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Seed priming of sorghum cultivars to tolerate salt stress

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Seed priming of sorghum cultivars to tolerate salt stress

M O Shihab[1], J H Hamza[2]

[1]Ministry of Agriculture, Planning & Follow-up Office, Department of AEZ Maps

[2]University of Baghdad, College of Agricultural Engineering Sciences, Department of Field Crops

Corresponding author: j.hamza@coagri.uobaghdad.edu.iq

Abstract. A laboratory experiment was carried out in the laboratories of College of Agricultural Engineering Sciences, University of Baghdad in 2017. Three factors were studied; *Sorghum bicolor* L. cultivars (Inqath, Rabeh and Buhoth70), primed and unprimed seed, and salt stress (0, 6, 9 and 12 dS.m^{-1}). The aim was to improve germination and seedling growth under salt stress. The results showed significant superiority of Buhoth70 cultivar compared to others, significantly superiority of primed seed compared to the unprimed and significant negative impact as long as increasing levels of salt stress at germination ratio, plumule length, dry seedling weight and seedling vigor index. The interaction between cultivars, priming and salt stress showed that primed cultivars seed were better than unprimed to tolerate the salt stress at the same level of stress. A significant positive correlation found between all traits studied. It can conclude there was a role to the genotype and seed priming could enhance seed performance to tolerate salt stress. It can recommend priming sorghum seeds before planting under salt stress.

1. Introduction

Saline stress negatively affects plant physiological and biological processes such as photosynthesis, respiration, carbohydrate and protein synthesis and water relationships, leading to low productivity [1]. Salt stress has a role in preventing, delaying and reducing seed germination. Salinity causes a decrease in the osmotic potential of the soil solution, leading to slow seed imbibition with water, leading to embryotoxicity with sodium chloride, or affecting the processes of protein synthesis within the seed and thus inhibition of germination [2]. Early stages of plant growth, including seed germination, are affected by increased salt stress compared to late growth stages [3]. Saline stress affects all processes related to nutrient uptake and embryo development [4], leading to protein demolition, chlorophyll reduction and inhibition of electron transport due to the formation of chlorophyllase enzyme or changes in the synthesis of plastids [5]. Seed priming is an efficient and effective technique used to improve crop performance and increase production when there are environmental problems such as salinity of irrigation water or soil, water scarcity and extreme temperature [6, 7, 8]. Seed priming led to increasing speed and ratio of germination in wheat [9], also increased the susceptibility of small seed to resistance to stressful environments such as salinity, drought and extreme temperatures, repairing and synthesis of nucleic acids, as well increased protein synthesis and cellular membrane maintenance in maize [10, 11, 12]. The mean time of emergence under a wide range of environmental conditions can be minimised when the seed stimulated with gibberellic acid [13]. The presence of gibberellic acid increases the percentage of germination through indirect participation in the decomposition of the main substances found in the endosperm to the most basic materials transferred to the embryo [14]. Soaking of maize seeds with gibberellic acid for 24 hours increased seed response speed to initiate germination and increased field emergence [15]. Seed soaking in gibberellic acid increased field emergence ratio because it has a role in stimulating cell division, elongation and promotion of seeds for germination [16]. Salicylic acid has a role in preventing oxidative damage by reducing the production of oxidizing radicals of compounds during saline stress, as well as improving and increasing the efficiency of photosynthesis and reducing the salinity effect on the phenotype and function of the plant under saline conditions [17, 18, 19]. Salicylic acid has contributed to reducing the effect of salt stress at the germination stage of rice seeds under several saline levels [20]. The study aimed to improve germination and seedling growth in sorghum under salt stress.

2. Material and methods

A laboratory experiment was carried out in the laboratories of College of Agricultural Engineering Sciences, University of Baghdad in 2017. Three factors were studied: 1st factor was three sorghum cultivars (Inqath, Rabeh and Buhoth70); 2nd factor was unprimed and primed seed which were primed by soaking it in 300+70 $mg.l^{-1}$ of gibberellic acid (GA3) and salicylic acid, respectively, for 12 hours; 3rd factor was salt stress (0, 6, 9 and 12 $dS.m^{-1}$) which prepared by dissolving specific quantities of sodium chloride in one liter of distilled water. The following tests were carried out.

2.1 Germination ratio (%)

200 pure seed were taken from the treated seed and planted, folded in blotting paper and placed in germinator at the temperature of 25 °C ±5). Normal seedlings calculated at the end of the 10th day of the standard germination test [21].

2.2 The radicle and plumule lengths (cm) and dry seedling weight (mg)

Radicle length measured after being separated from its point of contact with the seed and plumule after separating it from its point of contact with the hypocotyl. A ruler was used to measure the length of 10 normal seedlings at the end of the 10th day of standard germination. Radicle and plumule were put in a perforated paper bag inside an electric oven for drying at 80°C for 24 hours [22]to measure the seedling dry weight.

