.5-ml Eppendorf centrifuge tube. These samples were kept overnight at –70 °C and then centrifuged (25 000 *g*) for 10 min at –10 °C. Standards were also prepared following the same procedure. The supernatant was filtered and subjected to HPLC (high performance liquid chromatography) analysis.

Analysis of SA was performed with HPLC (Sykam GmbH, Kleinostheim, Germany) equipped with S-1121 dual piston solvent delivery system and S-3210 UV/VIS diode array detector. The elution system consisted of 100% methanol: 1% acetic acid (52:48 v/v) as solvent; run isocratically with a flow rate of 1.10 ml min^{-1} at 40 °C. Twenty microliters of filtered extracts were injected into a Hypersil ODS reverse-phase (C_{18}) column (4.6 × 250 mm, 5-μm particle size: Thermo Hypersil GmbH, Germany) fitted with a C_{18} guard column. Detection of SA was performed at 280 nm by co-chromatography

with authentic standards. The peak areas were recorded and calculated by a computer with SRI peak simple chromatography data acquisition and integration software (SRI Instruments, Torrance, California, USA). Quantification was done using the standard addition method by spiking control plant samples with SA solutions (ranging from 10 to 200 ng ml^{-1}). The recoveries for SA were calculated and the values of SA were corrected on internal standard.

Determination of polyamines

Fresh leaves (3rd leaf from top) were harvested from plants of each treatment. Fresh leaves (2.0 g) were finely chopped in 20 ml of cold 5% aqueous perchloric acid and were stored at –70 or at –20 °C.

For PAs extraction and HPLC analysis, benzoylation method was performed as described previously (Flores & Galston 1982) with some modifications. Leaf tissues were homogenized in a mortar in 20 ml of cold 5% perchloric acid containing 200 nmol of 1,6-hexanediamine as an internal standard. The homogenates were incubated at 4 °C for 30 min and then centrifuged at 13 000 *g* for 20 min. Aliquots of 1 ml of supernatant were added to 1 ml of 4 M NaOH with 10 μl of benzoyl chloride. The mixture was vigorously vortexed for 30 s and then incubated for 30 min at room temperature. The reaction was terminated by adding 2 ml of saturated NaCl. The benzoyl-polyamines were extracted with 3 ml of cold diethyl ether for 30 min. Then the samples were centrifuged at 2500 *g* for 5 min and the ether phase was collected and dried under N_2 flow. The residues were redissolved in 500 μl of methanol in a 1.5-ml Eppendorf centrifuge tube. Polyamine standards (Sigma Chemical Co., USA) were prepared similarly to plant samples. These samples were filtered and stored at –20 °C for HPLC analysis.

The polyamine contents were analyzed by HPLC (Sykam GmbH, Kleinostheim, Germany) equipped with a S-1121 dual piston solvent deliv-

Table 1. Mean squares from analysis of variance of data for growth, grain yield and hormonal contents of two cultivars of spring wheat raised from seeds primed with kinetin or benzylaminopurine (cytokinins). *, **, *** = significant at 0.05, 0.01, and 0.001 confidence levels, respectively. ns = non-significant, SA = salicylic acid, Put = putrescine, Spd = spermidine, Spm = spermine.

Source of variation	d.f.	Shoot dry weight	Grain yield plant^{-1}	SA
Salinity (S)	1	136.4***	39.93**	287983.6ns
Main Plot Error	3	0.323	0.387	31976.2
Varieties (V)	1	24.41***	0.677***	905400.4***
V × S	1	7.440**	0.166**	1142036.5**
Subplot Error	6	0.319	0.009	923.4
Treatments (T)	7	2.118***	2.261***	2041012.1**
T × S	7	0.345ns	0.129***	3971840.9**
T × V	7	0.742***	0.214***	4079569.6**
T × V × S	7	0.641**	0.098**	779616.2***
Error	84	0.179	0.029	26345.9
		Put	Spd	Spm
Salinity (S)	1	7704.7***	543145.1***	6256.6***
Main Plot Error	3	5.80	286.2	2.76
Varieties (V)	1	6.18ns	28324.9**	219.5***
V × S	1	307.1**	6304.8*	988.2***
Subplot Error	6	8.77	927.4	0.299
Treatments (T)	7	907.3***	61094.9***	971.8***
T × S	7	658.5***	31283.4***	1496.6***
T × V	7	1067.7***	23882.8***	823.1***
T × V × S	7	648.1***	27705.8***	304.1***
Error	84	2.66	488.2	19.70

