



Full Length Article

Seed Priming with Putrescine Improves the Drought Resistance of Maize Hybrids

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Abstract

Maize productivity is severely hampered owing to looming water deficit worldwide. Exogenous use of polyamines, especially putrescine, is of vital significance in alleviating the adversities of drought in crop plants. This study, consisted of two independent experiments was conducted in a glasshouse. In experiment I, conducted to screen the maize hybrids for drought resistance, maize hybrids Pioneer 30-Y-87, Pioneer 31-R-88, Pioneer 32-W-86, Pioneer 3025 and Pioneer 3062 were sown in 8 kg soil and compost (1:1) filled pots maintained at 80, 60, 40 and 20% water holding capacity (WHC) from sowing till crop harvest. Performance of maize hybrid Pioneer 31-R-88 was better in terms of seedling biomass, leaf area and leaf water status, so was selected as drought resistant; whereas, performance of Pioneer 30-Y-87 in terms of these traits was poor and was designated as drought sensitive. In experiment II, potential of putrescine seed priming in improving the drought resistance of maize was evaluated. Seeds of maize hybrids (selected from experiment I) Pioneer 30-Y-87 (drought sensitive) and Pioneer 31-R-88 (drought resistant) were soaked in water (control) or aerated solution of putrescine (0.1, 0.01 and 0.001 mM) for 10 h. Seeds were thoroughly rinsed, re-dried near to original weight with forced air and then sowed in 10 kg soil-filled earthen pots maintained at 80 and 40% WHC designated as well-watered and drought stress, respectively. Drought stress hampered the seedling emergence, reduced the seedling biomass and disrupted the plant leaf water status. Nonetheless, putrescine application improved the plant biomass components and leaf water status under well-watered and drought conditions. However, seed priming with 0.1 mM putrescine was the most effective in this regard. Similarly, both the maize hybrids differed significantly for seedling emergence, seedling vigor, leaf area and leaf water relations; however, maize hybrid Pioneer 31-R-88 performed better than the hybrid Pioneer 30-Y-87 both under well-watered and water deficit conditions. In conclusion, seed priming with 0.1 mM putrescine can effectively improve the drought resistance in hybrid maize. © 2014 Friends Science Publishers

Keywords: Drought; Putrescine; Seed priming; Maize; Water relations

Introduction

Crop production is severely affected by decline in water availability (Araus, 2004; Shahbaz *et al.*, 2009). Current scenario of global climate change predicts a future increase in the aridity and frequency of extreme events of water deficit in many areas of the world (IPCC, 2007). Limiting supply of water all over the world and increasing future food needs for fast growing population pressure are further aggravating the drought effects (Somerville and Briscoe, 2001).

Early season water deficit influences the germination and stand establishment owing to reduced water uptake during the imbibition phase of germination (Okçu *et al.*, 2005; Taiz and Zeiger, 2010). However, there exists huge genotypic variation in this regard (Farooq *et al.*, 2009a, 2013), which may be exploited to develop drought resistant genotypes. Moreover, exogenous application of certain chemicals and plant growth regulators can also be helpful in mitigating the adversities of drought.

Polyamines (PAs), plant phenolic substances of ubiquitous nature, play key role in plants under adverse environmental conditions (Borsani *et al.*, 2001; Farooq *et al.*, 2009a, b). These act as important co-factor for some of the enzymes affecting hormone-mediating signaling processes during plant development and the transition from the vegetative to reproductive phase (Barth *et al.*, 2006; Bae *et al.*, 2008). As PAs are cationic in nature, these may make association with membrane phospholipids, help in stabilizing the bilayer structure and reduce injuries to membrane upon exposure to environmental stresses (Basra *et al.*, 1997). Thus PAs may help to protect the plants from adversities of environmental stresses (Bouchereau *et al.*, 1999).

Seed priming is a controlled hydration technique, which permits the germination metabolism without the actual germination (Bradford, 1986). For priming, seeds are soaked in solutions of low water potential and are then rinsed thoroughly after removing from the priming solutions (Farooq *et al.*, 2010). Use of PAs as priming agents has

been quite effective in improving the performance of wheat (Iqbal and Ashraf, 2006), sunflower (Farooq *et al.*, 2007) and rice (Farooq *et al.*, 2009b) under less than optimum condition; however, amongst the PAs, putrescine was the most effective for wheat and sunflower. However, to best of our knowledge, potential of seed priming with putrescine in improving the drought resistance of maize is not reported. This was hypothesized that there exists genotypic variation amongst maize hybrids for drought resistance and seed priming with putrescine can improve the drought resistance of maize.

