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Does polyamine seed pretreatment modulate growth and levels of some plant growth regulators in hexaploid wheat (*Triticum aestivum* L.) plants under salt stress?

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ABSTRACT. The effect of seed presowing treatment with polyamines (2.5 mM putrescine, 5.0 mM spermidine and 2.5 mM spermine) on growth and internal levels of different plant growth regulators in two spring wheat (*Triticum aestivum* L.) cultivars MH-97 and Inqlab-91 was studied. The primed seeds of each treatment and non-primed seeds were sown in a field in which NaCl salinity of 15 dS m^{-1} was developed. Although all three polyamines were effective in improving grain yield in both cultivars under saline conditions, the effect of spermine (Spm) was more pronounced. All priming agents reduced leaf free abscisic acid (ABA) levels in MH-97 as compared with untreated plants under saline conditions, but putrescine (Put) priming caused a maximum increase in leaf ABA concentration in MH-97 under saline conditions. In contrast, in Inqlab-91, Put proved to be very effective in increasing free indoleacetic acid (IAA) concentration under saline conditions. Spermidine (Spd) and Spm were very effective in enhancing salicylic acid (SA) concentration in both MH-97 and Inqlab-91 under saline conditions. Leaf free Put concentration was higher in plants of MH-97 raised from seeds treated with Spd under saline conditions. In contrast, all priming agents decreased leaf Put concentration compared with non-priming in Inqlab-91 under saline conditions. Plants of both cultivars raised from seeds treated with Put had the maximum Spm levels under saline conditions. Overall, the beneficial effects of priming agents (polyamines) were cultivar specific. Physiologically, the beneficial effect of pre-sowing treatment with Spm on grain yield in both cultivars may be attributed to altered hormonal balance.

Keywords: Hormone priming; Plant hormones; Pre-sowing treatment; Salinity; Salt tolerance.

INTRODUCTION

Salt stress-induced imbalance in the hormonal levels of plants is well known (Debez et al., 2001; Wang et al., 2001). Abscisic acid (ABA) generally increases in response to salinity, and auxins decline (Zhang and Zhang, 1994; Wang et al., 2001). 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. Therefore, SA-diminished changes in phytohormone levels are suggested to be responsible for salt tolerance in some crop species (Shakirova et al., 2003). Moreover, the inhibitory effect of salt stress on plant growth involves an array of cellular processes, and all of these may be regulated by an altered hormone homeostasis under salt stress (Xiong and Zhu, 2002). For example, elevations in endogenous ABA levels could act as an internal inhibitor of shoot growth in some plants (Lee et al., 1996). However, adequate levels of ABA could regulate the export of organic substances from the leaf and presumably participate in the regulation of the metabolism of transportable assimilates (Kiseleva and Kaminskaya, 2002).

Polyamines (PAs), such as Spd and Spm and their obligate precursor Put, are polybasic amines that are implicated in many physiological processes in plants (Smith, 1985; Tiburcio et al., 1993; Galston and Kaur-Sawhney, 1995; Kumar et al., 1997). Because of their polycationic nature at a physiological pH, PAs occur

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in plant cells not only as free forms, but also as bound forms. The intracellular free PA pool depends on several processes including a) PA synthesis, b) PA degradation, c) PA conjugation, d) PA transport (Tiburcio et al., 1990; Slocum, 1991; Bagni and Torrigiani, 1992; Tiburcio et al., 1997) and e) interaction with other hormones like ABA (Lee et al., 1997). Recent molecular genetic analyses have shown that altered polyamine levels have profound effects on plant growth and development (Fritze et al., 1995; Kumar et al., 1996; Masgrau et al., 1997; Watson and Malmberg, 1998). Polyamines, being cationic in nature, can associate with anionic components of the membrane such as phospholipids thereby stabilizing the bilayer surface and retarding membrane deterioration (Roberts et al., 1986; Galston and Kaur-Sawhney, 1987; Kramer and Wang, 1989; Basra et al., 1994). It has been shown that PAs in chloroplasts can covalently bound to chlorophyll-protein complexes in thylakoids and Rubisco in the stroma (Del Duca et al., 1994). These ionic interactions are considered to increase stabilization of the subcellular compounds and membranes under stress (Roberts et al., 1986).

