Salt tolerance and regulation of gas exchange and hormonal homeostasis by auxin-priming in wheat

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Abstract – The objective of this work was to assess the regulatory effects of auxin-priming on gas exchange and hormonal homeostasis in spring wheat subjected to saline conditions. Seeds of MH-97 (salt-intolerant) and Inqlab-91 (salt-tolerant) cultivars were subjected to 11 priming treatments (three hormones x three concentrations + two controls) and evaluated under saline (15 dS m^{-1}) and nonsaline (2.84 dS m^{-1}) conditions. The priming treatments consisted of: 5.71, 8.56, and 11.42 × 10^{-4} mol L^{-1} indoleacetic acid; 4.92, 7.38, and 9.84 × 10^{-4} mol L^{-1} indolebutyric acid; 4.89, 7.34, and 9.79 × 10^{-4} mol L^{-1} tryptophan; and a control with hydroprimed seeds. A negative control with nonprimed seeds was also evaluated. All priming agents diminished the effects of salinity on endogenous abscisic acid concentration in the salt-intolerant cultivar. Grain yield was positively correlated with net CO_2 assimilation rate and endogenous indoleacetic acid concentration, and it was negatively correlated with abscisic acid and free polyamine concentrations. In general, the priming treatment with tryptophan at 4.89 × 10^{-4} mol L^{-1} was the most effective in minimizing yield losses and reductions in net CO_2 assimilation rate, under salt stress conditions. Hormonal homeostasis increases net CO_2 assimilation rate and confers tolerance to salinity on spring wheat.

Index terms: *Triticum aestivum*, hormonal balance, plant growth regulators, salinity, seed priming, stomatal regulation.

Tolerância ao sal e regulação de trocas gasosas e da homeostase hormonal pelo condicionamento osmótico com auxinas em trigo

Resumo – O objetivo deste trabalho foi avaliar os efeitos regulatórios do condicionamento osmótico com auxinas sobre as trocas gasosas e a homeostase hormonal em trigo de primavera, submetido a condições salinas. Sementes das cultivares MH-97 (intolerante ao sal) e Inqlab-91 (tolerante ao sal) foram submetidas a 11 tratamentos de condicionamento osmótico (três hormônios x três concentrações + dois controles) e avaliadas sob condições salinas (15 dS m^{-1}) e não salinas (2,84 dS m^{-1}). Os tratamentos com condicionamento consistiram de: ácido indolacético a 5,71, 8,56 e 11,42 x 10^{-4} mol L^{-1}; ácido indolbutírico a 4,92, 7,38 e 9,84 × 10^{-4} mol L^{-1}; e triptofano a 4,89, 7.34 e 9,79 × 10^{-4} mol L^{-1}; e sementes condicionadas em água (controle). Um tratamento com sementes não condicionadas também foi avaliado. Os agentes condicionadores diminuíram os efeitos de salinidade sobre a concentração de ácido abcísico endógeno, na cultivar intolerante ao sal. A produtividade de grãos correlacionou-se positivamente à taxa de assimilação líquida de CO_2 e à concentração de ácido indolacético; e negativamente ao ácido abcísico e às concentrações de poliaminas livres. De forma geral, o tratamento com triptofano a 4,89 × 10^{-4} mol L^{-1} foi o mais efetivo em amenizar perdas produtivas e reduções na assimilação líquida de CO_2, sob condições de estresse salino. A homeostase hormonal aumentou a taxa de assimilação líquida de CO_2 e conferiu tolerância à salinidade ao trigo de primavera.

Termos para indexação: *Triticum aestivum*, balanço hormonal, reguladores de crescimento, salinidade, condicionamento de sementes, regulação estomatal.

Introduction

Soil salinity is one of the biggest threats to global agriculture. A number of strategies have been used to overcome the salinity problem either by management or by inducing salt tolerance in plant (Flowers, 2004; Cuartero et al., 2006). Nonetheless, due to the genetic and physiological complexities of the salt tolerance trait (Flowers, 2004), the strategies to cope with salinity problem have come across a limited success. Furthermore, consumers are reluctant to accept transgenically modified food (Munns, 2005). In this scenario, seed priming has gained much popularity in inducing salt tolerance in relatively less salt-tolerant plants, since it is an easy technique, with low risk and low cost. Beneficial effects of seed priming have been

reported for many crops; however, its mechanisms, particularly at later growth stages of plants, remain largely unknown.

Plant hormones affect a wide spectrum of processes in plants under both normal and stressful environments. Exogenous application of plant growth regulators (PGR) have been shown to alleviate the drastic effects of abiotic stresses on germination and plant growth (Finch-Savage et al., 2004; Javid et al., 2011). Different stresses alter the endogenous levels of hormones, and thus affect plant growth. Therefore, plant response to salinity is not exclusively a function of the osmotic shock or ionic toxicity, but also appears to be a consequence of the effect of salt on hormonal balance.

In contrast to abscisic acid, auxins are mainly synthesized in the shoots. Although indoleacetic acid (IAA) biosynthesis follows several pathways, its most common precursor is tryptophan (TRP). Seed priming with auxins has been shown to alleviate the inhibitory effects of salt stress on germination and growth in wheat (Iqbal & Ashraf, 2007). Relatively very little, and often confusing information are available concerning the involvement of auxins in stomatal regulation (Pospíšilová, 2003). For instance, Assmann & Armstrong (1999) found a differential effect of low and high IAA concentration on stomatal conductance (g_s), whereas, in *Pisum sativum* and *Phaseolus vulgaris*, Eamus (1986) found CO_2 concentration-dependent effect of IAA on g_s. Auxin is known to antagonize abscisic acid (ABA) induced stomatal closure in *Arabidopsis* (Tanaka et al., 2006). Ethylene has been shown to mediate the auxin-induced stomatal opening in some other plants (Merritt et al., 2001; Iqbal et al., 2011). Therefore, changes in IAA concentrations might regulate stomatal opening and closing in a concentration-dependent manner.

