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Seed Priming: A Shotgun Approach for Alleviation of Salt Stress in Wheat

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Abstract

Salinity is a major abiotic constraint to sustainable crop production. Seed priming is a useful tool to enhance the performance of crops on saline soils. A field study was carried out to explore the potential of priming to improve salt tolerance in wheat cultivars MH-97 and SARC-1. Seeds were primed in distilled water (hydropriming) and aqueous solutions containing 50 mg L^{-1} of salicylic acid (SA), kinetin (Kin), ascorbate and 50 mM calcium chloride ($CaCl_2$) for 12 h. After priming, seeds were sown in normal (0.31 dS m^{-1}) and saline (10 dS m^{-1}) fields. Seed priming with ascorbate, SA and Kin effectively alleviated the salinity-induced damage in both wheat cultivars; however, ascorbate priming was the most effective. Seed priming with SA, Kin and ascorbate significantly decreased the uptake of Na^+ and Cl^- and enhanced the uptake of K^+ in leaves of both cultivars under salinity stress. These results suggest that priming with seed priming with ascorbate, SA and Kin are effective strategies to improve the wheat productivity under salinity stress.

Keywords: Plant hormones; Seed priming; Salinity; Salt tolerance; Wheat

Introduction

Salinity severely damages crop plants through osmotic and ionic stresses, which reduces shoot growth and photosynthetic rate (Munns and Tester, 2008). Salinity can also induce oxidative stress which causes molecular destruction to plant cells through the production of reactive oxygen species (ROS) (Apel and Hirt, 2004). Plants possess ROS scavenging enzymes such as catalase, glutathione peroxidase and superoxide dismutase and non-enzymatic compounds such as glutathione, ascorbic acid and carotenoids (Sairam *et al.,* 2005). Reactive oxygen species production under salinity and their scavenging by the activation of antioxidants have been well documented for crops like wheat (Sairam *et al.,* 2005; Wahid *et al.,* 2007), rice (Vaidyanathan *et al.,* 2003) and beans (Palma *et al.,* 2009). Although removal of ROS by activation of antioxidants is genetically controlled yet it can be improved by adopting physiological approaches (Azevedo Neto *et al.,* 2005).

A big amount has been spent on reclamation of saline soils with a low success rate owing to reduce availability of quality of water and low soil permeability. Hence, measures for effective utilization of saline lands for crop production needs be sort out. It involves the development of salt tolerant crop cultivars and management practices to mitigate salinity effects. Breeding crops for salinity tolerance is limited due to complex nature of traits for selection. Different approaches like conventional breeding and selection, transgenes production (Zhao *et al.,* 2006), exogenous application of osmolytes, osmoprotectants or plant hormones (Ashraf *et al.,* 2008) and pre-sowing seed treatments (El-Tayeb, 2005; Afzal *et al.,* 2006) have been employed for alleviation of salinity stress in crops.

Among various strategies, seed priming is the simplest and cost effective approach in combating salinity problems in field crops (Wahid *et al.,* 2007; Afzal *et al.,* 2008). Seed priming is a technique involving controlled soaking of seeds in aerated solution of water, osmotic or nutrients and then redrying in order to boost up seed metabolic activities before radicle emergence. Priming enhanced the seed germination through protein synthesis, repair of nucleic acid and membranes (Pandey, 1989; Fujikura and Karssen, 1995). Different priming approaches viz., hydropriming (Basra *et al.,* 2006), hormonal priming (Iqbal and Ashraf, 2006; Afzal *et al.,* 2006) and halopriming (Basra *et al.,* 2006; Afzal *et al.,* 2008, 2012) improved salinity tolerance in wheat. There is evidence that priming improves the

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activity of antioxidant compounds and ROS scavenging enzymes (Bailly *et al.*, 1998), hence seed priming may be employed to enhance the crop performance under salinity stress.

Plant hormones are likely candidates for playing a role in the induction of resistance to suboptimal environmental conditions. Salicylic acid and kinetin have gained much attraction due to their beneficial role under salinity stress (Gadallah, 1999; El-Tayeb, 2005). Incorporating these hormones during hydration may enhance the priming effects. Although many studies have evaluated the significant impact of seed priming on wheat performance under saline conditions (Iqbal and Ashraf, 2006; Afzal *et al.*, 2011, 2012; Jafar *et al*., 2012), but extensive field appraisal of seed priming is required before its dissemination to the farmers. Therefore current study was carried out to investigate the influence of different priming techniques on stand establishment, growth and yield of wheat plants grown under normal and saline fields.