Materials and Methods

The study comprised of two independent experiments was conducted in the glasshouse, University of Agriculture, Faisalabad, Pakistan. Both of experiments were laid out in completely randomized design in factorial arrangement with four replications. Seeds of maize hybrids Pioneer 30-Y-87, Pioneer 31-R-88, Pioneer 32-W-86, Pioneer 3025 and Pioneer 3062, used in this study, were obtained from Pioneer Seeds (Pvt.) Ltd. Sahiwal, Pakistan.

Experiment I

Seeds of maize hybrids "Pioneer 30-Y-87, Pioneer 31-R-88, Pioneer 32-W-86, Pioneer 3025 and Pioneer 3062 were sown on February 04, 2009 in plastic pots (30 cm × 18 cm) containing (8 kg) mixture (1:1) of soil and compost at 80, 60, 40 and 20% water holding capacity (WHC). Experimental soil was sandy loam having pH 7.92, EC= 1.6 dS m⁻¹ and organic matter 0.89%. Ten seeds were sown in each pot and then were thinned maintaining three plants in each pot one week after sowing. Fertilizer was applied as 0.48, 0.24 and 0.2 g NPK in each pot using urea (46% N), di-ammonium phosphate (18% N, 46% P₂O₅) and sulphate of potash (SOP) (50% K₂O) as sources. Half of nitrogen and whole quantity of phosphorus and potassium were applied as basal dose, while other half of nitrogen was applied one week before the harvest. Measured quantity of water was applied to maintain the targeted soil moisture level. Plants were harvested finally to record different observations three weeks after sowing.

Experiment II

Maize hybrids Pioneer 30-Y-87 (drought sensitive) and Pioneer 31-R-88 (drought resistant) selected from experiment I were used in this study. For priming, seeds of both maize hybrids were soaked in water or aerated solutions of 0.1, 0.01 and 0.001 mM putrescine for 10 h. After priming, seeds were rinsed thoroughly and re-dried near to their original weight and stored at 5°C until used. Fifteen seeds, of each hybrid, were sown in each earthen pot (30 cm × 28 cm) containing 10 kg mixture (1:1) of soil and compost maintained at 80 and 40% water holding capacity on January 19, 2010. Plants were thinned to maintain five plants in each pot one week after sowing. Fertilizers were

applied as 0.60, 0.38, 0.25 g NPK in each pot using urea (46% N), di-ammonium phosphate (18% N, 46% P₂O₅) and sulphate of potash (SOP) (50% K₂O) as sources. Half of nitrogen and whole phosphorus and potassium were applied as basal dose while other half of nitrogen was applied one week before the harvest. Measured quantity of water was applied to maintain the targeted soil moisture level. Plants were harvested to record different observations three weeks after sowing.

Observations

The experiments were visited daily and number of emerged seedlings was recorded according to the seedling evaluation handbook (Association of Official Seed Analysts, 1990). Final emergence percentage was taken as the ratio of number of emerged seedlings to total seeds sown in percentage. Mean emergence time (MET) was calculated following equation of Ellis and Robert (1981).

Plants were uprooted carefully, washed thoroughly; shoot and root were separated, and dried at 70°C in an oven till constant weight to get shoot and root dry weight. Leaf area of each plant selected was measured with the help of leaf area meter (Laser Area Meter CI-203). The third leaf from top (fully expanded youngest leaf) was excised at 6:30 am to 8:30 am to determine the leaf water potential with a pressure chamber (Soil Moisture Equipment Corp., Santa Barbara, CA, USA). The same leaf, as used for water potential measurement, was frozen in at -20°C for seven days, after which the frozen leaf material was thawed and the sap was extracted by pressing the material with a glass rod. The sap was used directly for the determination of osmotic potential in a vapor pressure osmometer (Vapro, 5520). The turgor potential was calculated as the difference between water potential and osmotic potential values.

Statistical Analysis

Data collected were analyzed statistically by using MSTAT-C software on computer. Least significance difference (LSD) test at 5% probability level was applied to compare the treatments means (Steel *et al.*, 1996).

