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Alleviation of salt stress in fine aromatic rice by seed priming

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Abstract

This study investigated the potential of seed priming for induction of salt tolerance in rice. The seeds of two fine aromatic rice *cv.* Shaheen Basmati (salt tolerant) and Basmati-2000 (salt sensitive) were soaked in aerated solutions of $CaCl_2$ (Ψ_s=-1.25 MPa), KCl (Ψ_s=-1.25 MPa), and H_2O_2 (50 mM) each for 36 h. Seeds soaked in distilled water and non-primed seeds were taken as control for comparison. Two experiments were conducted *in vitro* and primed seeds were exposed to 0, 40 and 80 mM NaCl in petri dishes and sand culture during germination and emergence respectively. Priming with $CaCl_2$ followed by KCl were more effective in inducing salt tolerance of both rice cultivars owing to enhanced germination capacity, speed of germination, seedling length and dry weight in saline medium. Incremental salinity reduced total chlorophyll percentage (TCP) by two fold in both cultivars but higher TCP was found at all salinity levels from $CaCl_2$ primed raised seedlings in salt tolerant cultivar. Decreased leaf Na^+ accumulation and increased K^+ uptake were found in $CaCl_2$ primed seedlings at moderate salinity in both cultivars but effect was more pronounced in Shaheen Basmati. Delayed germination and seedling growth with H_2O_2 pre-treatment indicates sensitivity of H_2O_2 primed seeds to salts and might be the result of higher Na^+ uptake and less chlorophyll contents in rice seedlings. Nonetheless, enhanced germination and seedling growth, high Na^+/K^+ exclusion indicates Shaheen Basmati tolerance to salinity than Basmati-2000 and seed priming with $CaCl_2$ seems more promising in induction of salinity tolerance in fine aromatic rice.

Keywords: salinity tolerance; seed priming; salts; rice.
Abbreviations: FGP- final germination percentage, MGT- mean germination time, T_{50}- time taken to 50% germination, TCP- total chlorophyll percentage.

Introduction

Soil salinity has become a severe threat to ensure food security in the developing world. Increasing salinity had significant impact on food production and more agriculture lands are expected to become salt affected due to climate change effect (Rengasamy, 2006). Cereals contribute mainly to food production and growing grain crops on saline soils require adoption of different strategies for sustainable crop production. The total worldwide area of land affected by salinity is about 190 million ha (FAO, 2010) and about 48 million ha of saline soil in South and South East Asia is unsuitable for rice cultivation by rice growers (Ponnamperuma and Bandyopadhya, 1980). Rice (*Oryza sativa* L.) being staple food for most of Asians is susceptible to salt stress (Munns and Tester, 2008) particularly during the early seedling stage (Li and Xu, 2007). Salinity affects the seed germination by creating osmotic stress due to reduced water uptake or through ionic imbalance due to toxic effects of sodium (Na^+) and chloride (Cl^-) ions (Hosseini et al., 2003). Salinity also upsets plant hormone level and reduces the utilization of seed reserves (Ahmad and Bano, 1992). Salinity induced stress inhibited seed germination constraints to achieve uniform seedling stand in rice (Almansouri et al., 2001) and ultimately diminishes economic yield and quality of produce (Ali et al., 2004). Rice is sensitive especially at young seedling stage, where varying degree of mortality occurs at 50 mM NaCl and about 50% of 14 days old seedlings may die in most salt sensitive varieties within ten days of salinity stress (Flowers and Yeo, 1981). Efficient strategies are required for effective utilization of saline lands for crop growth. Improvement of salinity tolerance in crop species is one potential strategy in overcoming salinity problems in agriculture (Flowers, 2004; Yamaguchi and Blumwald, 2005). Development of salt tolerant plants through conventional breeding programs is very slow due to the complexity of salt tolerance and lack of reliable traits for selection (Yamaguchi and Blumwald, 2005). Nonetheless, exogenous application of osmolytes, osmoprotectants or plant hormones through foliar or seed is a good option to alleviate the adverse effects of salinity stress on crops (Ashraf et al., 2008). Seed priming, a controlled hydration process followed by re-dyring is pragmatic approach to counteract the salinity effects in many crops because of its simplicity, low cost and effectiveness (Wahid et al., 2007; Afzal et al., 2011). The use of salts in priming solution or addition of signaling molecule like H_2O_2 can be used as adaptation strategy to improve salt tolerance in crops (Afzal et al., 2008a; Iqbal and Ashraf, 2007). Priming treatments are being used to shorten the time between planting and emergence and to protect seeds from biotic and abiotic factors during critical phase of seedling establishment. Such earlier and synchronized emergence often leads to uniform stands and improved yield (Farooq et al., 2006b; Afzal et al., 2006; Afzal et al., 2011). Improved salt tolerance from priming is the result of higher capacity for osmotic adjustment since plants from primed seeds accumulate Na^+ and Cl^- in roots and more sugars and organic acids in leaves (Cayela et al., 1996). Similarly seed priming improved salt tolerance in wheat at initial germination stage found was due to reduced oxidative damage, expression