Interest is growing in the possible involvement of PAs in the defense reactions of plants to various environmental stresses (Flores, 1990; Kumar et al., 1997; Bouchereau et al., 1999; Shen et al., 2000; Galston, 2001; He et al., 2002; Maiale et al., 2004). Many reports have indicated that the stress tolerance of plants is correlated with their capacity to enhance the synthesis of PAs upon encountering the stress (Evans and Malmberg, 1989; Chattopadhyay et al., 1997; Bouchereau et al., 1999; Mo and Pau, 2002; Kasinathan and Wingler, 2004; Kasukabe et al., 2004). Which of the three PAs plays central roles in stress responses of plants may depend on the plant species and the types of stress (Kasukabe et al., 2004). In many, but not all, cases, however, Spd is more closely associated with stress tolerance of plants than Put and Spm (Bouchereau et al., 1999; Li and Chen, 2000; Shen et al., 2000; He et al., 2002; Martínez-Téllez et al., 2002; Maiale et al., 2004). For example, salinity greatly enhanced the accumulation of Spm and Spd associated with a decrease in Put content in wheat cultivars (El-Shintinawy, 2000). Lefevre and Lutts (2000) observed that an ionic rather than osmotic component of salt stress is involved in the initial steps of PAs accumulation in rice. Lin and Kao (1995) showed contrasting results that increasing NaCl levels led to a decrease in free putrescine levels, but an increase in Spd levels in a salt-sensitive rice cultivar, cv. Taichung Native 1. In pea (Anderson and Martin, 1973) and barley (Smith, 1973), neither Put nor Spd accumulated when exposed to increasing salinity. Salt stress increased the levels of Put and decreased the concentration of Spm in wheat (Simon-Sarkadi et al*.,* 2002).

Even if PAs accumulate, this does not prove their involvement in stress protection, especially as the role of PAs may depend on their cellular localization and whether they are free, bound to proteins, or conjugated to phenolic acids (Bouchereau et al., 1999). However, a correlation between stress tolerance and polyamine levels has been demonstrated in a number of studies using a variety of plant materials, but the physiological rationale for stress-induced polyamine accumulation remains unknown. The function of PAs is presumed to be protective, with a role in scavenging free radicals being a favoured hypothesis (Mansour, 2000). These reports indicate that the individual PAs may have different roles during the response of plants to salt stress. Seed priming with appropriate concentrations of PAs has also been shown to be beneficial under salt stress in some crops (Mansour et al., 2002; Mishra and Sharma, 1994; Ali, 2000). Moreover, hydropriming is also known to be effective under saline conditions (Twitchell, 1955; Zheng et al., 1998; Roy and Srivastava, 1999; Junmin et al., 2000; Cassaro-Silva, 2002).

All these reports led us to hypothesize that pre-sowing seed treatment with PAs could alleviate the adverse effects of salt stress on the growth of spring wheat. Thus, the primary objective of the present study was to assess that up to what extent PAs applied as per-sowing seed treatment could ameliorate the effect of salt stress on two wheat cultivars differing in salt tolerance. In addition, it was determined that how far pre-sowing seed treatment with PAs can regulate the internal levels of PAs and other plant growth regulators in two genetically diverse wheat cultivars subjected to salt stress.

MATERIALS AND METHODS

The seeds of two moderately salt tolerant spring wheat cultivars, MH-97 and Inqlab-91, were obtained from the Wheat Section, Ayub Agricultural Research Institute, Faisalabad, Pakistan. Solutions of 2.5 mM, 5.0 mM and 2.5 mM each of Put, Spd and Spm were used for seed priming, respectively, following Mansour et al. (2002). Distilled water was also used for priming and as a control. Healthy wheat seeds (17 g for each treatment) were primed separately in 100 mL of solutions of PAs as well as in distilled water (DW) for 12 h at room temperature in plastic cups (250 mL). After presoaking, the seeds were surface dried on filter paper and then allowed to air-dry for 12 h at room temperature. The air-dried seeds were used for field experiments.

A field experiment was conducted at the Botanic Garden, University of Agriculture, Faisalabad (UAF) during winter 2003-2004. Eight blocks having a length, width and depth of 1097, 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 = 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}). After three weeks, when the moisture contents were suitable for germination, the primed seeds (P-seeds) of

each treatment and non-primed seeds (NP-seeds) were sown in rows keeping row to row spacing at 15 cm. All pre-sowing seed treatments were randomized in each block. The experiment was laid out in a split split plot design.