ery system and S-3210 UV/VIS detector. The elution system consisted of methanol: water (65:35 v/v) as solvent, run isocratically with a flow rate of 0.70 ml min^{-1} at 50 °C. Twenty microliters of benzoylated extracts were injected into a Hypersil ODS reverse-phase (C_{18}) column (4.6 × 250 mm, 5-μm particle size: Thermo Hypersil GmbH, Germany) and detected at 254 nm. The peak areas were recorded and calculated by a computer with SRI peak simple chromatography data acquisition and integration software (SRI Instruments, Torrance, California, USA).

Data analysis

First the data were analysed using the chi-squared test to determine whether the variances were homogeneous across experiments. In all cases variances were homogeneous and data from the repeated experiments were pooled. Data were analysed using analysis of variance (ANOVA) performed using a COSTAT computer package (CoHort Software, 2003, Monterey, California). The comparisons of means were done by COSTAT computer package, using Duncan's New Multiple Range (DMR) test.

Results

Salt stress caused a significant reduction in shoot dry biomass of plants of both cultivars ($P \leq 0.01$; Table 1). Although all priming agents affected shoot dry biomass similarly, plants derived from seeds treated with moderate concentration (150 mg l^{-1}) of Kin in the salt intolerant and all concentrations of Kin as well as distilled water (hydropriming) in the salt tolerant cultivar had greater shoot dry biomass when compared with untreated control under salt stress (Table 2).

Grain yield significantly decreased under salt stress in both cultivars ($P \leq 0.01$; Table 1). However, Kin pre-treatment significantly improved grain yield in both cultivars ($P \leq 0.01$). In

Table 2. Growth and grain yield (g $plant^{-1}$) attributes of two cultivars of spring wheat at 2.84 dS m^{-1} (control) or 15 dS m^{-1} NaCl (salt) when the plants were raised from seeds primed with cytokinins (n = 4). BAP, benzyladenine (benzyl aminopurine); Kin, kinetin. Means with the same letters do not differ significantly at $P \leq 0.05$ within each attribute.

Pre-sowing seed treatments		MH-97		Inqlab-91	
		Control	Salt	Control	Salt
Shoot dry biomass					
BAP	(100 mg l^{-1})	4.24^{fg}	3.07^{l}	6.69^{ab}	3.39^{hijkl}
	(150 mg l^{-1})	4.51^{f}	3.03^{l}	6.84^{a}	3.36^{ijkl}
	(200 mg l^{-1})	4.21^{fg}	2.917^{l}	5.29^{e}	3.39^{hijkl}
Kin	(100 mg l^{-1})	5.37^{de}	3.32^{jkl}	6.52^{abc}	3.88^{fghijk}
	(150 mg l^{-1})	5.96^{cd}	4.07^{fgh}	5.98^{cd}	4.03^{fghi}
	(200 mg l^{-1})	5.24^{e}	3.62^{ghijkl}	6.18^{abc}	4.25^{fg}
Distilled Water		5.17^{e}	3.39^{hijkl}	6.27^{abc}	3.99^{fghij}
Untreated		4.30^{fg}	2.96^{l}	6.09^{bc}	3.22^{kl}
LSD 5% = 0.595					
Grain yield					
BAP	(100 mg l^{-1})	3.19^{gh}	2.22^{jkl}	3.60^{ef}	2.27^{ijk}
	(150 mg l^{-1})	3.05^{h}	1.99^{l}	3.76^{de}	2.33^{ijk}
	(200 mg l^{-1})	3.21^{gh}	2.36^{ijk}	3.98^{bcd}	2.46^{ij}
Kin	(100 mg l^{-1})	3.97^{bcd}	3.24^{gh}	3.98^{bcd}	3.11^{h}
	(150 mg l^{-1})	4.11^{abc}	3.11^{h}	3.93^{cd}	3.17^{gh}
	(200 mg l^{-1})	4.29^{a}	3.11^{h}	4.21^{ab}	3.00^{h}
Distilled Water		3.60^{ef}	2.35^{ijk}	3.58^{ef}	2.52^{i}
Untreated		3.43^{fg}	2.12^{kl}	3.57^{ef}	2.25^{ijkl}
LSD 5% = 0.239					

contrast, seed treatment with 150 mg l^{-1} BAP showed adverse effects on grain yield in the salt intolerant cultivar under salt stress. Overall, the effect of priming agents on grain yield was similar on both cultivars under saline conditions.