In comparison with the information on endogenous hormonal contents in plant tissues, information still lack on the auxin-priming-induced changes in endogenous hormonal concentrations in plants under salt stress. Therefore, it is important to study the effects of natural auxins and their precursors (TRP) as priming agents on the endogenous content of auxins. Moreover, ABA and polyamines could provide valuable information on their mechanism of action on plant growth.

The objective of this work was to assess the regulatory effects of auxin-priming on gas exchange and hormonal homeostasis in spring wheat subjected to saline conditions.

Materials and Methods

Field experiments were conducted during the winter 2002 and 2003, in the Botanic Garden, University of Agriculture, Faisalabad, where the average photosynthetically active radiation (PAR) of the entire growth period was 1,098 $\mu mol\ m^{-2}\ s^{-1}$, and the maximum and minimum relative humidity was 79 and 32%, respectively. The average maximum and minimum temperatures during the growth period were 28±4°C and 12±3°C, respectively.

Seeds (17 g) of two spring wheat cultivars, MH-97 (salt intolerant) and Inqlab-91 (salt tolerant) were subjected to 11 priming treatments (three hormones x three concentrations + 2 controls) and evaluated in saline (150 mmol L^{-1} NaCl) and nonsaline conditions. Seeds were primed separately, imbibed for 12 hours in solutions of: 5.71, 8.56, and 11.42 × 10^{-4} mol L^{-1} IAA; 4.92, 7.38, and 9.84 × 10^{-4} mol L^{-1} IBA; and 4.89, 7.34, and 9.79 × 10^{-4} mol L^{-1} TRP, as well as in distilled water (control). A negative control without any kind of priming was also evaluated. After pre-soaking, seeds were surface-dried with filter paper and then air-dried for 12 hours at room temperature. Seeds were sown in seedbeds (732x137x46 cm of length, width and depth, respectively) lined with polythene sheets and filled with thoroughly mixed sandy loam soil (saturation percentage = 25.5; EC_e = 2.84 dS m^{-1}; pH = 7.56). It was used a randomized block design, arranged in split-split plots (hormones in the main plots, saline conditions in the subplots, and cultivars in the sub-subplots), with four replicates. After three weeks, the primed (air-dried for 12 hours) and nonprimed seeds of both cultivars, were sown in the soil having moisture contents 13% (Iqbal & Ashraf, 2007), keeping row-to-row spacing about 15 cm. Plants were irrigated carefully with tap water (EC_w = 0.65 dS m^{-1}), so as to minimize dilution in salinity at the irrigation points. Electrical conductivity of the soil was checked periodically and maintained whenever required.

At boot stage, 12 plants from each treatment (three plants per replicate) were uprooted, washed with distilled water, and analyzed for shoot fresh biomass. At maturity, four plants per replicate were used to record plant height, number of fertile tillers per plant, and components of grain yield.

Measurements of net CO_2 assimilation rate (A), transpiration rate (E), and stomatal conductance (g_s) were made from 10:00 to 15:00 h at the boot stage (four plants per replicate) on a recently matured top leaf, using an open system LCA-4 ADC portable infrared gas

analyzer (Analytical Development Company, Hoddesdon, England). Measurements were made with the following specifications/adjustments: leaf chamber area, 11.35 cm^2; ambient CO_2 concentration (C_{ref}), 343±5 µmol mol^{-1}; substomatal CO_2 concentration (C_i), 235±34 µmol mol^{-1}; leaf chamber temperature (T_{ch}), 39±4°C; leaf chamber gas flow rate (V), 389±3 mL min^{-1}; molar flow of air per unit leaf area (U_s), 223±1.6 mol m^{-2} s^{-1}; ambient pressure (P), 99.8±0.7 kPa; and light intensity at leaf surface (Q_{leaf}), which was up to 1,025±72 µmol m^{-2} s^{-1}.

Recently matured top leaves were used for the determination of different phytohormones at boot stage. Auxins (IAA, IBA) and ABA were extracted and purified as described by Kusaba et al. (1998), with some modifications (Iqbal et al., 2006). The quantification of plant hormones was done by high performance liquid chromatograph (HPLC) using authentic standards run through for the whole procedure, as previously described (Iqbal et al., 2006).

Fresh, recently matured top leaves (2 g) were sampled at boot stage, and ground in 20 mL of cold 5% (v/v) aqueous perchloric acid. For polyamines (putrescine, PUT; spermidine, SPD and spermine, SPM) extraction and quantification, benzoylation method was performed as previously described (Flores & Galston, 1982), with minor modifications.

Collected data were analysed using the GLM module of CoStat version 6.2 (CoHort Software, Monterey, CA, USA). Analysis of variance was done using a randomized complete block design with split-split plot arrangement (d.f. = 120). Correlations among grain yield, gas exchange characteristics, and hormonal concentrations were determined using XLSTAT version 7.5.2 (Addinsoft SARL, Paris, FR). Means were compared using LSD values calculated according to Duncan's multiple range test, at 5% probability.