At the boot stage, 12 plants from each treatment (three plants replicate^{-1}) were uprooted and washed. After being dried with filter paper, roots were carefully removed, and data for fresh weights of shoots were recorded. Samples were dried in an oven at 65ºC for two weeks, and shoot dry weights were recorded. At maturity, grain yield plant^{-1} (g) was recorded.

Determination of hormones (Auxins, Abscisic acid) and Salicylic acid

Fresh leaves (3rd leaf from top) were harvested from each treatment. Two grams of fresh leaves were finely chopped in 30 mL of 80% cold (-70ºC) aqueous methanol (4:1, v/v) supplemented with 20 mg L^{-1} butylated hydroxytoluene (BHT) and stored at -70ºC or -20ºC. Fresh leaves already frozen and stored were ground with a mortar and pestle using aqueous 80% methanol supplemented with BHT (MeOH-BHT) as extracting solvent. To check recoveries during extraction and purification, 191.6 ng of IAA, 300 ng NAA, 287.4 ng salicylic acid (SA), and 191.6 ng abscisic acid (ABA) were used as internal standards and were added before homogenization in each sample and blank. The homogenate was vortexed for 10 min and filtered with suction through Whatman #42 paper (Whatman Shanghai, China). The residues in the flask and on the filter were rinsed thrice with 10-mL aliquots of extracting solvent and twice with 100% MeOH. The extracts were combined and mixed and then divided into two equal samples (Kusaba et al., 1998). One sample was used for IAA and ABA analyses and the other for SA analysis. The extract was concentrated to an aqueous residue by rotary flask evaporation (RFE) at 35ºC. The sublimation losses were reduced by adding phosphate buffer (pH 7). The aqueous fraction was transferred to 50-mL polypropylene centrifuge tubes. The flask, used for RFE, was rinsed with 10 mL of hexane to extract chlorophylls and lipids from the aqueous residue. Similarly, the aqueous fraction was partitioned three times against hexane after adjusting the pH to 8. The pH of the aqueous residue was adjusted to 3 with HCl and again partitioned three times with hexane. The hexane fractions were then discarded. 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 so as to extract any traces of hormones. 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 microcentrifuge tube. These samples were kept overnight at -70ºC and then centrifuged (25,000 *g*) for 10 min at -10ºC. (A lower temperature helped to remove any residues easily.) Standards were also prepared following the same procedure. The supernatant was filtered and subjected to HPLC (high performance liquid chromatography) analysis.

Analysis of IAA, SA, and ABA was performed by HPLC system (Sykam GmbH, Kleinostheim, Germany), equipped with S-1121 dual piston solvent delivery system and S-3210 UV/VIS detector. Following Guinn et al. (1986) with some modifications, 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 40ºC. Twenty microliters of filtered extracts (using a Whatman nylon membrane filter of 0.45 µm pore size, Whatman Int. Ltd., Maldstone, England) were injected into a Hypersil ODS reverse-phase (C_{18}) column (4.6 × 250 mm, 5-µm particle size: Thermo Hypersil GmbH, Germany). Detection of different hormones 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). All values were corrected on internal standards with known concentrations of different hormones.

Determination of polyamines

Fresh leaves (3rd leaf from top) were harvested from plants of each treatment. Two grams of fresh leaves were finely chopped in 20 mL of cold 5% aqueous perchloric acid and were stored at -70ºC.

For polyamine extraction and HPLC analysis, the benzoylation method was performed as described previously (Flores and Galston, 1982) with some modifications. Leaf tissue (2 g fresh weight) was homogenized in 20 mL of cold 5% perchloric acid with a mortar and a pestle containing 200 nmol of 1, 6-hexanediamine as an internal standard. The homogenates were incubated at 4ºC 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 2,500 *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 microcentrifuge tube. Polyamine standards (Sigma, USA) were prepared similarly to plant samples. These samples were filtered and stored at -20ºC for HPLC analysis.