Salt stress did not affect leaf free SA concentrations (Table 1). However, different priming agents differed significantly in altering free SA concentration in leaves of both cultivars under both saline and non-saline conditions ($P \leq 0.001$). Pre-sowing treatment with 100 mg l^{-1} of Kin in the salt intolerant and 150 mg l^{-1} of Kin and BAP in the salt tolerant cultivar was the most effective in increasing leaf free SA concentrations under saline conditions (Table 3). Overall, the improvement in leaf SA levels due to different priming agents was more in the salt intolerant than in the tolerant cultivar under saline conditions.

Salinity significantly affected leaf Put concentration of both cultivars. However, the cultivars did not differ significantly (Table 1). The priming agents differed significantly in altering the Put concentrations in leaves of both wheat cultivars ($P \leq 0.001$). Generally, salt stress caused an increase in Put levels in the plants of the tolerant cultivar (Table 4). All priming agents lowered leaf free Put levels in plants of the tolerant cultivar as compared with those in plants raised from non-primed seeds under saline conditions. In contrast, all concentrations of BAP were effective in increasing leaves free Put concentration in the salt intolerant cultivar under saline conditions. Overall, the improvement in leaf free Put due to priming agents was greater in the salt intolerant than in the tolerant cultivar under saline conditions.

Different priming agents significantly altered leaf free Spd concentration in both cultivars under both saline and non-saline conditions ($P \leq 0.001$; Table 1). BAP was found to be a very effective priming agent in improving leaf Spd concentration in both cultivars under saline conditions (Table 4). Priming with moderate concentration of Kin decreased leaf free Spd in the salt intolerant and increased in the tolerant cultivar under salt stress.

Salt stress imposed on plants by the growth medium significantly altered the leaf Spm concentration in both cultivars ($P \leq 0.001$; Table 1). However, some priming agents improved leaf free Spm concentration in both cultivars (Table 4). The mild concentration of BAP was the most effective in increasing leaf free Spm concentrations under saline conditions in both cultivars. Cultivars also responded differently with respect to Spm accumulation under salt stress. For instance, salt stress promoted the accumulation of Spm in the leaves of plants raised from non-primed seeds of the salt intolerant cultivar, whereas the reverse was true for the salt tolerant cultivar.

Discussion

Growth regulators have been proposed to alleviate the salinity-induced dormancy in some plants (Khan & Rizvi 1994, Khan & Ungar 1997). It is

Table 3. Leaf free salicylic acid (ng g^{-1} fresh wt.) of two cultivars of spring wheat at 2.84 dS m^{-1} (control) or 15 dS m^{-1} NaCl (salt) when the plants were raised from seeds primed with cytokinins ($n = 4$). BAP, benzyladenine (benzyl aminopurine); Kin = kinetin. Means with the same letters do not differ significantly at $P \leq 0.05$ within each attribute.

Pre-sowing seed treatments		MH-97		Inqlab-91	
		Control	Salt	Control	Salt
BAP	(100 mg l^{-1})	2667.4^{fg}	2142.6^{ij}	1803.2^{lm}	1516.4^{no}
	(150 mg l^{-1})	1109.0^{qr}	2956.4^{de}	1396.1^{o}	2910.3^{def}
	(200 mg l^{-1})	3246.4^{c}	2433.8^{gh}	3273.9^{c}	1478.6^{no}
Kin	(100 mg l^{-1})	3647.7^{b}	3882.1^{a}	1367.3^{op}	2070.5^{jk}
	(150 mg l^{-1})	1301.8^{opq}	2846.7^{def}	1950.2^{jkl}	2796.6^{ef}
	(200 mg l^{-1})	1852.6^{klm}	2803.4^{ef}	2548.9^{gh}	2037.2^{jkl}
Distilled Water		2331.4^{hi}	1147.2^{pqr}	1832.6^{klm}	1659.51^{mn}
Untreated		919.6^{r}	1133.8^{pqr}	3069.3^{cd}	2019.9^{jkl}
LSD 5% = 228.2					