Materials and Methods

Seed of wheat cultivars, SARC-1 (moderately salt tolerant) and MH-97 (salt sensitive) was procured from Ayub Agricultural Research Institute, Faisalabad, Pakistan. For priming treatments, seeds of both cultivars were soaked in distilled water (hydropriming), 50 mM $CaCl_2$ (osmopriming), 50 mg L^{-1} ascorbate (AsA priming), 50 mg L^{-1} kinetin (Kin priming) or 50 mg L^{-1} salicylic acid (SA priming) for 12 h. The concentrations and soaking periods for the seed material used in the present study were adopted from a previous pre-optimization study (Afzal *et al.*, 2006). After respective priming treatment, seeds were washed with distilled water and were re-dried through forced air by keeping on blotting papers in the laboratory benches for two days. Non-primed and seeds soaked in water (hydropriming) were taken as control.

Experiments were conducted at two sites in Faisalabad, Pakistan. One trial was conducted at nearby farmer's field on saline sandy clay soil (EC > 10 dS m^{-1}, pH 9.8, while the other one was conducted on non-saline sandy clay soil (EC 0.31 dS m^{-1}, pH 7.9, organic matter 0.85%), at Agronomic Research Area, University of Agriculture, Faisalabad, Pakistan. Both experiments were replicated thrice in randomized complete block design with factorial arrangement. The net plot size was 2.0 m × 3.0 m. A single plot having 8 rows with row to row spacing 25 cm was used as experimental unit. Seed rate of 120 kg ha^{-1} was used. All other agronomic operations were kept standard and uniform for all treatments.

Emergence was recorded daily and mean emergence time (MET) and time to get 50% emergence (E_{50}) were calculated according to Ellis and Roberts (1981) and Farooq *et al*. (2005), respectively. Leaf area index (LAI) was calculated according to formula given by Hunt (1978).

Determination of Ions in Leaves

The penultimate leaf was detached at booting stage for extraction of leaf sap to find out Na^+ and K^+ (Gorham *et al.*, 1984). Sodium and potassium ions were determined with the help of flame Photometer (Model 410, Sherwood Scientific Ltd, UK). Chloride in the leaf sap was measured by using Chloride analyzer.

At maturity, yield and yield related traits were recorded after harvesting plants manually.

Data Analysis

A statistical package, MSTATC was used to analyze data following analysis of variance technique. Significant differences among treatment's means were identified using least significant difference test at 5% probability. Microsoft excel program was used for data graphics and to compute standard errors for treatments comparison.

Results

Salt stress significantly delayed the seedling emergence and emergence count per unit area in both wheat cultivars than normal conditions. Most of the seed priming treatments improved emergence potential and decreased E_{50} and MET under normal and saline conditions (Table 1). Under saline conditions, priming with ascorbate followed by hormonal priming (Kin and SA) maximally enhanced emergence, E_{50} and MET of both wheat cultivars. Emergence of SARC-1 was significantly better than MH-97. Although MET was increased in salt stressed wheat plants however, primed seeds of MH-97 took less time for emergence. On the other hand, primed seeds of SARC-1 took same time to emerge as non-primed seeds under saline conditions.

Salt stress inhibited ($P<0.001$) plant height of wheat cultivars. Nevertheless, cultivars responded differentially for this parameter and SARC-1 stood tall than MH-97. Under salinity, ascorbate priming significantly improved plant height than rest of the priming treatments. With this, kinetin and other priming agents were also effective in improving plant height of both cultivars (Table 2).

Productive tillers of both wheat cultivars were less under salinity than normal conditions (Table 2). Under non-saline conditions, the response of all priming treatments was variable for both cultivars. Nonetheless under salt stress, maximum number of fertile tillers were recorded in plots where seeds primed with ascorbate were sown. It was followed by SA and Kin.

Leaf area index (LAI) of plants of both cultivars was significantly affected by various seed priming treatments (Table 2). Under normal conditions, priming improved LAI of SARC-1 cultivar; while, hydropriming and halopriming failed to do so for MH-97. However, improvements in LAI by seed priming were more pronounced under salinity stress benefiting both wheat cultivars.

