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Enhancement of Antioxidant Defense System Induced by Hormonal Priming in Wheat

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Use of plant hormones as seed priming agents is known to improve the field performance especially under stressful conditions like salinity. A pot experiment was conducted to study the effects of seed priming of two wheat (*Triticum aestivum* L.) cultivars Auqab-2000 (salt tolerant) and MH-97 (salt sensitive) under saline (15 dS m^{-1}) and non-saline (2.75 dS m^{-1}) conditions. For priming seeds were soaked in aerated water (hydropriming), and solutions of kinetin (Kin; 25 mg L^{-1}), or salicylic acid (SA; 50 mg L^{-1}) for 12 h. All the priming treatments significantly reduced the adverse effects of salinity in terms of improving final emergence, growth and grain yield of both cultivars. Seed priming with SA and Kin improved salt tolerance in both wheat cultivars by the activation of antioxidants, i.e. superoxide dismutase (SOD) and catalase (CAT) to counterbalance the oxidative damage. Albeit, Na^+ and Cl^- contents increased due to salinity, all priming strategies lowered the accumulation of Na^+ and enhanced the accumulation of K^+ in leaves of both cultivars. The results suggest that priming with SA followed by kinetin successfully improved fitness of wheat plants exposed to salt stress.

Keywords: seed priming, ion concentration, plant hormones, salt stress, salt tolerance

Introduction

In addition to the known osmotic stress and ion toxicity effects, salt stress also induces oxidative damage by producing reactive oxygen species (ROS) such as hydroxyl radical, singlet oxygen, superoxide and hydrogen peroxide (Lee et al. 2001). Though the removal of ROS is genetically controlled, however, it can be improved by adopting physiological approaches (Azevedo Neto et al. 2005).

For many crops, among various strategies tested, the use of seed priming is the simplest, low cost and highly effective approach for combating the problems of salinity (Iqbal and Ashraf 2006; Wahid et al. 2007). Seed priming is a controlled hydration of seeds followed by redrying in order to boost up all the pre-germinative metabolic activities before radicle protrusion (Afzal et al. 2008). There is evidence that priming enhances the activi-

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ties of several antioxidative enzymes (SOD, CAT, POD etc.) and non-enzymatic antioxidants (e.g. glutathione and ascorbate). As a result, the lipid peroxidation is greatly reduced during germination (Bailly et al. 1998).

Plant hormones are likely candidates for playing a role in the transformation of stress related signals into changes in gene expression needed to effect appropriate adaptation to suboptimal environmental conditions. Salicylic acid (SA) and kinetin (Kin) have received much attention due to their beneficial role in plant responses to salinity stress. The ameliorative effects of SA and Kin have been well documented in inducing salt tolerance when applied exogenously (Gadallah 1999; Janda et al. 2007) or as a seed treatment (Iqbal and Ashraf 2006) in wheat. By incorporating these hormones during hydration may enhance the priming benefits. To our knowledge, enhanced antioxidant defense system in wheat that alleviates salinity stress by priming with SA or Kin has not been reported. Therefore the present study was undertaken to determine the effect of priming on growth, yield and the antioxidative mechanism of wheat seeds subjected to saline environment.

Materials and Methods

The study was carried out on relatively salt tolerant (Auqab-2000) and sensitive (MH-97) wheat (*Triticum aestivum* L.) cultivars which were obtained from Ayub Agricultural Research Institute, Faisalabad, Pakistan. For priming, seeds were soaked in aerated distilled water (hydropriming), or solutions of $CaCl_2$ (50 mM), Kin (25 mg L^{-1}) or SA (50 mg L^{-1}) for 12 h. The concentration and soaking period was previously optimized (Afzal et al. 2006). After respective priming treatment for specific period, seeds were washed with distilled water and were dried back near to original weight with forced air under shade (it took about 48 h). The laboratory temperature during the drying period was 27 ± 2ºC.