Results and Discussion

Higher concentrations of the priming agents (IAA, IBA and TRP) increased plant height in the salt-tolerant cultivar (Table 1). However, the lowest concentration (4.89×10^{-4} mol L^{-1}) of TRP was much effective in increasing plant height in the salt-intolerant cultivar. Priming agents had nonsignificant effects on shoot fresh biomass, compared to the control. Plants raised from seeds primed at low concentration of TRP, in the salt-tolerant cultivar, and in high concentration of IBA in the salt-intolerant cultivar, had more fertile tillers than the control. IAA is present in plants mostly in the form of IAA conjugates (Ludwig-Müller, 2011). Thus, the better growth induced by TRP could be attributed to TRP-dependent synthesis of free IAA, particularly at early seedling stage. Exogenous application of auxin has already been shown to increase growth in wheat under salt stress (Iqbal & Ashraf, 2007), and in barley under water stress (Ashraf et al., 2006).

Low concentration (4.89×10^{-4} mol L^{-1}) of TRP was much effective in increasing A (net CO_2 assimilation rate) in the two cultivars, under both saline and nonsaline conditions (Figure 1). Although the priming agents did not affect g_s (stomatal conductance) in the salt-tolerant cultivar, they significantly increased g_s in the salt-intolerant cultivar. The results suggested that TRP-dependent IAA synthesis might be involved in enhancing A through stomatal opening, under both control and salt stress. The auxin-mediated enhancement in g_s and A was already reported for *Vicia faba* (Merritt et al., 2001) and *Hordeum vulgare* (Ashraf et al., 2006). Exogenous application of TRP increased photosynthetic pigments (chlorophyll a and b) in wheat under salt stress (El-Bassiouny, 2005).

Seed priming in TRP (4.89×10^{-4} mol L^{-1}) increased E (transpiration rate) in the salt-intolerant cultivar, while all priming agents increased E in the salt-stressed plants of the salt-tolerant cultivar, when compared with the control. Interestingly, soil salinity reduced much more E in the salt-tolerant than in the salt-intolerant wheat cultivar. The reduction in E is considered an important adaptive mechanism of salinity-tolerance in wheat (Robinson, 1988). This mechanism causes reduction in salt uptake, which usually occurs passively in wheat through the transpiration stream (Yeo & Flowers, 1986). Although the salt-tolerant cultivar had more g_s than the salt-intolerant one, it exhibited lower E under salt stress. These results suggest that salt-tolerant cultivar has a better control over the stomata, under salt stress.

James et al. (2002) observed reduction in g_s before any apparent decline in leaf water potential in the salt-stressed wheat plants. Similarly, Sadeghi & Nazemosadat (2011) observed substantial decrease in g_s before any noticeable change in leaf water potential in tolerant wheat lines. In this situation, enhanced transport of photoassimilates towards developing seeds and reduced utilization of photoassimilates in the vegetative organs could have compensated the reduction of yield in the salt-tolerant wheat cultivar (Grieve et al., 1992; Iqbal & Ashraf, 2007). In cotton, foliar spray with IAA partially counteracted the effect of water deficit on g_s, A and E (Kumar et al., 2001). In contrast, IBA did not affect E and A in either wild type

or ABA-deficient mutants of tomato (Herde et al., 1997). Taken together, the results suggest that TRP-priming removed both stomatal and nonstomatal limitations, and thus increased A in genetically diverse wheat cultivars.

Priming agents did not increase free IAA concentrations in the leaves, in the salt-intolerant cultivar, in comparison with the control (Table 2). Generally, auxin biosynthesis (Normanly, 2010), auxin influx and efflux carriers (Krupinski

Table 1. Growth parameters of wheat plants (mean±SE, n = 4) at 2.8 dS m^{-1} (control) and 15.0 dS m^{-1} (salt), raised from primed seeds with indoleacetic acid, indolebutyric acid, and tryptophan solutions[(1)].