The polyamine contents were analyzed by HPLC (Sykam GmbH, Kleinostheim, Germany) equipped

with S-1121 dual piston solvent delivery 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 50ºC. 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

Analysis of variance (ANOVA) of data for all attributes was computed 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

Saline growth medium caused a significant reduction in fresh weights of shoots of plants of both cultivars ($P \leq 0.001$; Table 1). Plants derived from seeds treated with different priming agents did not improve shoot fresh weight in either cultivar.

Salt stress imposed in the growth medium caused a significant reduction in the grain yield of both cultivars ($P \leq 0.01$; Table 1). However, different priming agents caused a significant increase in grain yield ($P \leq 0.001$). Of the priming agents used, Spm was found very effective in enhancing the grain yield of both cultivars under salt stress (Table 2). Overall, in comparison with non-priming, all priming treatments caused a significant improvement in the grain yield of both cultivars. An exception was in the plants of Inqlab-91, raised from seeds treated with Put under saline conditions.

Saline substrate significantly altered the leaf free IAA contents of both wheat cultivars ($P \leq 0.001$; Table 3). Cultivars also differed significantly from one another. However, different priming agents altered leaf IAA concentrations differently in both cultivars. For example, distilled water proved to be the priming agent most effective at increasing free IAA concentration in MH-97 under saline conditions. In contrast, in Inqlab-91, Put, and Spd proved to be very effective at increasing the free IAA concentration under saline conditions and non-saline control, respectively (Table 4). Overall, the increase in free IAA concentration in MH-97 due to different priming agents was higher than that in Inqlab-91 under both saline and non-saline conditions.

Leaf free ABA concentrations of both cultivars changed significantly under salt stress. The effects of priming agents differed significantly in altering leaf ABA concentration of both cultivars ($P \leq 0.001$; Table 3). Except hydropriming, all priming agents reduced leaf free ABA levels in MH-97 as compared with untreated plants under saline conditions. However, Put priming increased leaf ABA concentrations the most in MH-97 under saline conditions.

Salt stress significantly changed the leaf free SA concentrations of both cultivars ($P \leq 0.001$; Table 3). Priming agents also showed different effects on free SA concentration in leaves of both cultivars ($P \leq 0.001$). The pattern of increase or decrease in leaf SA concentration in both cultivars due to salt stress was not consistent (Table 4). However, Spd was a very effective priming agent in enhancing SA concentration in MH-97 under saline conditions. In contrast, pre-treatment with Spm slightly

Table 1. Mean squares from analysis of variance of data for shoot fresh mass and grain yield per plant of two cultivars of hexaploid wheat raised from seeds primed with different polyamines.

Source of variation	Degrees of freedom	Shoot fresh mass	Grain yield $plant^{-1}$
Salinity (S)	1	3721.1***	26.90**
Main plot error	3	14.09	0.276
Cultivars (C)	1	6.045ns	0.121ns
C x S	1	0.559ns	0.319*
Subplot error	6	16.72	0.039
Treatments (T)	4	31.74*	0.578***
T x S	4	10.961ns	0.061**
T x C	4	25.44*	0.137***
T x C x S	4	26.86*	0.129***
Error	48	9.469	0.016

*, **, *** = significant at 0.05, 0.01, and 0.001 levels, respectively.
ns = non-significant.

Table 2. Shoot fresh mass and grain yield per plant of two cultivars of hexaploid wheat at 2.84 dS m^{-1} (control) or 15 dS m^{-1} NaCl (salt) when the seeds were primed with solutions of different polyamines. Values in parentheses are SE of means (based on four replicates).

Pre-sowing seed treatments	MH-97		Inqlab-91	
	2.84 dS m^{-1}	15 dS m^{-1}	2.84 dS m^{-1}	15 dS m^{-1}
Shoot fresh biomass (g $plant^{-1}$)				
Put (2.5 mM)	27.72 (1.56)	14.51 (2.94)	29.79 (0.89)	18.39 (0.87)
Spd (5.0 mM)	27.29 (1.87)	14.97 (2.21)	29.05 (0.77)	15.79 (0.81)
Spm (2.5 mM)	31.23 (0.53)	14.84 (2.61)	31.13 (0.82)	14.87 (0.76)
Distilled water	29.82 (0.25)	15.98 (1.71)	24.66 (0.80)	16.43 (0.56)
Untreated	27.14 (0.47)	15.28 (0.89)	28.95 (0.89)	14.64 (0.73)
LSD 5%		4.375		
Grain yield (g dry weight $plant^{-1}$)				
Put (2.5 mM)	3.55 (0.144)	2.66 (0.059)	3.75 (0.150)	2.24 (0.094)
Spd (5.0 mM)	3.98 (0.112)	2.93 (0.103)	3.75 (0.083)	2.50 (0.017)
Spm (2.5 mM)	3.69 (0.108)	3.05 (0.074)	3.84 (0.078)	2.56 (0.021)
Distilled water	3.60 (0.044)	2.25 (0.137)	3.58 (0.025)	2.52 (0.029)
Untreated	3.43 (0.059)	2.12 (0.150)	3.57 (0.094)	2.25 (0.049)
LSD 5%		0.178		