Raising of seedlings and imposition of stress in soil culture

The soil having EC 2.54 dS m^{-1} was collected from a field, passed through 2 mm sieve and filled in glazed earthen pots @ 12 kg per pot. The desired salinity (15 dS m^{-1}) in pots of saline treatment was developed by mixing required amount of NaCl in the soil before filling in the pots. The recommended doses of NPK fertilizers (120 : 60 : 60 kg ha^{-1}) were applied in the form of urea, single super phosphate and potassium sulfate, respectively. The pots were irrigated with tap water (EC 0.90 dS m^{-1}). The primed and non-primed seeds were sown at a depth of 1 cm in each pot. After two weeks of emergence, six seedlings were randomly selected in each pot and the remaining plants were uprooted. Emergence was recorded daily according to the Seedling Evaluation Handbook of Association of Official Seed Analysts (1983). The leaf next to flag leaf, was detached at booting stage, quickly rinsed in distilled water and blotted with tissue paper, for leaf sap extraction to determine Na^+, K^+ and Cl^- (Gorham et al. 1984). At maturity, plants were harvested, threshed and yield parameters were recorded.

Determination of antioxidant enzyme activities

Measurements of antioxidants were made at the boot stage on a fully expanded third leaf (from the top) of each plant. For the estimation of CAT, known amount of frozen (–80ºC) plant material was homogenized in 600 µl medium composed of 50 mM phosphate buffer, pH 7.0 and 1 mM dithiothreitol (DTT). The samples were subjected to centrifugation at 12,000 *g* for 5 min. The supernatant was removed and CAT level was determined as the decrease in absorbance at 240 nm for 3 min by using a spectrophotometer (Spectronic 21 D, Milton Roy) (Dixit et al. 2001). SOD activity was assayed in leaves by using the photochemical NBT method as described by Dixit et al. (2001). The photoreduction of NBT (formation of purple formazan) was measured at 560 nm and an inhibition curve was prepared against different volumes of extract. One unit of SOD was defined as that being present in the volume of extract that caused inhibition of the photo-reduction of NBT by 50%. The ascorbic acid concentration in shoots was determined by using the method as described by Kampfenkel et al. (1995). The assay mixture contained 100 µL sample, 1.9 mL distilled water and 1 mL DCIP solution. The absorbance was measured at 520 nm by using spectrophotometer.

Data analysis

Data were analyzed by ANOVA using a statistical package, MSTATC. A completely randomized design was employed for growth, yield and antioxidant experiments with each response analyzed independently. Significant differences were identified using Fisher's protected least significant difference at 5% confidence interval. In case of antioxidants, standard errors were computed using Microsoft Excel program for comparison of treatment mean values.

Results

Growth attributes

Salt stress significantly ($P < 0.05$) restrained emergence, plant height and fertile tillers per plant of both wheat cultivars (Table 1). All seed priming treatments improved emergence and plant height under both non-saline and saline conditions. However, priming with salicylic acid (SA) followed by kinetin (Kin) maximally enhanced final emergence and plant height of both wheat cultivars under saline and non-saline environment. Stand establishment of salt tolerant Auqab-2000 was significantly better than MH-97. The response of priming treatments was different with respect of the number of fertile tillers per plant in both cultivars. During non-saline conditions, all priming agents failed to improve this attribute in Auqab-2000 while both SA and Kin priming significantly enhanced plant height in case of MH-97. Under stressful environment, maximum number of fertile tillers per plant were reported in plants raised from seeds primed with SA followed by Kin and distilled water.

Table 1. Influence of seed priming on growth attributes of two wheat cultivars at 2.74 dS m^{-1} (control) and 15 dS m^{-1} (saline) conditions

Priming treatments	2.74 dS m^{-1}		15 dS m^{-1}	
	Auqab-2000	MH-97	Auqab-2000	MH-97
	Final emergence (%)			
Untreated	86.83 c	85.00 d	77.50 f	70.00 g
Hydropriming	93.75 b	92.33 b	77.75 f	78.00 f
Kin (25 mg^{-1} L)	92.50 b	94.00 b	81.00 e	81.00 e
SA (50 mg^{-1} L)	96.50 a	96.00 a	84.83 d	83.50 d
	LSD 5% = 1.761			
	Plant height (cm)			
Untreated	58.33 d	54.97 efg	48.83 k	45.33 l
Hydropriming	59.00 c	55.90 e	54.08 gh	50.33 j
Kin (25 mg^{-1} L)	62.33 a	58.10 d	54.58 fg	52.33 i
SA (50 mg^{-1} L)	62.67 a	61.09 b	55.58 ef	53.33 h
	LSD 5% = 0.964			
	Fertile tillers per plant			
Untreated	1.99 c	1.90 d	1.50 j	1.49 j
Hydropriming	1.99 c	1.86 e	1.56 h	1.52 i
Kin (25 mg^{-1} L)	1.99 c	2.29 a	1.68 g	1.57 h
SA (50 mg^{-1} L)	2.00 c	2.21 b	1.76 f	1.75 f
	LSD 5% = 0.017			