Pre-sowing seed treatments	Salt-intolerant (MH-97)		Salt-tolerant (Inqlab-91)	
	Control	Salt	Control	Salt
		Plant height (cm)		
Indoleacetic acid				
5.71×10^{-4} mol L^{-1}	82.3±1.42	61.0±1.27	75.2±1.39	62.8±1.20
8.56×10^{-4} mol L^{-1}	82.9±1.46	67.8±1.81	74.4±1.41	65.2±1.34
11.4×10^{-4} mol L^{-1}	80.3±1.52	63.9±1.32	76.8±1.38	66.3±1.29
Indolebutyric acid				
4.92×10^{-4} mol L^{-1}	79.8±1.53	59.0±1.23	80.5±1.41	60.2±1.31
7.38×10^{-4} mol L^{-1}	82.0±1.45	67.8±1.39	77.6±1.42	64.5±1.37
9.84×10^{-4} mol L^{-1}	84.1±1.46	69.7±1.43	78.5±1.38	65.3±1.52
Tryptophan				
4.89×10^{-4} mol L^{-1}	77.8±1.50	74.1±1.58	80.0±1.46	61.1±1.23
7.34×10^{-4} mol L^{-1}	81.2±1.55	72.1±1.43	82.7±1.63	59.4±1.17
9.79×10^{-4} mol L^{-1}	82.5±1.41	67.3±1.46	83.9±1.52	66.5±1.40
Distilled water	83.4±1.43	71.6±1.47	80.5±1.38	58.5±1.32
Untreated	75.4±1.38	57.1±1.20	77.6±1.42	49.9±1.13
LSD 5% = 0.704				
		Shoot fresh weight (g)		
Indoleacetic acid				
5.71×10^{-4} mol L^{-1}	24.1±1.68	14.8±0.78	26.8±2.05	14.9±0.76
8.56×10^{-4} mol L^{-1}	24.2±1.52	15.8±0.83	30.6±3.08	11.6±0.59
11.4×10^{-4} mol L^{-1}	24.3±2.03	17.7±0.93	32.1±3.36	13.8±0.70
Indolebutyric acid				
4.92×10^{-4} mol L^{-1}	26.0±1.94	13.5±0.71	25.3±1.76	15.7±0.79
7.38×10^{-4} mol L^{-1}	28.6±2.79	14.4±0.76	26.5±1.45	16.8±0.85
9.84×10^{-4} mol L^{-1}	25.5±2.24	14.8±0.78	27.2±2.22	14.5±0.74
Tryptophan				
4.89×10^{-4} mol L^{-1}	28.7±2.77	15.5±0.79	31.7±1.88	13.8±0.69
7.34×10^{-4} mol L^{-1}	29.6±3.05	17.0±0.87	28.4±1.76	14.1±0.72
9.79×10^{-4} mol L^{-1}	27.9±2.71	16.5±0.89	29.7±1.38	14.0±0.71
Distilled water	29.8±2.09	15.9±0.84	24.6±2.61	16.4±0.83
Untreated	27.1±1.87	15.2±0.80	28.9±1.71	14.6±0.74
LSD 5% = 1.62				
		Fertile tillers per plant		
Indoleacetic acid				
5.71×10^{-4} mol L^{-1}	2.02±0.16	1.67±0.26	2.00±0.14	1.37±0.04
8.56×10^{-4} mol L^{-1}	1.79±0.09	1.83±0.07	1.92±0.13	1.20±0.08
11.4×10^{-4} mol L^{-1}	1.70±0.15	1.58±0.16	2.05±0.18	1.20±0.08
Indolebutyric acid				
4.92×10^{-4} mol L^{-1}	1.79±0.09	1.33±0.00	2.43±0.20	1.20±0.08
7.38×10^{-4} mol L^{-1}	1.65±0.16	1.54±0.08	1.99±0.11	1.37±0.04
9.84×10^{-4} mol L^{-1}	1.79±0.09	2.17±0.07	2.00±0.10	1.37±0.04
Tryptophan				
4.89×10^{-4} mol L^{-1}	2.09±0.16	1.58±0.28	2.05±0.11	1.71±0.14
7.34×10^{-4} mol L^{-1}	1.65±0.16	1.50±0.22	2.32±0.13	1.71±0.14
9.79×10^{-4} mol L^{-1}	2.02±0.16	1.67±0.26	1.98±0.15	1.20±0.08
Distilled water	1.79±0.09	1.21±0.08	2.13±0.09	1.37±0.14
Untreated	2.09±0.16	1.00±0.00	2.04±0.09	1.08±0.05
LSD 5% = 0.147				

[(1)]LSD, least significant difference; distilled water, hydropriming; untreated, nonprimed seeds.