Table 1. Influence of seed priming on germination attributes of two fine rice cultivars at 0 mM (control) and 40 and 80 mM NaCl (saline) conditions at germination.

Priming treatments	Shaheen Basmati			Basmati-2000		
	0 mM	40 mM	80 mM	0 mM	40 mM	80 mM
	Final germination (%)					
Untreated	96.67 ab	93.33 abc	86.67 c	96.67 ab	96.67 ab	93.33 abc
Hydropriming	93.33 abc	93.33 abc	86.67 c	86.67 c	73.33 d	66.67 d
$CaCl_2$ (2.2%)	100 a	100 a	100 a	100 a	100 a	100 a
KCl (2.2%)	93.33 abc	96.67 ab	96.67 ab	93.33 abc	93.33 abc	93.33 abc
H_2O_2 (50 mM)	96.67 ab	93.33 abc	90.00 bc	100 a	93.33 abc	93.33 abc
				LSD 5% = 9.58		
	Mean germination time (days)					
Untreated	4.35 klm	4.55 fghi	4.79 bc	4.43 jk	4.68 de	4.50 hij
Hydropriming	4.24 no	4.34 lm	4.58 fgh	4.22 op	4.47 ij	4.59 efg
$CaCl_2$ (2.2%)	4.14 p	4.44 j	4.68 de	4.32 mn	4.57 fgh	4.42 jkl
KCl (2.2%)	4.43 jkl	4.63 ef	4.87 b	4.51 ghij	4.76 cd	4.43 jk
H_2O_2 (50 mM)	4.54 fgh	4.74 cd	4.98 a	4.62 ef	4.87 b	4.57 fgh
				LSD 5% = 0.09		
	Time taken to 50% germination (days)					
Untreated	0.64 lmn	0.87 f-j	1.02 d-g	0.72 j-m	0.83 h-k	0.98 e-h
Hydropriming	0.57 mno	0.637 mn	0.78 i-l	0.48 no	1.20 bcd	1.35 b
$CaCl_2$ (2.2%)	0.40 o	0.80 jkl	0.95 e-i	0.65 lmn	0.90 e-j	1.05 c-f
KCl (2.2%)	0.69 klm	0.92 e-i	1.07 cde	0.77 i-l	0.85 g-k	1.00 e-h
H_2O_2 (50 mM)	0.85 g-k	1.08 cde	1.23 bc	0.93 e-i	1.69 a	1.84 a
				LSD 5% = 0.18		

Means with the same letters among treatments at various salinity levels don't differ significantly at P<0.05

of stress proteins and further activation of metabolic repair (Wahid et al., 2007; Afzal et al., 2011). Seed priming improved germination rates and uniformity of growth following reduced emergence time and increased yields are reported in many field crops including rice (Farooq et al., 2006b; Afzal et al., 2006; Afzal et al., 2011). But such enhancements are often found under non-saline conditions (Farooq et al., 2006a; 2006b) and few studies are available for alleviation of adverse salinity effects in rice during germination and early seedling growth by seed priming (Xu et al., 2011). We hypothesized that seed priming might induce salt tolerance in rice during the primary germination stage. In pursuit of the aim, the effect of salinity stress on germination, chlorophyll content, ionic contents of aromatic fine rice genotypes and possible protection through seed priming strategies at initial seedling stage were investigated.