Kin, Kinetin; SA, Salicylic acid. Means with the same letters do not differ significantly at $P < 0.05$

Yield and yield components

Salt caused a significant ($P < 0.001$) reduction in number of grains per ear for both cultivars, however, priming significantly reversed it in both cultivars under saline or non-saline conditions. Maximal number of grains per ear were recorded in plants raised from seeds primed with SA in both cultivars (Table 2). During salinity stress, all priming agents significantly affected 100-grain weight in both cultivars as compared to non-priming with the following order SA>Kin>hydropriming. None of the priming improved this attribute when wheat plants of both cultivars were grown without stress conditions. A reduction in grain yield of both cultivars was found as a result of salt stress ($P < 0.001$). However, Auqab-2000 gave more grain yield than MH-97. Albeit, all priming techniques significantly increased grain yield of both cultivars, however, maximum grain yield was reported in plants raised from seeds primed with SA under stressful or non-stressful environments.

Ionic analysis

Salinity stress significantly increased Na^+ and Cl^- levels in plants of both cultivars (Table 3). The plants raised from primed seeds (compared with non-primed seeds) exhibited re-

Table 2. Influence of seed priming on grain yield and yield components of two wheat cultivars at 2.74 dS m^{-1} (control) and 15 dS m^{-1}(saline) conditions

Priming treatments	2.74 dS m^{-1}		15 dS m^{-1}	
	Auqab-2000	MH-97	Auqab-2000	MH-97
	Number of grains per spike			
Untreated	35.01 f	31.75 hi	22.75 l	23.00 l
Hydropriming	41.75 d	37.75 e	25.50 k	27.25 j
Kin (25 mg^{-1} L)	48.50 b	42.00 d	31.25 hi	30.25 i
SA (50 mg^{-1} L)	59.33 a	44.75 c	32.25 gh	33.50 fg
	LSD 5% = 1.640			
	100-grain weight (g)			
Untreated	2.480 c	2.720 b	1.390 hi	1.220 I
Hydropriming	2.937 a	2.600 bc	1.750 ef	1.370 hi
Kin (25 mg^{-1})	2.940 a	2.550 bc	1.830 e	1.460 gh
SA (50 mg^{-1})	3.003 a	2.653 bc	2.013 d	1.620 fg
	LSD 5% = 0.1822			
	Grain yield (g/pot)			
Untreated	3.913 c	3.24 e	2.55 h	2.27 i
Hydropriming	4.473 b	3.88 c	3.44 de	2.58 gh
Kin (25 mg^{-1} L)	4.67 ab	4.1 c	3.46 de	2.79 fg
SA (50 mg^{-1} L)	4.75 a	4.53 ab	3.54 d	2.89 f
	LSD 5% = 0.2168			

Kin, Kinetin; SA, Salicylic acid. Means with the same letters do not differ significantly at $P < 0.05$

duced Na^+ and Cl^- levels in both cultivars. Priming with SA was the most effective in lowering toxic ions in both cultivars under saline or non-saline conditions. It was also noted that priming treatments increased K^+ levels in both cultivars. Priming with SA played a key role in increasing K^+ levels of both cultivars. Similar trend was observed in case of non-saline conditions, however, hydropriming failed to improve K^+ level in MH-97 plants. Higher K^+ levels were observed in salt tolerant than salt sensitive cultivar (Table 3).

Antioxidant defense system

The saline medium significantly altered antioxidant defense system of both cultivars (Fig. 1). Under both normal and saline conditions, hormonal priming (SA and Kin) maximally improved CAT (Fig. 1a), SOD (Fig. 1b), and ascorbic acid contents (Fig. 1c) in both cultivars. It was further noted that Auqab-2000 was more responsive to priming than MH-97 in improving antioxidants under stressful environment. Hydropriming failed to improve activities of antioxidants and ascorbic acid contents in both cultivars.