Results

Experiment I

Drought stress significantly influenced the emergence, seedling growth and water relations of all tested maize hybrids. Substantial decrease in final emergence, mean emergence time, shoot and root dry weights, leaf area and plant water status was observed with increase in the intensity of drought stress (Tables 1, 2). However, there was significant variation amongst the tested hybrids in this regard (Tables 1, 2). Maize hybrid Pioneer 31-R-88 performed better in terms of stand establishment, shoot and root dry weights, leaf area and water relations; whereas

Table 1: Influence of soil moisture regimes on final emergence percentage, mean emergence time, root dry weight and shoot dry weight of various maize hybrids

Treatments	Final emergence percentage (%)				Mean emergence time (days)				Root dry weight (g)				Shoot dry weight (g)			
	*80	**60	***40	****20	80	60	40	20	80	60	40	20	80	60	40	20
Pioneer 3025	93.50 ab	90.75 bc	86.00 ef	77.75 h	5.53 i	7.63 de	8.21 cd	8.79 bc	0.46 ab	0.22 cde	0.09 d-g	0.03 fg	0.30 abc	0.19 de	0.06 fg	0.03 fg
Pioneer 31-R-88	96.25 a	91.50 bc	88.50 c-f	81.50 gh	5.01 i	6.25 gh	7.39 e	8.22 cd	0.53 a	0.49 a	0.24 cd	0.07 efg	0.37 a	0.26 bcd	0.08 fg	0.04 fg
Pioneer 32-W-86	91.75 abc	86.50 def	81.25 gh	79.00 h	6.25 gh	7.56 de	8.40 c	9.39 b	0.44 ab	0.44 ab	0.13 d-g	0.02 g	0.25 bcd	0.20 cde	0.03 fg	0.02 fg
Pioneer 30-Y-87	90.25 bcd	81.00 gh	80.25 h	73.02 i	6.52 g	7.67 de	8.70 c	10.19 a	0.39 ab	0.21 cde	0.08 efg	0.01 g	0.24 bcd	0.13 ef	0.03 fg	0.02 fg
Pioneer 3062	90.25 bcd	89.75 b-e	87.50 c-f	79.50 h	5.70 hi	6.57 fg	7.23 ef	8.58 c	0.45 ab	0.34 bc	0.17 def	0.01 g	0.31 ab	0.20 cde	0.04 fg	0.02 fg
LSD (P≤0.05)	I× H = 4.02				I× H = 0.69				I× H = 0.14				I× H = 0.10			

Table 2: Influence of different soil moisture regimes on leaf area, water potential, osmotic potential and turgor potential of various maize hybrids

Treatments	Leaf area (cm ²)				Water potential (-MPa)				Osmotic potential (-MPa)				Turgor potential (MPa)			
	*80	**60	***40	****20	80	60	40	20	80	60	40	20	80	60	40	20
Pioneer 3025	195.78 ab	99.95 cde	35.05 efg	11.16 fg	0.40 d	0.67 a-d	1.00 abc	1.07 abc	0.78 bcd	0.82 a-d	1.14 abc	1.19 abc	0.38 b	0.15 def	0.14 def	0.11 f
Pioneer 31-R-88	245.05 a	164.90 bc	36.76 efg	23.47 efg	0.38 d	0.51 bcd	0.96 abc	1.03 abc	0.74 bcd	0.76 cd	1.12 abc	1.18 abc	0.47 a	0.23 b-f	0.23 b-f	0.15 def
Pioneer 32-W-86	172.79 abc	142.95 bcd	26.12 efg	9.31f g	0.39 d	0.65 a-d	1.00 abc	1.05 abc	0.77 bcd	0.80 a-d	1.19 abc	1.21 abc	0.38 b-e	0.21 b-f	0.15 def	0.14 def
Pioneer 30-Y-87	142.52 bcd	79.27 def	23.20 efg	7.12 f g	0.51 bcd	1.01 abc	1.24 ab	1.34 a	0.83 bcd	0.97 a-d	1.39 abc	1.45 a	0.32 b-f	0.15 def	0.12 ef	0.11 f
Pioneer 3062	211.77 ab	156.90 bcd	36.07 efg	10.26 f g	0.45 cd	0.74 a-d	1.07 abc	1.31 a	0.79 bcd	0.96 a-d	1.20 abc	1.43 a	0.34 bc	0.20 b-f	0.13 def	0.12 ef
LSD (P≤0.05)	I× H = 78.73				I× H = 0.74				I× H = 0.86				I× H = 0.16			

*, **, ***, **** = 80, 60, 40 and 20% water holding capacity

Figures sharing same letter, for a parameter, did not differ significantly at P ≤ 0.05

emergence and growth of hybrid Pioneer 30-Y-87 strongly impeded with decrease in available water (Table 1). Therefore, hybrid Pioneer 31-R-88 was designated as drought resistant, and hybrid Pioneer 30-Y-87 was taken as drought sensitive one.