Results

Germination

Salinity significantly reduced germination and early seedling growth of both rice cultivars while seed priming improved germination and related attributes i.e. final germination percentage (FGP), mean germination time (MGT) and time taken to 50% germination (T_{50}) in comparison to non-primed control (Table 1). Halopriming with $CaCl_2$ following KCl improved final germination percentage and reduced germination time in both cultivars under salinity than untreated control and other priming treatments. However, response was similar within priming treatments for salt sensitive and with hydropriming in salt tolerant cultivar. Minimum final germination was found for salt sensitive at high salinity while reduced germination time for salt tolerant Shaheen Basmati as indicated by lower T_{50} values (Table 1). Under saline condition, hydropriming took less time to germinate and were followed by $CaCl_2$ halopriming in Shaheen Basmati and no significant improvement was recorded in Basmati-2000 for T_{50} and MGT (Table 1). Root length; shoot length and seedling dry weight of both cultivars was also decreased with increasing salinity while halopriming with $CaCl_2$ following KCl improved root and shoot lengths and seedling dry weight than untreated control under normal as well saline conditions in both cultivars (Table 2). Nevertheless, osmopriming with H_2O_2 behaved similar to control for most of germination and seedling growth attributes (Table 2).

Emergence

Response of seed priming treatments for seedling emergence was also similar and seed treatments significantly affected emergence and seedling growth attributes under salinity. MET, E_{50} and FEP were decreased with increase in salinity level in both cultivars (Table 3). Maximum seedling emergence percentage was recorded in both cultivars by halopriming with KCl following $CaCl_2$ at high salinity level in salt tolerant cultivar and was statistically similar to untreated control at both salinity levels in salt sensitive type. However, reduced MET and E_{50} values were found for hydropriming followed by $CaCl_2$ halopriming for salt tolerant Shaheen Basmati as compared to other priming treatments including control. While reduced MET and E_{50} values were found for halopriming with KCl following $CaCl_2$ in salt sensitive Basmati-2000 at all salinity level (Table 3). Halopriming with $CaCl_2$ or KCl also improved root, shoot lengths and seedling dry weight in both rice types when exposed to moderate and high saline conditions. However maximum seedling growth was recorded in both cultivars with $CaCl_2$ halopriming and salt tolerant cultivar performed better than salt sensitive in terms of root and shoot lengths and dry weight of seedlings (Table 4).

Ionic and biochemical analysis

Salinity significantly affected the Na^+ uptake in both cultivars and seed priming reduced the uptake of Na^+ ions. With increasing in salinity level, Na^+ uptake also increased and leaf Na^+ concentration also increased at highest salinity level in all the treatments for both the cultivars (Figure 1). Nonetheless, halopriming with $CaCl_2$ reduced root Na^+ uptake and leaf accumulation of salt tolerant cultivar than salt sensitive type