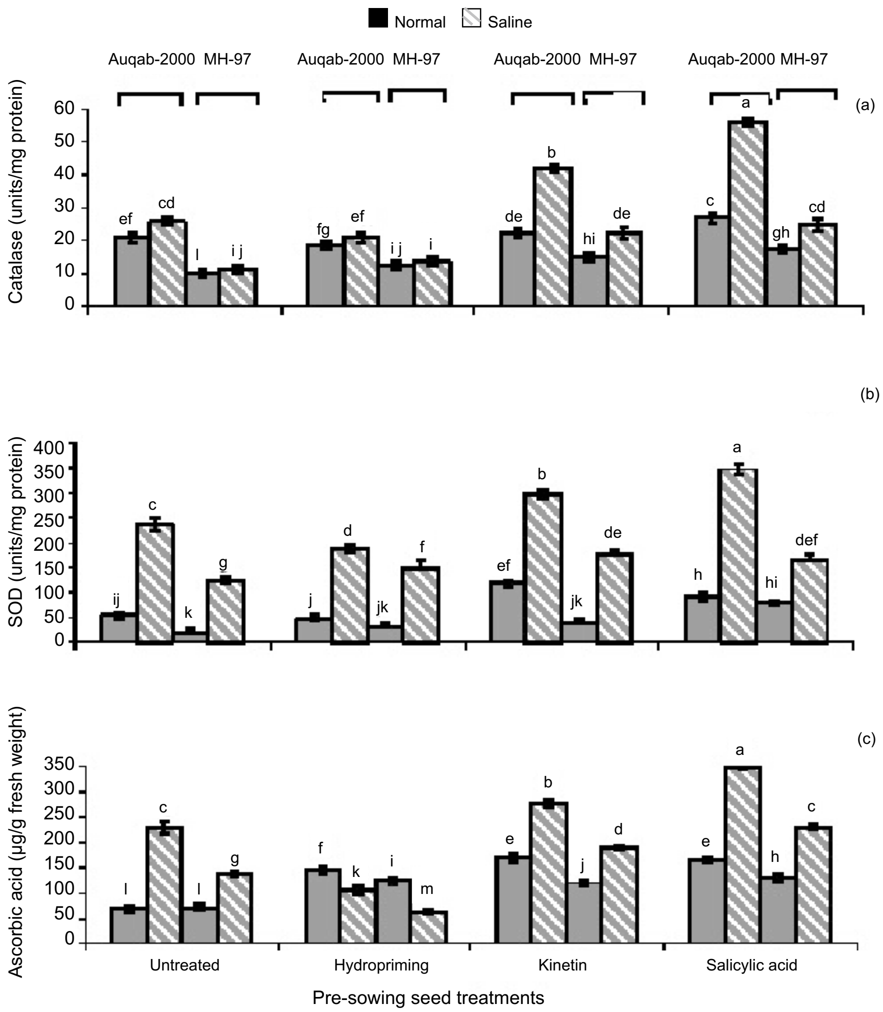

Figure 1. Changes in antioxidant enzymes and ascorbic acid contents of two wheat cv. Auqab-2000 and MH-97 under normal or saline conditions after seed priming. Means denoted by different letters are significantly different at $P \leq 0.05$, as determined by Duncan's multiple range test

Discussion

Hormonal priming with SA and Kin produced no visible symptoms of toxicity; rather it alleviated the deleterious effects of salinity on seed emergence and seedling growth, as evidenced by a significantly higher plant height and number of fertile tillers of salt stressed plants (Table 1). Likewise, the adverse effects of salinity on wheat plants and their improvement by seed conditioning on small scale are well established. Despite this, studies to show the improvement in antioxidant defense system under salinity stress conditions as

Table 3. Influence of seed priming on ionic contents (mol m^{-3}) of two wheat cultivars at 2.74 dS m^{-1} (control) and 15 dS m^{-1} (saline) conditions

Priming treatments	2.74 dS m^{-1}		15 dS m^{-1}	
	Auqab-2000	MH-97	Auqab-2000	MH-97
		Na^+		
Untreated	536.5 i	577.0 g	636.5 e	701.3 a
Hydropriming	508.7 j	504.5 k	601.0 f	679.0 b
Kin (25 mg-1 L)	401.0 n	496.8 l	541.9 h	650.8 c
SA (50 mg-1 L)	350.0 o	491.0 m	601.0 f	648.5 d
		LSD 5% = 1.611		
		K^+		
Untreated	592.7 d	523.5 g	359.0 n	300.0 o
Hydropriming	662.5 c	501.0 h	446.5 m	358.5 n
Kin (25 mg^{-1} L)	692.5 b	535.3 f	474.1 j	465.0 l
SA (50 mg^{-1} L)	696.3 a	576.2 e	484.2 i	470.6 k
		LSD 5% = 1.728		
		Cl^-		
Untreated	276.0 g	338.5 d	320.0 e	363.3 b
Hydropriming	187.5 m	301.0 f	237.5 j	376.0 a
Kin (25 mg^{-1} L)	176.0 n	262.5 h	263.5 h	361.2 c
SA (50 mg^{-1} L)	220.0 k	212.5 l	251.2 l	251.0 l
		LSD 5% = 1.792		