Salt stress caused up to 50% reduction in number of

Table 1: Influence of seed priming techniques on emergence and seedling vigor of two wheat cultivars under normal and saline conditions

	Normal (0.31 dS m^{-1})		Saline (15 dS m^{-1})	
	SARC-1	MH-97	SARC-1	MH-97
Emergence count (m^{-2})				
Untreated	215 cd	162 f	93 ij	82 j
Hydropriming	240 ab	173 ef	134 gh	120 h
Ascorbate (50 mg L^{-1})	227 bc	207 d	140 g	134 gh
$CaCl_2$ (50 mM)	211 cd	180 e	132 gh	101 i
Salicylic acid (50 mg L^{-1})	246 a	198 d	127 gh	121 h
Kinetin (50 mg L^{-1})	205 d	161 f	142 g	120 h
LSD 5% = 17.29				
Time to 50% emergence (days)				
Untreated	10.36 hi	12.04 g	17.38 b	18.64 a
Hydropriming	7.95 l	10.58 hi	15.41 def	16.28 c
Ascorbate (50 mg L^{-1})	8.87 jk	10.57 hi	14.63 f	14.87 ef
$CaCl_2$ (50 mM)	9.10 j	10.49 hi	14.77 ef	15.80 cd
Salicylic acid (50 mg L^{-1})	7.81 l	10.71 h	15.59 de	14.84 ef
Kinetin (50 mg L^{-1})	8.22 kl	09.87 i	15.34 def	15.21 def
LSD 5% = 0.74				
Mean emergence time (days)				
Untreated	19.33 e	18.67 e	24.67 bc	26.33 a
Hydropriming	17.00 f	16.00 fgh	23.00 bc	23.67 bc
Ascorbate (50 mg L^{-1})	16.33 fgh	17.00 f	22.67 cd	22.00 cd
$CaCl_2$ (50 mM)	15.67 fgh	16.63 fg	23.00 bc	23.00 bc
Salicylic acid (50 mg L^{-1})	16.00 fgh	15.67 fgh	23.00 bc	23.67 bc
Kinetin (50 mg L^{-1})	14.67 h	16.67 fg	23.33 bc	23.00 bc
LSD 5% = 1.64				

Table 2: Influence of seed priming on growth attributes of two wheat cultivars under normal and saline conditions

Priming treatments	Normal (0.31 dS m^{-1})		Saline (15 dS m^{-1})	
	SARC-1	MH-97	SARC-1	MH-97
Plant height (cm)				
Untreated	67.87 d-g	78.97 b	57.03 jk	50.37 l
Hydropriming	68.95 cde	79.23 b	65.20 fgh	59.03 ij
Ascorbate (50 mg L^{-1})	72.50 c	84.02 a	65.75 e-h	62.15 hi
$CaCl_2$ (50 mM)	68.55 def	85.45 a	62.35 hi	54.55 k
Salicylic acid (50 mg L^{-1})	71.00 cd	86.62 a	65.33 fgh	57.53 jk
Kinetin (50 mg L^{-1})	71.03 cd	84.57 a	64.43 gh	57.20 jk
LSD 5% = 3.745				
Number of fertile tillers (m^{-2})				
Untreated	378.00 d-g	353.00 ghi	192.70 l	202.70 l
Hydropriming	432.00 ab	390.30 cde	309.70 jk	341.00 hij
Ascorbate (50 mg L^{-1})	449.70 a	390.30 cde	368.00 e-h	352.00 ghi
$CaCl_2$ (50 mM)	403.30 bcd	387.00 c-f	332.30 ij	284.00 k
Salicylic acid (50 mg L^{-1})	399.30 cde	351.00 ghi	342.00 hij	329.70 ij
Kinetin (50 mg L^{-1})	402.00 bcd	414.7 bc	357.00 f-i	338.70 hij
LSD 5% = 32.47				
Leaf Area Index				
Untreated	6.17 i	8.05 d	4.39 l	5.01 k
Hydropriming	7.12 f	7.99 d	6.17 i	6.65 g
Ascorbate (50 mg L^{-1})	8.81 b	8.47 c	7.24 ef	7.13 f
$CaCl_2$ (50 mM)	7.35 e	8.10 d	5.72 j	6.41 gh
Salicylic acid (50 mg L^{-1})	7.37 e	9.27 a	5.75 j	6.95 g
Kinetin (50 mg L^{-1})	7.50 e	9.23 a	5.11 k	6.26 hi
LSD 5% = 0.214				

grains per spike of both cultivars in the present study. However, seed priming techniques were quite effective and significantly improved this attribute. Maximum number of grains per spike in both cultivars was recorded in plots, where seed primed with ascorbate were sown during salt stress (Table 3). Besides normal conditions, all priming agents also affected 1000-grain weight in both cultivars as compared to non-primed control under salinity and order of improvement was SA>Kin>$CaCl_2$> hydropriming.