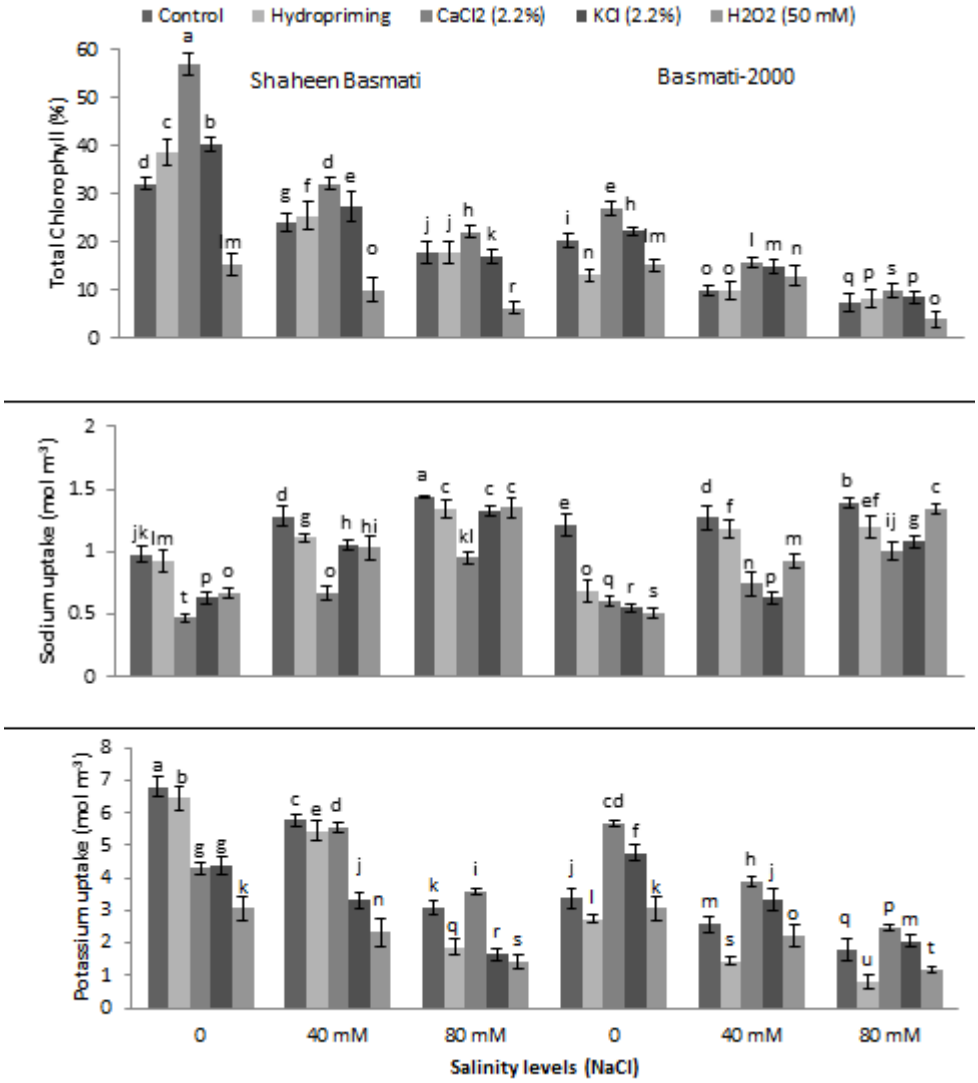

Fig 1. Total chlorophyll percentage (TCP) and leaf Na^+ and K^+ concentration of fine rice cultivars seedlings as affected by seed priming under normal (0 mM) and saline (40 and 80 mM NaCl) conditions.

with higher Na^+ values. While maximum root and leaf Na^+ uptake was observed for untreated control and H_2O_2 priming. Likely, K^+ uptake increased in seedlings raised from $CaCl_2$ haloprimed seeds in both salt tolerant and sensitive cultivars. Although with increase in salinity level, K^+ uptake was reduced in both cultivars but highest K^+ accumulation was observed in Shaheen Basmati than Basmati-2000 (Figure 1). Total chlorophyll percentage (TCP) also decreased with increasing salinity and comparatively higher TCP was found for salt tolerant than salt sensitive cultivar. Among the priming treatments, maximum total chlorophyll contents was obtained for halopriming with $CaCl_2$ and was followed by KCl in both cultivars in comparison to other priming treatments including untreated control (Figure 1).

Discussion

It is well documented that salinity reduces the germination as well as seedling growth in crop plants and seed priming ameliorates salinity affects during early seedling growth (Ashraf and Harris, 2004; Afzal et al., 2006). Seed priming with $CaCl_2$ or KCl has already been reported to improve rice performance under normal conditions (Farooq et al., 2009). In present study, these seed priming agents were also found very effective in alleviating the deleterious effects of salinity on seed germination and seedling growth as evident from significantly curtailed MGT, T_{50} or minimal reduction in seedling growth i.e. root, shoot lengths and seedling dry weight (Table 1-2). The higher germination percentage in seeds primed with $CaCl_2$ is according to Ashraf and Rauf (2001) for wheat and Afzal et al. (2008b) for maize who reported an increase in germination percentage of plants raised from seeds primed with calcium salt under salinity stress. Seeds primed with $CaCl_2$ had an advantage in maintaining germination under saline conditions perhaps due to the influence of Ca^{2+} on membranes (Shannon and Francois, 1977) and enhanced antioxidant proteins like Cu/Zn SOD and other stress induced dehydrins proteins (Hameed et al., 2010). Calcium thus protects plants from adverse affects of salt stress and improves the growth of plants under saline conditions as found in this study.

Table 2. Influence of seed priming on early seedling growth of two fine rice cultivars at 0 mM (control) and 40 and 80 mM NaCl (saline) conditions at germination.