Table 3. Mean squares from analysis of variance of data for IAA, ABA, and SA and polyamine concentrations of two cultivars of hexaploid wheat raised from seeds primed with different polyamines.

Source of variation	Degrees of freedom	IAA	ABA	SA
Salinity (S)	1	792614.2***	100509.1***	8461635.1***
Main plot error	3	421.2	548.2	9414.9
Cultivars (C)	1	19438.5*	50605.4***	12246589.0***
C x S	1	73564.6**	94511.6***	5254614.9***
Subplot error	6	2821.9	12.7	39618.8
Treatments (T)	4	52291.8***	11815.3***	5132013.4***
T x S	4	78157.3***	9782.8***	1312861.3***
T x C	4	76262.8***	19010.7***	2249342.3***
T x C x S	4	87476.9***	23998.0***	1849103.4***
Error	48	1327.4	649.8	40559.5
		Putrescine	Spermidine	Spermine
Salinity (S)	1	4450.3**	385946.6***	3081.3ns
Main plot error	3	46.8	118.4	574.9
Cultivars (C)	1	133.6ns	3027.8ns	188.2ns
C x S	1	705.4**	26786.5*	2513.1**
Subplot error	6	38.3	2609.3	96.6
Treatments (T)	4	635.7***	25959.7***	2191.4***
T x S	4	902.5***	31620.2***	2628.6***
T x C	4	1238.4***	5230.5ns	1547.7***
T x C x S	4	809.4***	8347.2ns	549.6ns
Error	48	19.8	3251.5	271.3

*, **, *** = significant at 0.05, 0.01, and 0.001 levels, respectively; ns = non-significant.
IAA, indoleacetic acid; ABA, abscisic acid; SA, salicylic acid.

increased leaf SA concentration in Inqlab-91 under saline conditions.

Salt stress of the growth medium significantly affected leaf Put concentration of both cultivars, but the cultivars also did not differ significantly (Table 3). However, the priming agents differed significantly in altering the Put levels in both wheat cultivars ($P \leq 0.01$). Leaf free Put concentration was higher in plants of MH-97 raised from seeds treated with Spd under saline conditions (Table 5). Of all priming agents, Put was the most effective at increasing leaf Put concentration in Inqlab-91 under saline conditions. In contrast, the remaining priming agents decreased leaf Put content compared with non-priming in Inqlab-91 under saline conditions.

Different priming agents altered leaf free Spd concentration under both saline and non-saline conditions, but cultivars also did not differ significantly. Similarly, the difference among priming agents in altering the Spd levels of plants of both cultivars was also insignificant (Tables 3 and 5).

Salt stress imposed in the growth medium had no significant effect on leaf spermine (Spm) concentration in either cultivar, and neither cultivar differed significantly from the other. However, priming agents differed significantly in altering free Spm concentration in both cultivars (Tables 3 and 5). For example, plants of both cultivars raised from seeds treated with Put had the maximum Spm levels under saline conditions.

DISCUSSION

It should now be evident that growth regulator balance can be changed at high salinities, and this effect can be partially alleviated with application of exogenous growth promoter substances (Gadallah, 1999; Khan et al., 2000; Debez et al., 2001). In the present study, priming with Put alleviated the adverse effects of salt stress on hormonal balance in the plants of Inqlab-91. For example, put pre-treatment considerably increased leaf ABA and IAA concentrations in Inqlab-91 under saline conditions. In contrast, in MH-97, it decreased free ABA levels compared with the untreated plants under saline conditions. The increased ABA levels can regulate the pathways of photoassimilate utilization in leaves by partitioning carbon flows either to the synthesis of high molecular weight compounds (cellulose, hemicellulose, and proteins), used for cell growth in leaves, or to

Table 4. IAA, ABA and SA concentrations (ng g^{-1} fresh weight) of two cultivars of hexaploid wheat at 2.84 dS m^{-1} (control) or 15 dS m^{-1} NaCl (salt) when the seeds were primed with solutions of different polyamines. Values in parentheses are SE of means (based on four replicates).