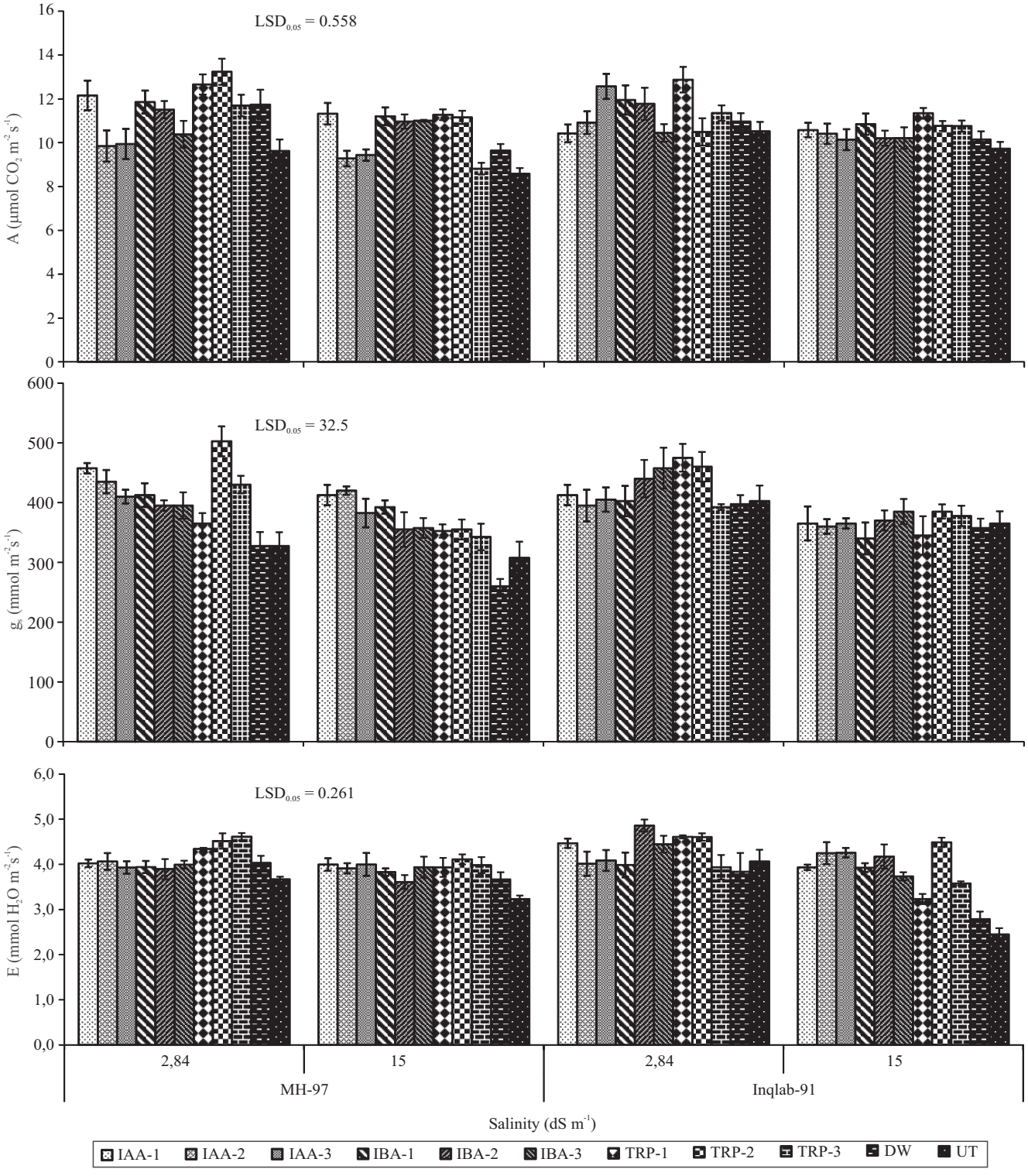

Figure 1. Gaseous exchange characteristics of wheat plants raised from primed seeds with auxins, or tryptophan, and sown under both control (2.82 dS m^{-1}) and saline (15.0 dS m^{-1}) conditions. IAA-1-3, indoleacetic acid (5.71, 8.56, and 11.42 × 10^{-4} mol L^{-1}, respectively); IBA-1-3, indolebutyric acid (4.92, 7.38 and 9.84 × 10^{-4} mol L^{-1}, respectively); TRP-1-3, tryptophan (4.89, 7.34 and 9.79 × 10^{-4} mol L^{-1}, respectively); DW, soaking in distilled water (hydropriming); UT, untreated (nonprimed) seeds; A, net CO_2 assimilation rate; g_s, stomatal conductance; E, transpiration rate.

& Jonsson, 2010), as well as diverse interacting hormones affect free auxin gradients in space and time (Liu et al., 2013). In addition, the potential of plants to conjugate IAA and their capacity to hydrolyse them varies at different developmental stages, as well as under different stresses (Ludwig-Müller et al., 1996). In this context, our results suggested that auxin-priming either altered IAA biosynthesis or accelerated the IAA metabolism for conjugate formations,

Table 2. Free hormonal concentrations (ng g^{-1} fresh weight) in leaves of wheat plants (mean±SE, n = 4), at 2.8 dS m^{-1} (control) and 15.0 dS m^{-1} (salt), raised from primed seeds with indoleacetic acid, indolebutyric acid, and tryptophan solutions[(1)].

Pre-sowing seed treatments	Salt-intolerant (MH 97)		Salt-tolerant (Inqlab 91)	
	Control	Salt	Control	Salt
		Indoleacetic acid		
Indoleacetic acid				
5.71×10^{-4} mol L^{-1}	419.6±32.1	132.2±18.6	303.8±16.0	145.1±08.4
8.56×10^{-4} mol L^{-1}	483.5±07.0	114.6±11.1	149.5±20.1	68.9±14.7
11.4×10^{-4} mol L^{-1}	289.0±30.6	126.2±04.0	282.6±04.2	150.8±24.8
Indolebutyric acid				
4.92×10^{-4} mol L^{-1}	294.1±25.8	159.9±32.3	195.0±27.3	198.8±16.2
7.38×10^{-4} mol L^{-1}	153.6±06.1	92.7±04.9	138.6±01.3	116.3±30.0
9.84×10^{-4} mol L^{-1}	358.2±40.9	154.5±11.7	111.2±23.9	145.6±16.6
Tryptophan				
4.89×10^{-4} mol L^{-1}	251.2±03.6	120.1±16.6	221.3±14.2	96.5±16.8
7.34×10^{-4} mol L^{-1}	318.4±11.9	190.7±11.1	266.5±01.3	165.6±08.1
9.79×10^{-4} mol L^{-1}	216.4±30.3	125.8±06.4	134.9±23.6	96.9±38.0
Distilled water	421.7±18.7	180.9±16.6	561.4±49.1	88.2±01.8
Untreated	472.5±19.5	138.1±35.4	288.9±21.3	101.5±01.6
LSD 5% = 25.6				
		Indolebutyric acid		
Indoleacetic acid				
5.71×10^{-4} mol L^{-1}	214.5±40.6	265.2±32.5	134.3±15.7	94.1±27.5
8.56×10^{-4} mol L^{-1}	71.0±20.2	252.6±39.4	267.7±42.8	88.9±15.4
11.4×10^{-4} mol L^{-1}	263.4±61.9	355.3±62.0	165.5±22.5	70.8±16.0
Indolebutyric acid				
4.92×10^{-4} mol L^{-1}	214.6±01.3	299.6±07.2	119.4±04.9	141.1±31.2
7.38×10^{-4} mol L^{-1}	361.1±46.3	215.8±18.9	305.6±26.4	199.4±23.2
9.84×10^{-4} mol L^{-1}	214.1±06.6	147.4±10.8	131.5±17.7	202.3±10.7
Tryptophan				
4.89×10^{-4} mol L^{-1}	32.2±05.8	166.0±04.6	165.3±24.2	47.3±09.6
7.34×10^{-4} mol L^{-1}	213.9±32.5	293.1±35.7	276.6±18.3	193.2±20.1
9.79×10^{-4} mol L^{-1}	51.5±01.9	22.3±04.2	157.6±01.7	23.7±0.22
Distilled water	98.5±21.3	91.8±62.5	45.4±07.4	192.2±20.0
Untreated	262.4±12.2	43.1±08.2	162.7±31.6	217.1±03.7
LSD 5% = 25.0				
		Abscisic acid		
Indoleacetic acid				
5.71×10^{-4} mol L^{-1}	66.5±10.7	175.1±12.4	57.8±07.0	313.5±12.3
8.56×10^{-4} mol L^{-1}	105.9±13.7	134.4±05.0	88.2±09.3	150.5±07.7
11.4×10^{-4} mol L^{-1}	60.9±05.5	138.6±02.8	90.6±11.6	179.5±09.3
Indolebutyric acid				
4.92×10^{-4} mol L^{-1}	152.9±12.4	167.0±05.5	111.5±16.9	228.9±14.7
7.38×10^{-4} mol L^{-1}	73.9±08.3	102.7±02.1	65.6±15.3	133.6±10.5
9.84×10^{-4} mol L^{-1}	98.6±11.5	131.4±06.3	105.4±06.4	305.5±08.3
Tryptophan				
4.89×10^{-4} mol L^{-1}	109.1±11.9	251.7±19.5	87.9±08.2	195.9±12.5
7.34×10^{-4} mol L^{-1}	152.9±10.2	139.0±03.9	127.3±07.6	127.8±09.1
9.79×10^{-4} mol L^{-1}	207.6±07.5	195.3±08.9	84.3±07.8	209.5±05.6
Distilled water	119.2±01.6	323.4±17.2	93.2±07.0	102.9±10.2
Untreated	96.4±08.6	358.5±14.7	162.1±11.8	127.3±09.6
LSD 5% = 19.9				