Table 3: Influence of seed priming on grain yield and yield components of two wheat cultivars under normal and saline conditions

Priming treatments	Normal (0.31 dS m^{-1})		Saline (15 dS m^{-1})	
	SARC-1	MH-97	SARC-1	MH-97
Number of grains per spike				
Untreated	46.30 f	51.50 e	23.73 m	23.33 m
Hydropriming	55.73 a	54.40 a-d	26.77 l	27.73 kl
Ascorbate (50 mg L^{-1})	55.10 ab	54.83 abc	30.23 j	34.70 g
$CaCl_2$ (50 mM)	53.33 d	53.73 bcd	32.03 h	30.73 ij
Salicylic acid (50 mg L^{-1})	53.90 bcd	54.77 abc	32.73 h	31.90 hi
Kinetin (50 mg L^{-1})	53.57 cd	54.80 abc	33.17 h	28.80 k
LSD 5% = 1.233				
1000-grain weight (g)				
Untreated	30.23 f-i	37.17 bc	25.17 m	25.50 lm
Hydropriming	32.00 ef	38.03 ab	25.97 klm	26.50 j-m
Ascorbate (50 mg L^{-1})	35.97 cd	38.37 ab	30.10 f-i	30.3 7 fgh
$CaCl_2$ (50 mM)	33.34 de	37.07 bc	27.77 ijkl	25.50 lm
Salicylic acid (50 mg L^{-1})	32.97 de	40.27 a	29.07 ghi	28.90 g-j
Kinetin (50 mg L^{-1})	31.27 efg	39.53 ab	29.20 ghi	28.17 h-k
LSD 5% = 2.546				
Grain yield (kg ha^{-1})				
Untreated	2549 h-k	3279 d	1412 m	2089 l
Hydropriming	3095 def	3796 c	2109 l	2680 g-j
Ascorbate (50 mg L^{-1})	2844 e-h	3883 bc	2383 ijk	3124 de
$CaCl_2$ (50 mM)	2946 efg	3670 c	2383 jkl	2889 efg
Salicylic acid (50 mg L^{-1})	2854 e-h	4211 a	2449 jk	3016 def
Kinetin (50 mg L^{-1})	2798 f-i	4125 ab	2310 kl	3008 def
LSD 5% = 322				

Reduced grain yield was observed in both cultivars during salinity stress. Albeit all priming techniques significantly increased the grain yield of both cultivars, ascorbate priming inflicted maximum grain yield under saline and non-saline conditions.

Salinity stress significantly increased Na^+ and Cl^- levels in plants of both wheat cultivars (Table 4). Wheat plants of both cultivars that developed from primed seeds accumulated comparatively lower Na^+ levels than those derived from non-primed seeds. Except hydropriming, all priming treatments averted Na^+ and Cl^- levels in both cultivars under saline conditions.

Soil salinity caused marked decrease in K^+ uptake in the leaves of both wheat cultivars. All priming treatments played a key role in increasing K^+ level of both cultivars. Similar trend was observed in case of non-saline conditions. Higher K^+ level were observed in SARC-1 than MH-97. Ascorbate and SA priming reduced the effect of salinity by maximally increasing K^+ level in both cultivars (Table 4).

Discussion

Seed priming can lessen the deleterious effect of salinity on emergence, seedling establishment and ultimately enhances yield of wheat crop (Wahid *et al.,* 2007; Ashraf *et al.,* 2008). The efficiency of seed priming can be enhanced by using

Table 4: Influence of seed priming on ionic contents (mg kg^{-1} dry weight) of two wheat cultivars normal and saline conditions

Priming treatments	Normal (0.31 dS m^{-1})		Saline (15 dS m^{-1})	
	SARC-1	MH-97	SARC-1	MH-97
	Na^+			
Untreated	117.3 d	110.5 defg	145.5 b	132.7 c
Hydropriming	104.5 fghi	103.4 ghi	163.2 a	129.3 c
Ascorbate (50 mg L^{-1})	94.60 j	99.20 ij	113.7 de	116.0 de
$CaCl_2$ (50 mM)	105.4 fghi	102.8 ghi	130.6 c	117.4 d
Salicylic acid (50 mg L^{-1})	99.33 ij	105.1 fghi	108.3 efgh	111.9 de
Kinetin (50 mg L^{-1})	102 .23 hij	108.3 efgh	115.9 de	113.8 de
	LSD 5% = 7.888			
	K^+			
Untreated	320.3 efg	317.2 fg	92.30 kl	76.50 l
Hydropriming	356.2 bcd	344.5 cde	115.2 ijk	93.70 kl
Ascorbate (50 mg L^{-1})	388.5 a	380.9 ab	119.03 ij	105.5 ijk
$CaCl_2$ (50 mM)	367.1 abc	330.4 ef	120.7 ij	91.80 kl
Salicylic acid (50 mg L^{-1})	332.7 def	290.4 h	124.3 i	113.2 ijk
Kinetin (50 mg L^{-1})	315.5 fgh	301.1 gh	94.70 kl	96.80 jkl
	LSD 5% = 25.54			
	Cl^-			
Untreated	231.5 i	362.5 d	362.0 d	450.2 a
Hydropriming	225.3 i	300.0 g	350.0 de	400.6 c
Ascorbate (50 mg L^{-1})	175.5 k	210.5 j	265.5 h	300.0 g
$CaCl_2$ (50 mM)	300.0 g	316.0 f	320.0 f	420.3 b
Salicylic acid (50 mg L^{-1})	190.0 j	230.5 i	260.0 h	340.0 e
Kinetin (50 mg L^{-1})	200.0 j	260.5 h	300.5 g	352.5 de
LSD 5% = 12.07				