possible that under high salt concentrations, the amount of naturally occurring cytokinins may be suppressed and pre-sowing treatment induces their synthesis under saline conditions. In addition, the quantitative and qualitative responses of plants to different cytokinins may differ considerably under saline conditions as had been observed in the present studies. Generally, pre-sowing seed treatment with different cytokinins alleviated the inhibitory effect of salt stress on both wheat cultivars; Kin was the more effective of the priming agents in both cultivars under saline conditions. It was earlier reported that salinity stress can reduce CKs export from the root to the shoot in many plants (Rao & Rao 1985, Kuiper *et al*. 1988, 1990). However, an adequate cytokinin supply is essential for normal plant development (Mok 1994, Schmülling *et al*. 1997, Takei *et al*. 2002); it can explain why exogenous application of kinetin had overcome the effects of salinity stress on the growth and grain yield of wheat cultivars under saline conditions. These results are in agreement with those of some earlier studies (Salama & Awadalla 1987, Farida *et al*. 2003). An improvement in wheat productivity (grain yield) due to increased rates of translocation of photosynthates from leaves to grains caused by hormone pre-treatment has been also suggested by Ray and Choudhuri (1981) and Aldesuquy and Ibrahim (2001).

Table 4. Leaf free polyamines (μg g^{-1} fresh wt.) of two cultivars of spring wheat at 2.84 dS m^{-1} (control) or 15 dS m^{-1} NaCl (salt) when the plants were raised from seeds primed with cytokinins (n = 4). BAP, benzyladenine (benzyl aminopurine); Kin, kinetin. Means with the same letters do not differ significantly at $P \leq 0.05$ within each attribute.

Pre-sowing seed treatments		MH-97		Inqlab-91	
		Control	Salt	Control	Salt
Putrescine (Put)					
BAP	(100 mg l^{-1})	10.05^{mn}	41.425^{c}	7.74^{no}	21.33^{h}
	(150 mg l^{-1})	24.72^{g}	31.42^{e}	17.155^{ij}	16.385^{j}
	(200 mg l^{-1})	33.13^{e}	56.965^{b}	9.67^{mn}	35.51^{d}
Kin	(100 mg l^{-1})	7.615^{no}	25.645^{fg}	13.87^{k}	13.9^{k}
	(150 mg l^{-1})	15.015^{jk}	16.665^{ij}	11.32^{lm}	16.69^{ij}
	(200 mg l^{-1})	7.93^{no}	10.045^{mn}	6.705^{o}	27.42^{f}
Distilled Water		10.72^{m}	16.48^{j}	13.4^{kl}	26.6^{fg}
Untreated		9.175^{mno}	19.06^{hi}	10.195^{mn}	81.14^{a}
LSD 5% = 2.29					
Spermidine (Spd)					
BAP	(100 mg l^{-1})	201.59^{e}	175.08^{ef}	142.645^{fghi}	301.245^{d}
	(150 mg l^{-1})	115.495^{hij}	436.925^{a}	91.61^{jk}	335.28^{bc}
	(200 mg l^{-1})	136.77^{ghi}	413.735^{a}	193.925^{e}	364.19^{b}
Kin	(100 mg l^{-1})	74.435^{kl}	114.065^{ij}	79.37^{kl}	148.6^{fghi}
	(150 mg l^{-1})	64.72^{kl}	65.345^{kl}	29.43^{m}	355.365^{bc}
	(200 mg l^{-1})	54.975^{lm}	125.615^{ghi}	157.195^{fg}	327.88^{cd}
Distilled Water		89.09^{jkl}	145.585^{fghi}	144.445^{fghi}	136.18^{ghi}
Untreated		151.5^{fgh}	342.185^{bc}	175.675^{ef}	200.1^{e}
LSD 5% = 31.07					
Spermine (Spm)					
BAP	(100 mg l^{-1})	10.6^{hij}	84.455^{a}	5.935^{j}	42.595^{b}
	(150 mg l^{-1})	18.325^{efg}	37.72^{b}	5.99^{j}	11.495^{fghij}
	(200 mg l^{-1})	18.955^{ef}	8.43^{ij}	12.725^{fghij}	22.03^{de}
Kin	(100 mg l^{-1})	15.935^{efgh}	38.315^{b}	5.84j	27.06^{cd}
	(150 mg l^{-1})	11.83^{fghij}	16.675^{efgh}	16.75^{efgh}	16.765^{efgh}
	(200 mg l^{-1})	7.52^{ij}	40.86^{b}	18.175^{efg}	29.19^{c}
Distilled Water		5.635^{j}	7.4^{ij}	14.455^{fghi}	11.38^{ghij}
Untreated		6.865j	18.13^{efg}	39.3^{b}	26.06^{cd}
LSD 5% = 6.241					