[(1)]LSD, least significant difference; distilled water, hydropriming; untreated, nonprimed seeds; IAA, indoleacetic acid; IBA, indolebutyric acid; ABA, abscisic acid.

which caused reduction in free IAA concentration in leaves, in the salt-intolerant cultivar compared to the control.

Plants raised from IAA-primed (11.42×10^{-4} mol L^{-1}) seeds had higher IBA concentration in the salt-intolerant cultivar. In contrast, IBA (4.92×10^{-4} mol L^{-1}) increased free IAA concentration in the leaves, in the salt-stressed plants of the salt-tolerant cultivar. The differential behaviour might be due to differences in auxin-metabolism in the two cultivars. IAA is synthesized both from TRP (TRP-dependent pathway) and indolic TRP precursor (TRP-independent pathway). Even though IAA exists mostly in the form of IAA conjugates (Ludwig-Müller, 2011), salinity could alter IAA concentration in different plants (Javid et al., 2011). The homeostasis between IBA and IAA concentration has been shown to improve survival during drought and salt stress (Tognetti et al., 2010).

Plants of the salt-tolerant cultivar raised from auxin-primed seeds had higher free ABA in the leaves. However, the reverse was true in case of the salt-intolerant cultivar, in comparison with control. Both auxin and ABA act antagonistically to control/modulate different processes in plants (De Smet et al., 2003). Although pre-sowing seed treatments affected ABA levels differently (Table 2), they did not affect grain weight in both cultivars (Figure 2). However, priming with low concentrations (4.89–7.34×10^{-4} mol L^{-1}) of TRP increased fertile tillers per plant and, thus, effectively alleviated the drastic effect of salinity on grain yield, in both cultivars subjected to salt stress.

In the present study, plants of both cultivars raised from TRP-primed seeds had higher free putrescine (PUT), spermidine (SPD) and spermine (SPM) in leaves, in comparison with the control under salt stress (Figure 3).