antioxidants or plant hormones as priming agents that might alleviate the adverse effects of salinity on crops. Ascorbate priming and hormonal priming with SA or Kin induced earlier and synchronized emergence under saline or non-saline conditions as is evident from the data for MET, E_{50} and emergence count in both cultivars (Table 1). The order of response by priming was ascorbate>kinetin> SA>hydropriming. Early emergence (lower MET) by priming under saline conditions might be due to improved repair and synthesis of nucleic acids or protein during priming, which resulted in higher germination (Bray *et al.*, 1989; Soon *et al.*, 2000). Alleviation of toxic effects of salinity stress in wheat by priming have been reported by many scientists (Harris *et al.*, 1999; Kamboh *et al.*, 2000; Basra *et al.*, 2006).

In present study, priming with ascorbate, SA and Kin alleviated the adverse effects of salinity on growth, as evident from significantly higher plant height, number of fertile tillers and LAI of salt stressed wheat plants (Table 2). It is noteworthy that growth and grain yield under salinity were maximally enhanced by seed priming with ascorbate, SA and kinetin (Table 3). Although $CaCl_2$ improved growth and grain yield as compared to non-primed seeds under saline conditions yet its response was similar to hydropriming and less effective than other priming treatments (Table 2 and 3). The role of SA for promotion of growth and counteraction of stress induced growth inhibition is well reported for various crop species (Zhou *et al.*, 1999; Shakirova *et al.*, 2003; El-Tayeb, 2005). This improvement in growth and grain yield can be correlated with an earlier and higher emergence and improved yield contributing factors (grains per spike and 1000-grain weight) (Iqbal and Ashraf, 2006). Priming with ascorbate exerted some favorable effects on growth and transpiration of wheat seedlings counteracting the damaging effects of salts (Al-Hakimi and Hamada, 2001). Exposure of wheat plants to SA upregulated cell division in apical zones of seedling roots and thus increased plant growth and ultimately wheat productivity (Shakirova *et al.*, 2003). Kinetin is implicated in mobilization of storage reserves for utilization during germination (Hocart *et al.*, 1990).

Many studies have shown that high salinity may distress nutrient-ion activities in the plants, making plants susceptible to osmotic, ionic stresses along with to nutritional disorders that reduce yield and quality of plants (Essa, 2002; Munns and Tester, 2008). However, priming techniques (ascorbate, SA and Kin) maximally decreased the uptake of Na^+, Cl^- and increased the uptake of K during salinity stress (Table 4). Reduced Na^+ uptake from SA priming is an indication of less membrane injury, higher relative water content and productivity (El-Tayeb, 2005). More uptake of beneficial elements and less uptake of toxic mineral elements is generally considered as better salt tolerance strategy of crops (Greenway and Munns, 1980; Shannon and Grieve, 1999). For example, priming with ascorbate, SA and Kin in our study, alleviated NaCl stress on growth and grain yield, decreased Na^+ and Cl^- accumulation and slightly improved uptake of K^+ concentration in leaf indicating that pre-soaking of seeds with growth regulators increased salt tolerance causing increased absorption of essential nutrients and restricted absorption of toxic elements. Due to influence of Ca^{2+} on membranes, seeds primed with $CaCl_2$ protected wheat plants from adverse effects of salt stress and improved the growth of plants under saline conditions by decreasing uptake of toxic ions.

In conclusion, seed priming treatments (Kin, SA, $CaCl_2$ and ascorbate) used in the present study were effective in inducing salt tolerance in wheat by improving early stand establishment, growth and ionic homeostasis. Among all priming agents, priming with ascorbate was relatively non-toxic, less expensive and the most effective in alleviating salt stress in both wheat cultivars under field conditions. Ascorbate priming can therefore be recommended to farmers for the enhancement of the performance of wheat under saline conditions.

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