Pre-sowing seed treatments	MH-97		Inqlab-91	
	2.84 dS m^{-1}	15 dS m^{-1}	2.84 dS m^{-1}	15 dS m^{-1}
Free IAA				
Put (2.5 mM)	209.2 (2.51)	164.1 (26.54)	121.9 (4.74)	182.9 (10.37)
Spd (5.0 mM)	85.4 (0.11)	125.1 (13.13)	633.2 (12.19)	94.8 (6.97)
Spm (2.5 mM)	231.2 (4.74)	119.6 (15.43)	273.7 (8.45)	112.9 (6.70)
Distilled water	421.8 (18.83)	180.9 (18.79)	561.4 (49.02)	88.2 (1.05)
Untreated	472.5 (19.29)	138.1 (35.93)	288.9 (21.48)	101.5 (1.74)
LSD 5%		51.79		
Free ABA				
Put (2.5 mM)	183.6 (16.82)	231.5 (12.80)	59.5 (19.78)	187.1 (7.59)
Spd (5.0 mM)	31.5 (0.88)	178.5 (2.38)	173.8 (1.34)	110.7 (0.37)
Spm (2.5 mM)	116.9 (9.91)	137.9 (9.40)	145.2 (18.85)	122.6 (10.60)
Distilled water	119.3 (0.90)	329.4 (13.94)	93.3 (3.47)	102.9 (5.91)
Untreated	96.5 (9.53)	368.5 (21.15)	168.1 (19.50)	127.3 (5.56)
LSD 5%		36.24		
Free SA				
Put (2.5 mM)	1588.4 (10.4)	1634.6 (31.0)	1524.9 (117.9)	1572.1 (34.7)
Spd (5.0 mM)	1851.5 (74.3)	2851.5 (39.7)	3929.5 (204.7)	1959.6 (62.2)
Spm (2.5 mM)	2434.3 (51.6)	1668.8 (128.2)	4744.4 (90.9)	2574.5 (71.2)
Distilled water	2331.5 (63.2)	1147.2 (81.0)	2332.6 (207.6)	1659.5 (57.7)
Untreated	919.6 (90.1)	1133.8 (108.9)	3069.3 (61.5)	2019.9 (63.1)
LSD 5%		286.3		

Table 5. Polyamine concentrations (µg g^{-1} fresh weight) of two cultivars of hexaploid wheat at 2.84 dS m^{-1} (control) or 15 dS m^{-1} NaCl (salt) when the seeds were primed with solutions of different polyamines. Values in parentheses are SE of means (based on four replicates).

Pre-sowing seed treatments	MH-97		Inqlab-91	
	2.84 dS m^{-1}	15 dS m^{-1}	2.84 dS m^{-1}	15 dS m^{-1}
Putrescine				
Put (2.5 mM)	8.66 (0.35)	22.37 (0.92)	10.52 (1.14)	24.24 (6.77)
Spd (5.0 mM)	28.16 (1.32)	35.71 (3.69)	15.17 (2.07)	17.99 (0.36)
Spm (2.5 mM)	15.88 (0.82)	23.87 (4.36)	8.04 (0.30)	15.14 (0.28)
Distilled water	10.72 (0.85)	16.48 (0.66)	13.40 (1.51)	21.60 (4.09)
Untreated	9.18 (0.20)	19.06 (0.50)	8.70 (1.44)	81.14 (2.77)
LSD 5%		6.32		
Spermidine				
Put (2.5 mM)	43.9 (15.5)	308.9 (24.8)	58.5 (4.35)	355.1 (80.1)
Spd (5.0 mM)	108.1 (0.37)	228.3 (27.7)	61.6 (17.6)	182.7 (18.7)
Spm (2.5 mM)	67.9 (3.91)	303.2 (66.5)	141.7 (24.6)	169.5 (71.6)
Distilled water	79.1 (11.3)	145.6 (18.9)	134.4 (12.6)	136.2 (15.2)
Untreated	151.5 (0.46)	342.2 (5.00)	175.7 (6.59)	240.1 (27.9)
LSD 5%		(S x V x T) = ns		
Spermine				
Put (2.5 mM)	6.31 (1.35)	44.47 (8.51)	4.19 (0.06)	25.42 (5.81)
Spd (5.0 mM)	6.80 (0.58)	12.53 (0.23)	2.80 (0.13)	5.89 (1.28)
Spm (2.5 mM)	6.54 (0.21)	17.73 (3.33)	8.72 (0.28)	9.13 (0.32)
Distilled water	5.63 (0.21)	7.40 (0.39)	14.46 (1.96)	6.38 (1.01)
Untreated	6.86 (0.38)	18.13 (0.36)	20.19 (6.48)	26.06 (2.74)
LSD 5%		(S x V x T) = ns		