Priming treatments	Shaheen Basmati			Basmati-2000		
	0 mM	40 mM	80 mM	0 mM	40 mM	80 mM
	Root length (cm)					
Untreated	4.18 f	3.95 h	3.64 i	3.58 i	3.61 i	3.20 l
Hydropriming	4.78 d	4.40 e	4.10 g	3.92 h	3.92 h	3.653 i
$CaCl_2$ (2.2%)	5.64 a	5.13 b	4.83 cd	4.18 f	3.32 k	3.460 j
KCl (2.2%)	5.09 b	4.88 c	4.20 f	3.36 k	3.12 m	2.53 o
H_2O_2 (50 mM)	3.48 j	3.07 m	2.94 n	3.15 m	2.98 n	2.95 n
				LSD 5% = 0.078		
	Shoot length (cm)					
Untreated	5.28 f	5.05 h	4.73 i	4.67 i	4.703 i	4.29 l
Hydropriming	5.87 d	5.490 e	5.19 g	5.01 h	5.010 h	4.74 i
$CaCl_2$ (2.2%)	6.73 a	6.22 b	5.92 cd	5.27 f	4.41 k	4.55 j
KCl (2.2%)	6.18 b	5.97 c	5.29 f	4.45 k	4.21 m	4.04 n
H_2O_2 (50 mM)	4.57 j	4.16 m	3.62 o	4.24 lm	4.07 m	4.04 n
				LSD 5% = 0.79		
	Seedling dry weight (g)					
Untreated	0.17 d	0.11 ij	0.08 lm	0.13 klm	0.09 kl	0.06 no
Hydropriming	0.15 ef	0.12 i	0.08 lm	0.13 gh	0.09 kl	0.06 no
$CaCl_2$ (2.2%)	0.22 a	0.17 d	0.14 efg	0.20 b	0.17 cd	0.14 ef
KCl (2.2%)	0.20 b	0.17 d	0.14 efg	0.18 c	0.15 e	0.12 hi
H_2O_2 (50 mM)	0.13 fg	0.10 jk	0.07 mn	0.12h i	0.08 lm	0.05 o
				LSD 5% = 0.012		

Means with the same letters among treatments at various salinity levels don't differ significantly at P<0.05

Another reason for increase in germination percentage in case of $CaCl_2$ and KCl halopriming might be due to enhanced oxygen uptake and mobilization of nutrients from cotyledon to embryonic axis (Farooq et al., 2006b). Poor germination and seedling growth with H_2O_2 pretreatment might be the reason of high H_2O_2 concentration used in this study which became toxic and injured cells when applied externally (Sairam et al., 2002). Halopriming with $CaCl_2$ significantly improved emergence and seedling growth in Shaheen Basmati whereas as $CaCl_2$ and KCl proved better in case of Basmati-2000 which could be related to dormancy breakdown of rice seeds due to enhanced seed K and Ca concentration and amylase activity (Farooq et al., 2006b). Better root, shoot length and seedling dry weight raised from primed seeds might be due to earlier start of emergence as indicated by lower values of MET and E_{50} under varying salinity levels (Table 3-4). Kathiresan et al. (1984) also found similar findings and reported maximum root and shoot growth; seedling height and field emergence in sunflower seeds in response to priming with $CaCl_2$. Priming with H_2O_2 failed to improve emergence and seedling growth in rice cultivars which is inconsistent with Wahid et al. (2007) who reported improved salt tolerance in wheat by alleviation of salt stress and oxidative damage by H_2O_2 pre-treatment. Salinity caused water deficit due to excessive ions might be toxic for mobilization of reserves, radicle emergence and early growth of seedlings (Dood and Donovan, 1999). However, halopriming with $CaCl_2$ or KCl reduced the Na^+ uptake of plants and/or increased the uptake of K^+ compared to control under salinity (Figure 1). Similar findings were coined by Iqbal and Ashraf (2007) in wheat. The lower K^+ and higher uptake of Na^+ is generally considered as important physiological criteria of salt tolerance (Ashraf and Harris, 2004). Albeit, build up of high Na^+ in the shoots might be due to high substrate salinity but $CaCl_2$ halopriming followed by KCl alleviated NaCl stress on early seedling growth and improved salt tolerance is associated with increased absorption of essential nutrients and restricted absorption of toxic elements (Chhipa and Lal, 1995; Wahid and Shabbir, 2005). These findings explicitly indicate that $CaCl_2$ and KCl triggered changes primarily related to the enhancement of chlorophyll contents to meet plant nutritional requirement to thrive under salinity. In conclusion, $CaCl_2$ followed by KCl enhanced capacity of rice cultivars to withstand salinity. Improved early seedling growth, higher chlorophyll contents and nutrient partitioning are important manifestations of seed priming with Ca and K salts. However, H_2O_2 pretreatment failed to alleviate the adverse effects of salinity at germination level. The above findings suggest that Shaheen Basmati is putative salt tolerant cultivar than Basmati-2000 and require further dissection of salt tolerance mechanisms when exposed to salinity stress. Seed priming with Ca and K salts require field appraisal before its recommendation to the growers in rice-wheat system to improve rice performance under saline conditions.