2.3 Seedling vigor index (SIV)

It calculated by the equation of Abdul-Baki and Anderson [23];
SVI = germination ratio × (length of radicle + length of plumule).

2.4 Statistical analysis

Data were collected and analyzed statistically by using the GenStat program V.12.1. Analysis of variance runs according to the completely randomized design with four replications. Means compared by using the least significant difference at $p<0.05$ (LSD 5%). Simple correlation analysis was performed between the studied traits [24].

3. Results and discussion

The results of variance analysis (Table 1) showed significant differences in the studied traits as affected by cultivars, priming and salt stress and their combinations; except the effect of cultivars × priming at seedling dry weight and seedling vigour index, and priming × salt stress at radicle and plumule lengths, and cultivars × priming × salt stress at dry seedling weight, radicle and plumule lengths, and seedling vigour index which were insignificant.

Table 1. Squares mean (MS) according to variance analysis for effect of cultivars, priming and salt stress in the studied traits in sorghum

Source of variance	df	Germination ratio	Radicle length	Plumule length	Dry seedling weight	Seedling vigor index
C	2	168.792*	11.5989*	107.385*	0.0094684*	714,921*
P	1	4,187.042*	16.5834*	83.4588*	0.007758*	2,289,493*
S	3	907.375*	20.3812*	72.8428*	0.0015619*	1,466,149*
C × P	2	162.042*	1.3299*	2.4803*	0.0000708	9,230
C × S	6	163.125*	1.7669*	3.6267*	0.0003895*	37,911*
P × S	3	26.486*	0.1732	1.4632	0.0003261*	44,039*
C × P × S	6	26.486*	0.185	0.4441	0.000082	4,165
Error	72	7.903	0.2914	0.6777	0.0001108	7,823
CV		4	19	12.4	13.8	12.9
SE		2.811	0.5398	0.8232	0.01052	88.45

[C] cultivars; [P] priming; [S] salt stress; [CV] coefficient variance; [SE] standard error; * Significant at $p<0.05$

3.1 Germination ratio (%)

The cultivar of Buhoth70 was superior compared to others; while Inqath cultivar gave the lowest mean (Table 2). This may be due to the variation of these cultivars in the process of seed imbibition and absorption of water and fill their tissues to restore the active growth resulting from the rupture of the seed coat and emergence of radicle and plumule. The treatment of primed seed was significantly superior compared to the unprimed (Table 2). These results are consistent with the concept that seed treatment with growth regulators improves germination ratio [25]. The germination ratio decreased significantly with the increase in salt stress levels. The control treatment gave the highest mean, while the lowest mean returned to the 12 $dS.m^{-1}$ (Table 2). The reason for the low germination ratio may be due to the slow of seed imbibition, which causes delayed degradation of the endosperm content as well as the role of salinity in reducing or inhibiting the activity of the essential gibberellins in the activation of hydrolysis enzymes, especially the alpha-amylase enzyme. The results of the binary combinations of cultivars and priming showed that all cultivars had been improved their performance as a result of priming their seeds compared to the unprimed (Table 2). The combination (Inqath ×primed seed) was significantly superior compared to others and gave the highest mean without differing significantly with the combination (Buhoth70 × primed seed); while the combination (Inqath x unprimed seed) had the lowest mean (Table 2). The results of the binary combinations between cultivars and salt stress showed that the cultivars differed in their ability to tolerate salt stress at the same salt stress levels (Table 2). The combination (Buhoth70 × 0) significantly exceeded most other combinations and gave the highest mean without differing significantly with the combinations (Buhoth70 x 6) and (Inqath × 0), while the lowest mean was due to the combination (Buhoth70 × 12) (Table 2). The results of combinations between priming and salt stress showed that priming had improved seed performance to tolerate salt stress compared to the unprimed at the same stress level (Table 2). The combination (primed seed × 0) significantly exceeded the others and gave the highest mean, while the lowest mean returned to the combination (unprimed seed x 12) (Table 2). The triple combination of cultivars, priming and salt stress significantly surpassed the others and gave the highest mean without significant differences with the combinations (Buhoth70 × primed seed × 0), (Inqath × primed seed × 0), (Buhoth70 x primed seed × 6), while the lowest mean was due to the combination (Inqath x unprimed seed × 12) (Table 2).