the synthesis of transportable forms of carbohydrates (Kiseleva and Kaminskaya, 2002). Similarly, auxins play a critical role in the regulation of plant growth and development, including cell division and differentiation (Thimann, 1977; Dietz et al., 1990; Normanly, 1997; Davies, 1995; Pospíšilová, 2003). Thus, the improvement in growth only in Inqlab-91 due to priming with Put seems to have been due to increased leaf ABA and IAA levels under salt stress.

Generally, salinity caused a marked increase in Put levels in both cultivars. Both arginine decarboxylase (ADC) and ornithine decarboxylase (ODC) are responsible for salt (NaCl) induced increase in Put biosynthesis (Friedman et al., 1989; Lee and Chen, 1998). However, plants show diverse responses to high saline conditions with respect to PA biosynthesis: some systems show increased PA levels while others show smaller increases or even decreases (Krishnamurthy and Bhagwat, 1989; Lin and Kao, 1995; Lee and Chen, 1998). In the present study, in comparison with plants raised from untreated seeds, pre-treatment with Put increased endogenous free Put levels only in Inqlab-91 under saline conditions. Serrano et al. (1997, 1998) have hypothesized that the increase in PAs levels in response to stress is immediate, indicating it is a consequence of stress rather than a protective mechanism. After a period of time, even if the stress situation continues, PA levels decrease and resemble those from non-stressed plants. However, many contrasting reports have suggested the change in PAs under salt stressed conditions to be a protective mechanism (Roberts et al., 1986; Kramer and Wang, 1989; Basra et al., 1994; Basra et al., 1997; Kasukabe et al., 2004).

Pre-sowing seed treatment with Spd also improved the grain yield of both cultivars under saline conditions. However, the salinity induced decrease in leaf free IAA was not alleviated by pre-sowing treatment with Spd in either cultivar. It also caused a decrease in leaf ABA levels and an increase in SA levels in both cultivars except Inqlab-91, in which pre-sowing treatment with Spd did not ameliorate salt-induced reduction in leaf SA levels under saline conditions. SA is an endogenous growth regulator which controls plant growth and development (Schettel and Balke, 1983), photosynthesis and transpiration rates (Pancheva et al., 1996), and ion uptake and transport (Harper and Balke, 1981; Uzunova and Popova, 2000). SA is also known to reverse salt stress-induced decreases

in total and reducing sugar contents by providing a pool of compatible osmolytes in the presence of sodium (Tari et al., 2002). In the present study, the greater improvement in grain yield in MH-97 could be attributed to increased accumulation of SA under saline conditions. Furthermore, the pattern of accumulation of PAs was not consistent in both cultivars due to pre-sowing treatment with Spd under saline conditions. For example, Spd proved to be the most effective of all priming agents in accumulating leaf free Put levels in MH-97 while it failed to cause accumulation of leaf Put contents in Inqlab-91 under saline conditions. Pre-sowing seed treatment with Spd did not affect leaf free Spd and Spm levels in either cultivar under saline conditions. This contravenes the findings reported in some earlier studies, e.g., salt-tolerant rice cultivars (Krishnamurthy and Bhagwat, 1989), *Limonium* sp. (Bouchereau et al., 1999) and *Bromus* spp. (Santa-Cruz et al., 1997). It may be possible that high levels of binding of Put and Spd to proteins in actively dividing young tissues and lower levels in mature non-dividing tissues might have resulted in lower leaf free Put levels in Inqlab-91 and non-significant levels of Spd and Spm in both cultivars under saline conditions. Such accumulation and non-accumulation of Spd and Spm has already been reported in tissues of *Chrysanthemum* (Aribaud et al., 1995).