Materials and methods

Plant material and experimental details

Rice seeds *cv.* Shaheen Basmati (salt tolerant) and Basmati-2000 (sensitive) obtained from Rice Research Institute Kala Shah Kako, Lahore, Pakistan were used in the study (Bashir et al., 2007). For priming, 250 g seeds of each cultivar were soaked in respective solution of each osmoticum using 1:3 (w/v) ratio for 36 h. For hydropriming, seeds were soaked in distilled water and in case of osmopriming, 2.2% $CaCl_2$ or KCl solution (Ψs=-1.25 MPa) and of 50 mM H_2O_2 were used at room temperature (25±2 ^{0}C). After priming, seeds were rinsed three times in distilled water and re-dried under shade to its original moisture level and weight (Farooq et al., 2009).

Table 3. Influence of seed priming on germination attributes of two fine rice cultivars at 0 mM (control) and 40 and 80 mM NaCl (saline) conditions at seedling emergence.

Priming treatments	Shaheen Basmati			Basmati-2000		
	0 mM	40 mM	80 mM	0 mM	40 mM	80 mM
	Final emergence (%)					
Untreated	93.33 ab	80.00 bc	41.67 h-l	93.33 ab	75.55 cd	37.77 h-m
Hydropriming	46.00 hij	35.83 i-m	31.77 klm	37.77 h-m	33.67 j-m	25.50 m
$CaCl_2$ (2.2%)	60.00 efg	45.07 h-k	62.22 d-g	75.56 cd	73.33 cde	31.90 klm
KCl (2.2%)	86.67 abc	64.44 def	64.45 def	95.55 a	86.66 abc	44.44 h-k
H_2O_2 (50 mM)	24.44 m	34.91 j-m	29.35 lm	51.11 fgh	48.890 ghi	33.33 j-m
				LSD 5% = 13.51		
	Mean emergence time (days)					
Untreated	4.90 klm	5.10 f-i	5.34 bc	4.98 jk	5.23 de	5.05 hij
Hydropriming	4.80 no	4.89 lm	5.13 fgh	5.06g-j	5.31 cd	5.15 efg
$CaCl_2$ (2.2%)	4.69 p	5.00 j	5.24de	4.88 mn	5.13 fgh	4.98 jkl
KCl (2.2%)	4.98 jkl	5.18 ef	5.42 b	4.77 op	5.02 ij	4.99 jk
H_2O_2 (50 mM)	5.09 f-i	5.29 cd	5.53 a	5.17 ef	5.42 b	5.12 fgh
				LSD 5% = 0.09		
	Time taken to 50% emergence (days)					
Untreated	0.62 lmn	0.85 f-j	0.99 d-g	0.70 j-m	0.80 h-k	1.02 c-f
Hydropriming	0.54 mno	0.61 lmn	0.76 i-l	0.45 no	1.17 bcd	1.32 b
$CaCl_2$ (2.2%)	0.38 o	0.77 i-l	0.92 e-i	0.62 lmn	0.87 e-j	0.95 e-h
KCl (2.2%)	0.66 klm	0.89 e-i	1.04 cde	0.74 i-l	0.82 g-k	0.97 e-h
H_2O_2 (50 mM)	0.82 g-k	1.05 cde	1.20 bc	0.90 e-i	1.67 a	1.82 a
				LSD 5% = 0.18		

Means with the same letters among treatments at various salinity levels don't differ significantly at P<0.05

Table 4 Influence of seed priming on early seedling growth of two fine rice cultivars at 0 mM (control) and 40 and 80 mM NaCl (saline) conditions at seedling emergence.