Experiment II

Final emergence, mean emergence time, root and shoot dry weights, leaf area, leaf water potential and osmotic potential, of tested maize hybrids, were significantly influenced by drought (Table 3). Maize hybrids also differed significantly for above parameters except mean emergence time (Table 3). However, seed priming with putrescine significantly influenced all the above parameters. Interaction of drought, hybrids and putrescine was only significant for final emergence, and shoot and root dry weights (Table 3). Drought stress significantly delayed the seedling emergence and decreased the leaf area, leaf water potential and osmotic potential in maize hybrids (Table 4). Likewise, maize hybrid Pioneer 31-R-88 had more leaf area, leaf water potential, osmotic potential and turgor potential than the hybrid Pioneer 30-Y-87 (Table 4). Seed priming with putrescine significantly decreased the mean emergence time and improved the leaf area, leaf water potential and osmotic potential in maize hybrids (Table 4). In this regard, seed priming with 0.1 mM putrescine was the most effective treatment (Table 4).

Seed priming with putrescine improved the final emergence in both the tested hybrids under drought stress. However, no improvement in final emergence of Pioneer 30-Y-87 was recorded from seed priming with putrescine under well-watered conditions (Table 5). Seed priming with putrescine also improved the root and shoot dry weights of both the tested hybrids under well-watered and drought

conditions. In all cases, seed priming with 0.1 mM of putrescine was the most effective treatment (Table 5).

Discussion

Water is pre-requisite for seed germination and imbibition of water and is considered as first step of germination. Decrease in soil moisture contents not only delayed the seedling emergence but also decreased the final emergence percentage in all tested maize hybrids (Tables 1, 4, 5). The process of germination starts with water imbibitions followed by active metabolism and seedling emergence (Farooq *et al.*, 2010). With decrease in soil moisture contents, rate of imbibition in germinating seeds is reduced, which cause delay or total failure of germination (Lee and Kim, 2000). However, genotypes differ in this regard (Table 1); certain genotypes are better able to extract soil moisture and thus can complete the germination process in a better way (Hampton and Tekrony, 1995). Better and earlier germination give a better start to the growing plants, which enable them to have better growth even during later developmental stages as indicated by higher shoot and root dry weights and leaf area of drought resistant maize hybrid Pioneer 31-R-88 (Table 1). Maintenance of plant water status, even with limited water supply also provide the evidence of better plant (Farooq *et al.*, 2009a) as is indicated by hybrid Pioneer 31-R-88 (Table 1).

Seed priming with putrescine not only improved the emergence rate and final emergence but also increased the seedling vigor as indicated by higher root and shoot dry weight and leaf area (Tables 4, 5). Improvement in germination indicates the involvement of putrescine in germination metabolism as evidenced previous for wheat (Farooq *et al.*, 2011). Improved root and shoot dry weights and leaf area from seeds primed with putrescine

Table 3: Analysis of variance for FEP, MET, root dry weight, shoot dry weight, leaf area, WP, OP and TP as affected by putrescine applied through seed treatment of maize hybrids under different soil moisture regimes

SOV	df	FEP	MET	Root dry weight	Shoot dry weight	Leaf area	WP	OP	TP
Irrigation (I)	1	1002.57**	118.25**	0.04**	0.01**	2106.22**	0.19**	0.25**	0.01 ^{ns}
Hybrids (H)	1	451.47**	0.13 ^{ns}	0.15**	0.01**	1026.38**	0.10**	0.03**	0.02*
I×H	1	13.11*	10.66**	0.00 ^{ns}	0.00*	354.14*	0.02*	0.00 ^{ns}	0.03*
Putrescine (Put)	3	26.43**	1.06*	0.03**	0.00**	1298.33**	0.04**	0.02**	0.00*
I×Put	3	0.89 ^{ns}	0.10 ^{ns}	0.00*	0.00**	98.97 ^{ns}	0.00 ^{ns}	0.00**	0.00 ^{ns}
H×Put	3	3.96*	0.22 ^{ns}	0.00**	0.00**	6.74 ^{ns}	0.00 ^{ns}	0.00 ^{ns}	0.00 ^{ns}
I×H×Put	3	11.58*	0.26 ^{ns}	0.00*	0.00**	20.62 ^{ns}	0.00 ^{ns}	0.00 ^{ns}	0.00 ^{ns}