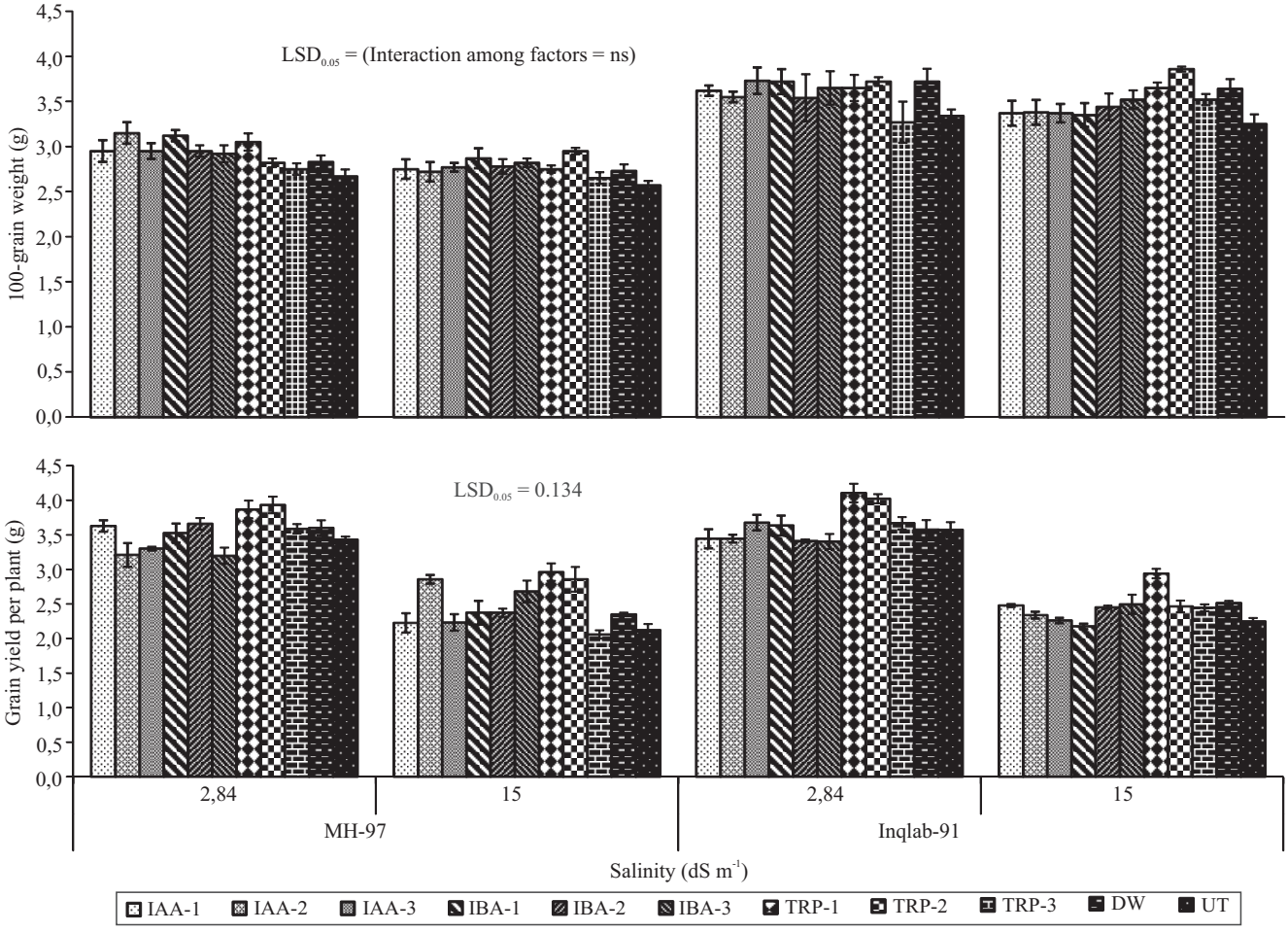

Figure 2. Grain weight and yield of wheat plants raised from primed seeds with auxins, or tryptophan, sown under both control (2.82 dS m^{-1}) and saline (15.0 dS m^{-1}) conditions. IAA-1-3, indoleacetic acid (5.71, 8.56 and 11.42×10^{-4} mol L^{-1}, respectively); IBA-1-3, indolebutyric acid (4.92, 7.38 and 9.84×10^{-4} mol L^{-1}, respectively); TRP-1-3, tryptophan (4.89, 7.34 and 9.79×10^{-4} mol L^{-1}, respectively); DW, soaking in distilled water, hydropriming; UT, untreated (nonprimed) seeds.

The increase in SPM and a decrease in PUT in leaves suggested that TRP accelerated the conversion of PUT into SPD and SPM, which in turn was associated with better plant growth under salt stress. Plant capability to convert PUT into SPD and SPM closely relates to their developing ability under stress conditions (Chattopadhyay et al., 2002). The exogenously applied auxin could also alter polyamines transport (Bagni & Pistocchi, 1991), thus affecting the tissue-specific polyamines concentrations.

Significant positive correlations of grain yield with different gas exchange characteristics, such as A (r = 0.466), g_s (r = 0.474) and E (r = 0.398), was observed in the present study (Table 3). Plant ability to increase growth and grain yield under salt stress was highly