Priming treatments	Shaheen Basmati			Basmati-2000		
	0 mM	40 mM	80 mM	0 mM	40 mM	80 mM
	Root length (cm)					
Untreated	11.91 c	9.93 e	7.06 l	10.83 d	8.69 ghi	5.75 no
Hydropriming	11.21 d	9.23 f	6.36 m	10.13 e	8.20 ij	5.05 pq
$CaCl_2$ (2.2%)	13.89 a	11.16 d	9.04 fgh	12.81 b	8.56 hi	7.24 kl
KCl (2.2%)	12.15 c	10.17 e	7.30 kl	11.07 d	7.75 jk	5.99 mn
H_2O_2 (50 mM)	10.18 e	8.20 ij	5.33 op	9.10 fg	7.53 kl	4.74 q
				LSD 5% = 0.52		
	Shoot length (cm)					
Untreated	14.78 bc	12.80 de	8.55 lm	9.22 jkl	7.85 mn	6.36 o
Hydropriming	14.08 c	12.10 ef	8.00 mn	10.37 h	7.52 n	5.63 p
$CaCl_2$ (2.2%)	16.76 a	13.19 d	9.93 hij	11.24 g	8.57 lm	6.55 o
KCl (2.2%)	15.02 b	11.54 fg	7.45 n	8.87 kl	7.90 mn	6.32 op
H_2O_2 (50 mM)	13.05 d	9.59 ijk	7.55 n	9.95 hi	7.78 n	6.13 op
				LSD 5% = 0.72		
	Seedling dry weight (g)					
Untreated	62.00 b	40.33 def	32.00 hi	28.00jkl	21.00 op	18.00 pqr
Hydropriming	41.00 de	33.83 gh	26.00 lm	20.50opq	18.00 pqr	17.00 qr
$CaCl_2$ (2.2%)	81.67 a	64.33 b	45.33 c	61.67b	26.67 klm	21.66 nop
KCl (2.2%)	42.00 cd	38.00 ef	25.00 lmn	30.00ijk	24.00 mno	18.00 pqr
H_2O_2 (50 mM)	37.00 fg	40.67 def	17.00 qr	41.00 de	31.33 hij	21.17 op
				LSD 5% = 3.77		

Means with the same letters among treatments at various salinity levels don't differ significantly at P<0.05

Germination and seedling vigor evaluation

Germination and seedling vigor evaluation of the rice seeds was carried out in accordance with the International Rules for Seed Testing (ISTA, 2011). Four replicates of 25 seeds of each cultivar were placed in 12 cm diameter petri dishes on two layers of filter paper at 25°C in a growth chamber. The seeds were partially submerged to 4 mL of saline (40 and 80 mM NaCl) or non-saline (distilled water) solutions in each petri dish. All petri dishes were placed in plastic container and wrapped with polythene bags to avoid moisture loss. A seed was scored germinated when radicle was visible. Seed germination count was made every 24 h, and terminated when maximum germination was achieved. Time to 50% germination (T_{50}) was calculated according to the formulae of Coolbear et al. (1984). Mean germination time (MGT) was calculated according to Ellis and Roberts (1981). Emergence test was conducted in plastic trays (2.5 cm depth x 30 cm

width) filled with acid washed and air dried sand (Afzal et al., 2006). Saline (40 and 80 mM NaCl) or non-saline solution (distilled water) was applied to all the plastic trays according to the requirement. 25 seeds of each cultivar were sown per replicate. On seventh day after emergence, five randomly seedlings were carefully removed from sand and evaluated for root and shoot lengths per replicate. Dry weight was determined after oven drying at 70^{o}C for 48 h.

Ionic and biochemical analysis

For leaf sap extraction to determine Na^+ and K^+, the seedlings after emergence were quickly rinsed in distilled water and blotted with tissue paper (Gorham et al., 1984). Sodium and potassium were determined using Flame Photometer (Sherwood 410). For chlorophyll determination fresh and healthy young seedlings of 0.5 g was grinded in 10 mL of 80% acetone. From this 1 ml aliquot was taken and total volume of 5 ml was made by adding 4 ml of acetone. Poured it in the cuvettes and read at 663 and 645 OD's using UV-spectrophotometer (ORI Germany). Total chlorophyll percentage (TCP) was calculated according the formula given by Nagata and Yamashita (1992).

Statistical analysis

The experiments regarding germination and seedling vigour evaluation were laid out in completely randomized design (CRD) with three factors factorial (seed treatments x salinity levels x cultivars) having four replications. The data were analyzed by ANOVA using a statistical package, MSTATC. Significant differences were identified using Fisher's protected least significant difference at 5% confidence interval.

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