Salicylic acid is already shown to increase the resistance of plants to salinity (Shakirova *et al*. 2003) and other abiotic stresses (Senaratna *et al*. 2000, Bezrukova *et al*. 2001). In our studies, pre-sowing treatment with mild and moderate concentration of Kin that increased grain yield in the salt intolerant and tolerant cultivars also altered leaf SA concentration in both the cultivars under salt stress. For example, mild concentration of Kin was the most effective pre-treatment that boosted SA concentration in the leaves of the salt intolerant cultivar. Similarly, plants of the tolerant cultivar raised from seeds primed with moderate concentration of Kin accumulated SA in the leaves and also produced greater grain yield under saline conditions. In contrast, although moderate concentration (150 mg l^{-1}) of BAP increased leaf free SA concentration in both cultivars, it did not alter grain yield under salt stress. Thus, the results of our studies do not suggest the protective role of SA accumulation in the leaves on the growth of the plants of either cultivar under salt stress.

Recent studies show that SA is involved in the plant responses to salt and osmotic stress by playing a role in the reactive oxygen species-mediated damage caused by high salt and osmotic conditions (Borsani *et al*. 2001) possibly through signalling and gene regulation. However, SA-diminished changes in phytohormones levels are also suggested to be responsible for salt tolerance in wheat seedlings (Shakirova *et al*. 2003). However, the results of present studies do not support the hypothesis that priming-induced changes in hormonal balance (at least in the case of leaf free SA concentration) are associated with better growth and development of plants under salt stress (Kessler 1961, Shah & Loomis 1965).

Pretreatment with different cytokinins altered leaf free PAs in both cultivars under saline conditions. Generally, BAP-priming boosted the concentrations of PAs in the leaves of plants of either cultivar under salt stress. In contrast, plants of both cultivars raised from Kin-primed seeds did not accumulate PAs (Put, Spd and Spm) in the leaves except for Spm in the salt tolerant cultivar under salt stress. There are many reports that suggest the protective role of PAs accumulated in plants under salt stressed conditions (Roberts *et al*. 1986, Kasukabe *et al*. 2004). The present studies suggested that a lower accumulation of PAs in the leaves may be an indicator for Kin-priming induced salt tolerance in wheat plants. In many but not all cases, however, Spd is more closely associated with stress tolerance of plants than are Put and Spm (Bouchereau *et al*. 1999, He *et al*. 2002).

Hydropriming also increased growth of plants of both cultivars as compared with that of plants raised from non-primed seeds under saline conditions. However, it only increased grain yield in the salt tolerant cultivar under salt stress. It caused a considerable decrease in the leaf free PAs (Spd and Spm) in both cultivars under salt stress. There are some reports which exhibit the considerable effectiveness of hydro-priming on germination and later growth in different plant species under both saline and non-saline conditions, e.g., *Acacia tortilis* (Rehman *et al*. 1998), maize, rice and chickpea (Harris *et al*. 1999), wheat (Kamboh *et al*. 2000). However, hydropriming was earlier shown to be ineffective to improve germination and growth in wheat (Chaudhri & Wiebe 1968), and Kentucky bluegrass (*Poa pratensis*) (Pill & Necker 2001).

In conclusion, Kin was the most effective in alleviating the adverse effect of salt stress, particularly on grain yield in both cultivars. Based on performance regarding grain yield under salt stress, it could be concluded that Inqlab-91 is not a more salt tolerant cultivar than MH-97. The mechanism by which Kin-induced increase in grain yield remains unclear. Overall, due to the beneficial effects on growth and grain yield of differently adapted wheat cultivars, pre-sowing seed treatment with moderate concentration of Kin is recommended for wheat cultivars to alleviate the inhibitory effect of salt stress on growth and yield, when grown under saline conditions.

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