Spm was the most effective of all the polyamines for seed priming because it maximized improvement in grain yield in both cultivars under both saline and non-saline conditions. Priming of seeds of Inqlab-91 with Spm proved to be effective in reducing the drastic effect of salt stress on plant hormonal balance. For example, salinity-induced reduction in SA was alleviated by Spm priming of Inqlab-91 under saline conditions. However, leaf free ABA and SA levels in MH-97 were not affected by pre-soaking with Spm under saline conditions. Thus, the increase in grain yield is probably due to the increased rate of translocation of photosynthates from leaves to grains caused by pre-sowing Spm treatment. Such increases in grain yield following hormone pre-treatments has already been found in wheat (Lovell, 1971; Dewdeny and Mcwha, 1978; Ray and Choudhuri, 1981; Aldesuquy and Ibrahim, 2001).

Hydro-priming did not show a pronounced effect on shoot growth or grain yield in either cultivar under saline conditions, except for Inqlab-91, which showed improved grain yield. Failure of hydro-priming to improve germination and growth has already been reported in wheat (Chaudhri and Wiebe, 1968) and Kentucky bluegrass (*Poa pratensis*) (Pill and Necker, 2001). However, some reports do 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., wheat (Kamboh et al., 2000), and *Acacia tortilis* (Rehman et al., 1998). Hydro-priming also failed to show a consistent effect on hormone homeostasis in both cultivars. For example, it increased leaf free IAA in MH-97, but decreased it in Inqlab-91, under saline conditions. Moreover, the decrease or increase in leaf free ABA and SA due to hydropriming was not pronounced in either cultivar under saline conditions.

In conclusion, of the different priming agents used in this study, Spm was most effective at alleviating the adverse effect of salt stress on grain yield in both wheat cultivars. Although all other priming agents were also effective in inducing salt tolerance in wheat cultivars, the beneficial effects of these priming agents were cultivar specific. Physiologically, the beneficial effect of pre-sowing treatment with Spm on grain yield in both cultivars may be attributed to altered hormonal balance.

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以多元胺預先處理種子能否調節發芽後處於鹽逆境六倍體小麥之生長及若干植物生長調節物質之含量高低？

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種子發芽前，分別以多元胺 (2.5 mM putrescine, 5.0 mM spermidine 及 2.5 mM spermine) 預先處理對之後幼苗之生長及內在生長調節物質之量的影響以兩種春小麥 (*Triticum aestivum* L.) 品系 MH-97 及 Inqlab-91 測試。實驗組及對照組之種子均種在鹽度為 15 dS m^{-1} 之實驗田。雖然對兩種供試小麥之穀粒產量受鹽害之減低情形，三種多元胺均可改善；spermine 之效果最好。三種多元胺均對 MH-97 葉內自由型離層酸 (ABA) 之減少 (實驗組比對對照組) 均有所改善，但以 putrescine 之效果最佳。相對地，putrescine 在品系 Inqlab-91 內則最有效地增加葉內自由型 indoleacetic acid, spermidine 及 spermine 則在兩種供試品系處於鹽逆境下有效地增強葉中之水楊酸 (salicylic acid) 含量。以 spermidine 預處理之種子，之後在鹽逆境下葉中自由型之 putrescine 則以 MH-97 較對照組為高。相反地，在品系 Inqlab-91 所有三種多元胺之種子處理均導致之後發芽幼苗在鹽逆境下均比對照組有較低之葉中自由型 putrescine，兩種品系之種子經 putrescine 預處理後，之後發芽之植物體在鹽逆境下均有最大量之葉中自由型 spermidine。整體而言，多元胺之種子預處理其好處依品種而異。自生理觀點而言，種子以 spermine 預處理之所以在鹽逆境下能改善兩種供試品種之穀粒產量，可能歸因於植物激素的平衡之改變。

關鍵詞：植物生長調節物質預處理；植物生長調節物質；發芽前之預處理；鹽度；高鹽耐受性。