Kin, Kinetin; SA, Salicylic acid. Means with the same letters do not differ significantly at $P < 0.05$

a result of treatment with SA and Kin are scanty. It is noteworthy that growth and grain yield of salt stressed seedlings was significantly enhanced due to seed priming especially SA application (Tables 1 and 2). This increase in grain yield was found to be associated with an earlier and higher emergence and improved yield contributing factors (number of grains per ear and 100-grain weight) (Iqbal and Ashraf 2006). Presumably seed enhancements result in the initiation of early metabolic processes of emergence (Bewley and Black 1982). There are also reports those suggest that Kin is implicated in mobilization of storage reserves for utilization during germination (Hocart et al. 1990) that might be the reason of its effectiveness as priming agent.

The results of present study also show that hormonal priming with SA or Kin alleviated NaCl stress on growth and grain yield, decreased Na^+ and Cl^- accumulation and slightly improved uptake of K^+ (Table 3) indicating that pre-soaking of seeds with growth regulators increased salt tolerance causing increased absorption of essential nutrients and restricted absorption of toxic elements (Chipa and Lal 1993). Pretreatment of SA induced a reduction in Na^+ absorption and toxicity and increased K^+ contents further reflecting low membrane injury, high water content and dry matter production (El-Tayeb 2005).

The enhancement was shown to be closely related with the genetic background of cultivars (sensitive/tolerant), level of salt stress (NaCl concentration and duration) and pre-sowing seed treatments. Therefore, it is likely that efficient scavenging system against

ROS in both wheat cultivars due to pre-sowing seed treatments is a key component for tolerance to NaCl stress (Wahid et al. 2007). Strongest activities of CAT and SOD (Fig. 1a and 1b) along with ascorbic acid contents (Fig. 1c) were exposed in wheat plants raised from seeds treated with SA followed by Kin compared with that of hydropriming and non-priming treatments. An enhanced activity of antioxidant system in plants under stress is often related to the improvement of stress tolerance (Wang and Li 2006). Enhanced activity of certain antioxidant enzymes with SA application under stress conditions including salinity has been observed (Stevens et al. 2006; Janda et al. 2007). The present results and those obtained by Al-Hakimi and Hamada (2001) provide an evidence of the role played by the salicylic acid in plant adaptation mechanisms under salinity stress. There are reports which indicate that salicylic acid is a key player to improve the acclimation to different abiotic stressors especially salt stress probably due to the increased activation of aldose reductase, aldehyde oxidase and ascorbate peroxidase enzymes and to the accumulation of osmolytes, such as sugars, sugar alcohol or proline (Tari et al. 2002; Szepesi et al. 2009; Tari et al. 2010). Higher ascorbic acid contents of both wheat cultivars in the present study further confirmed by Cao et al. (2009) who reported that higher glutathione/oxidized glutathione ratio and ascorbic acid/dehydroascorbate ratio in plants by SA application during salinity stress might be the key reason for their salt tolerance. It is also possible that priming with Kin that increased grain yield in both wheat cultivars might be boosted SA concentration in the plants that ultimately improved antioxidant system (SOD and CAT) in salt stressed wheat plants (Iqbal and Ashraf 2006).

In conclusion, seed priming treatments used in the present study were effective in inducing salt tolerance in wheat, however, hormonal priming with SA proved be the most effective in alleviating salt stress on grain yield of both wheat cultivars. The activation of antioxidants in wheat plants due to SA and Kin has beneficial effects on growth and grain yield by prevention of oxidative damage and enhanced capacity of wheat to withstand high salinity. Further, the beneficial effect of priming of both cultivars under salt stress can be attributed increased K^+ accumulation and decreased Na^+ and Cl^- uptake in the salt stressed plants.

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