SOV = source of variation, df = degree of freedom, ** = significant at $P \leq 0.01$, * = significant at $P \leq 0.05$, ns = non significant, FEP = final emergence percentage, MET = mean emergence time, WP = water potential, OP = osmotic potential, TP = turgor potential

Table 4: Influence of seed priming with putrescine on MET, leaf area, water potential, osmotic potential and turgor potential of maize hybrids under different soil moisture regimes

Treatments	MET (days)	Leaf area (cm ²)	Water potential (-MPa)	Osmotic potential (-MPa)	Turgor potential (MPa)
Irrigation					
80 % WHC	5.29 b	98.16 a	0.69 b	1.26 b	0.57
40 % WHC	8.43 a	84.91 b	0.82 a	1.41 a	0.59
LSD ($P \leq 0.05$)	0.26	3.92	0.02	0.03	Ns
Hybrids					
Pioneer 30-Y-87	6.91	86.91 b	0.80 a	1.36 a	0.56 b
Pioneer 31-R-88	6.80	96.16 a	0.71 b	1.31 b	0.60 a
LSD ($P \leq 0.05$)	Ns	3.92	0.02	0.03	0.02
Putrescine					
Control	7.19 a	78.61 d	0.82 a	1.38 a	0.57 b
0.1 mM	6.50 c	103.44 a	0.68 d	1.29 d	0.61 a
0.01 mM	6.75 bc	94.82 b	0.74 c	1.33 c	0.58 ab
0.001 mM	6.99 ab	89.25 c	0.78 b	0.35 b	0.57 b
LSD ($P \leq 0.05$)	0.36	5.54	0.03	0.04	0.03

Figures sharing same letter did not differ significantly at $P \leq 0.05$, MET = mean emergence time, WHC = water holding capacity

Table 5: Influence of seed priming with putrescine on FEP, root dry weight and shoot dry weight of maize hybrids under different soil moisture regimes

Treatments	FEP (%)				Root dry weight (g)				Shoot dry weight (g)			
	80% WHC		40% WHC		80% WHC		40% WHC		80% WHC		40% WHC	
	Pioneer 30-Y-87	Pioneer 31-R-88	Pioneer 30-Y-87	Pioneer 31-R-88	Pioneer 30-Y-87	Pioneer 31-R-88	Pioneer 30-Y-87	Pioneer 31-R-88	Pioneer 30-Y-87	Pioneer 31-R-88	Pioneer 30-Y-87	Pioneer 31-R-88
Control	91.52 cd	92.38 cd	79.30 i	87.76 f	0.23 h	0.34 e	0.18 i	0.27 g	0.10 fgh	0.12 def	0.08 i	0.09 hi
Put 0.1 mM	92.53 c	99.20 a	83.96 g	89.10 ef	0.36 d	0.44 a	0.31 f	0.42 b	0.13 c	0.18 a	0.11 ef	0.13 cd
Put 0.01 mM	91.31 cd	98.47 a	81.39 h	88.96 ef	0.27 g	0.42 b	0.24 h	0.33 e	0.12 cde	0.15 b	0.10 ghi	0.11 ef
Put 0.001 mM	90.63 de	96.30 b	80.60 hi	88.14 f	0.27 g	0.38 c	0.26 j	0.32 ef	0.11 efg	0.13 c	0.09 hi	0.10 fgh
LSD ($P \leq 0.05$)	1.83				0.02				0.02			

Figures sharing same letter did not differ significantly at $P \leq 0.05$, FEP = final emergence percentage, WHC = water holding capacity

may be attributed to putrescine-triggered increase in cell division within the apical meristem, which caused an increase in plant growth (Huang and Villanueva, 1992; Cvikrova *et al.*, 1999). Polyamines (putrescine, spermidine, spermine) are now being increasingly regarded as plant growth stimulant and secondary messenger in signaling pathways (Davies, 2004; Liu *et al.*, 2007; Kusano *et al.*, 2008), which modulate the plant development (Ali, 2000). This regulatory role, however, becomes more important under abiotic stresses like drought (Farooq *et al.*, 2009a, b). Interestingly increase in putrescine concentration, in priming solution, increased the maize performance (Tables 4, 5).

In conclusion, drought stress suppressed the seedling emergence and growth of maize; however, maize hybrid Pioneer 31-R-88 was better able to perform well. Seed

priming with 0.1 mM putrescine was quite effective in improving the resistance against drought in hybrid maize.

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