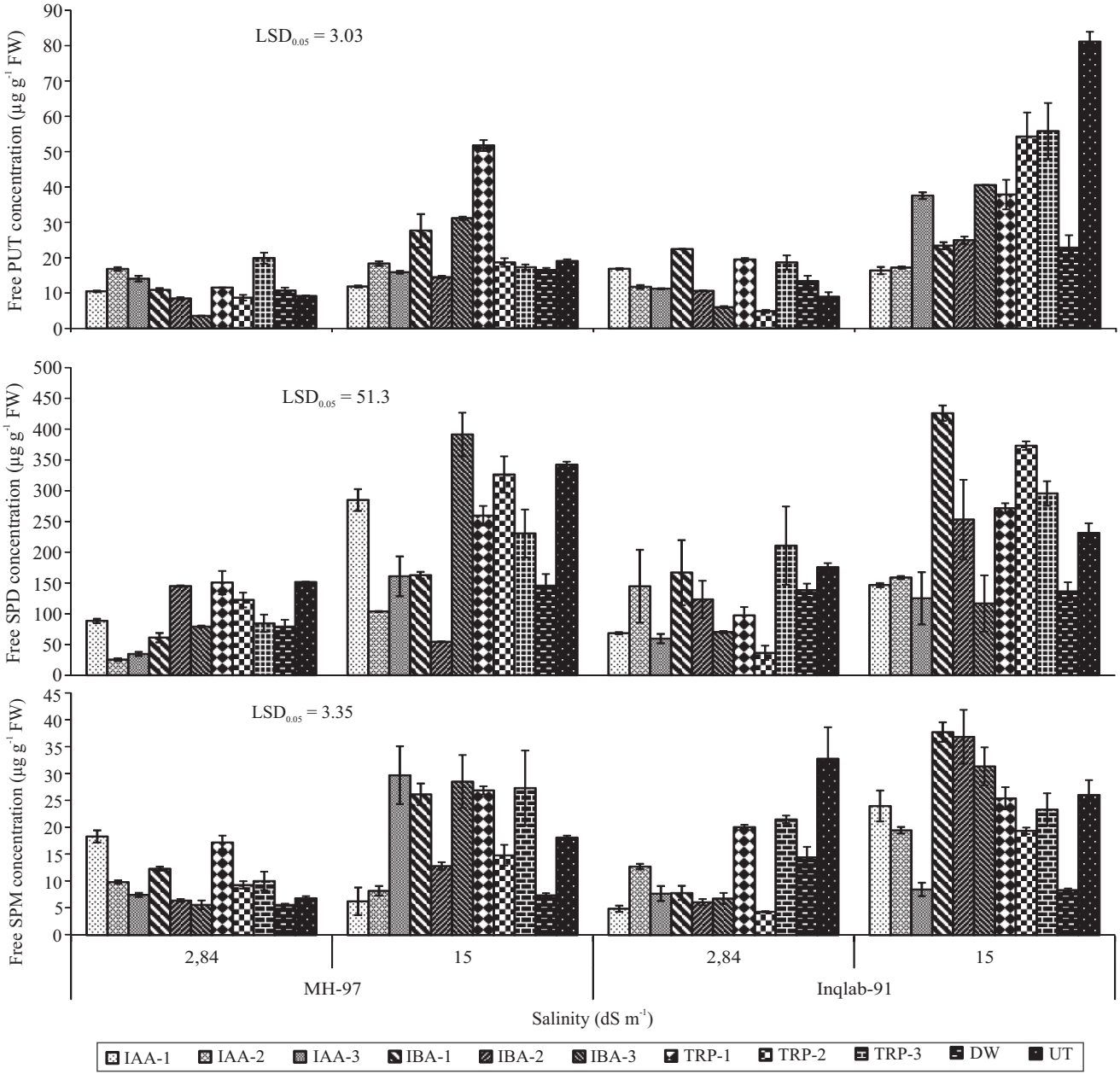

Figure 3. Leaf-concentrations of free putrescine (PUT), spermidine (SPD), and spermine (SPM) of wheat plants raised from primed seeds with auxins, or tryptophan, sown under both control (2.82 dS m^{-1}) and saline (15.0 dS m^{-1}) conditions. IAA-1-3, indoleacetic acid (5.71, 8.56 and 11.42 × 10^{-4} mol L^{-1}, respectively); IBA-1-3, indolebutyric acid (4.92, 7.38 and 9.84 × 10^{-4} mol L^{-1}, respectively); TRP-1-3, tryptophan (4.89, 7.34 and 9.79 × 10^{-4} mol L^{-1}, respectively); DW, soaking in distilled water, hydropriming; UT, untreated (nonprimed) seeds.

Table 3. Pearson's correlation coefficient among grain yield, gas exchange characteristics, and hormonal concentrations of two spring wheat cultivars grown under both saline and nonsaline conditions.

Variable[(1)]	GYP	A	E	gs	GW	IAA	IBA	ABA	PUT	SPD
A	0.466*									
E	0.398*	0.337*								
gs	0.474*	0.374*	0.324*							
GW	0.217*	0.162	0.157*	0.212*						
IAA	0.519*	0.165	0.191*	0.171*	-0.066					
IBA	0.086	0.047	0.039	0.202*	-0.125	-0.087				
ABA	-0.514*	-0.252*	-0.195*	-0.390*	-0.204*	-0.296*	-0.352*			
PUT	-0.442*	-0.129	-0.373*	-0.207*	0.146	-0.388*	-0.167*	0.259*		
SPD	-0.464*	-0.106	-0.237*	-0.296*	-0.071	-0.369*	-0.117	0.324*	0.417*	
SPM	-0.398*	-0.100	-0.183*	-0.181*	0.019	-0.297*	-0.133	0.337*	0.431*	0.542*

[(1)]GYP, grain yield per plant; A, net CO_2 assimilation rate; E, transpiration rate; gs, stomatal conductance; GW, 100-grain weight; IAA, indoleacetic acid; IBA, indolebutyric acid; ABA, abscisic acid; PUT, putrescine; SPD, spermidine; and SPM, spermine. *Significant at 5% probability, by two-tailed test.

correlated with free IAA (r = 0.519) in the leaves. In contrast, free ABA in the leaves showed a highly negative correlation (r = -0.514) with grain yield per plant, in both cultivars under salt stress. Endogenous concentrations of different polyamines (PUT, SPD and SPM) were negatively correlated (r = -0.442, r = -0.464, r = -0.398, respectively) with grain yield. Thus, hormonal balance, rather than the real concentrations of hormones, played an important role in salt-tolerance of spring wheat.

Conclusions

1. Hormonal homeostasis is a key factor responsible for priming-induced salt-tolerance in spring wheat.

2. Auxin-priming-mediated hormonal homeostasis enhances CO_2 assimilation rate, which results in better growth and higher grain yield, and confers tolerance to salinity in spring wheat.

3. Seed priming at low concentration of tryptophan was the most effective priming agent for minimizing losses in grain yield and assimilation rate due to salt stress, in genetically diverse spring wheat cultivars.

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