Table 2. Germination ratio (%) by the effect of cultivars, priming and salt stress in sorghum

Seed priming	Cultivars	Salt stress (dS.m^{-1}) 0	6	9	12	Seed priming × Cultivars
Primed seed	Buhoth70	83	82	78	64	76.8
	Inqath	83	78.5	72	75	77.1
	Rabeh	83.5	74	69	74	75.1
Unprimed seed	Buhoth70	74	73	71	54	68
	Inqath	72	61	53.5	51	59.4
	Rabeh	67	63	59	59	62
LSD 5%		4				2
						Seed priming
Seed priming × Salt stress	Primed seed	83.2	78.2	73	71	76.3
	Unprimed seed	71	65.7	61.2	54.7	63.1
LSD 5%		2.3				1.1
						Cultivars
Cultivars × Salt stress	Buhoth70	78.5	77.5	74.5	59	72.4
	Inqath	77.5	69.8	62.8	63	68.3
	Rabeh	75.3	68.5	64	66.5	68.6
LSD 5%		2.8				1.4
Salt stress		77.1	71.9	67.1	62.8	
LSD 5%		1.6				

3.2 Radicle length (cm)

The cultivar of Inqath was significantly superior compared to others, and Rabeh cultivar gave the lowest mean (Table 3). The treatment of primed seed was significantly superior compared to the unprimed (Table 3), which may be due to the priming effectiveness in increasing the division and expansion of cells and differentiation. The length of radicle decreased significantly with the increase in saline stress levels, and the control treatment gave the highest mean, while the lowest mean was due to the treatment of the 12 dS.m^{-1} (Table 3). This is maybe due to the negative effect of salinity on the activity of growth regulators and the inhibition of division and elongation processes. The results of the binary combinations of cultivars and priming showed that all cultivars had been improved their performance as a result of priming their seeds compared to the unprimed (Table 3). The combination (Inqath × primed seed) significantly was superior compared to others and gave the highest mean, while the lowest mean belonged to the combination (Rabeh × unprimed seed) (Table 3). The results of the binary combinations of cultivars and salt stress showed that the cultivars differed in their ability to tolerate salt stress at the same salt stress level (Table 3). The combination (Inqath x 0) significantly exceeded all other combinations and gave the highest mean, while the lowest mean was due to the combination (Rabeh x 12) (Table 3). A high significant positive correlation observed between the radicle length and the germination ratio (0.639) (Table 5).

Table 3. Radicle length (cm) by the effect of cultivars, priming and salt stress in sorghum

Seed priming	Cultivars	Salt stress ($dS.m^{-1}$) 0	6	9	12	Seed priming × Cultivars
Primed seed	Buhoth70	3.8	3.5	2.9	2.1	3.1
	Inqath	5.5	5.2	2.8	2.5	4
	Rabeh	3.5	3	2.2	2.1	2.7
Unprimed seed	Buhoth70	3.5	3.1	2.4	1.7	2.7
	Inqath	4.4	3.5	2.2	1.4	2.8
	Rabeh	2.2	2	1.4	1.2	1.7
LSD 5%			NS			0.4
						Seed priming
Seed priming × Salt stress	Primed seed	4.3	3.9	2.6	2.2	3.3
	Unprimed seed	3.4	2.9	2	1.4	2.4
LSD 5%			NS			0.2
						Cultivars
Cultivars × Salt stress	Buhoth70	3.6	3.3	2.6	1.9	2.9
	Inqath	4.9	4.3	2.5	1.9	3.4
	Rabeh	2.9	2.5	1.8	1.6	2.2
LSD 5%			0.5			0.3
Salt stress		3.8	3.4	2.3	1.8	
LSD 5%			0.3			

NS Non-significant at $p<0.05$

3.3 Plumule length (cm)

Buhoth70 cultivar was superior compared to the others; while Inqath cultivar gave the lowest mean (Table 4). This maybe belongs to the genotype differences basically in their nature of speed, division and growth of cells. The treatment of primed seed was significantly superior compared to the unprimed (Table 4). The effect of priming may be due to the acceleration of germination and the emergence of the radicle and plumule, giving it a higher chance of growth during the time. This supported by the positive correlation between this trait and the germination ratio (Table 5). There was an inverse linear relationship between salt stress levels and mean plumule length, significantly decreasing the length of plumule with increasing of saline stress levels. The control treatment gave the highest mean, while the lowest mean returned to 12 $dS^* m^{-1}$ treatment (Table 4). The reason may be due to the negative effect of salinity on the activity of growth regulators, and inhibition of division and elongation, which caused the reduction of length. The results of the binary combinations of cultivars and priming showed improvement in cultivars performance by priming their seed compared to the unprimed (Table 4). The combination (Buhoth70 x primed seed) significantly exceeded the others and gave the highest mean; while the combination (Inqath × unprimed seed) gave the lowest mean (Table 4). The results of the binary combinations of cultivars and salt stress showed that the cultivars differed in their ability to tolerate salt stress at the same salt stress level (Table 4). The combination (Buhoth70 × 0) significantly exceeded the others and gave the highest mean, while the lowest mean belonged to the combination (Inqath x 12) (Table 4). A highly significant correlation observed between plumule length and each one of germination ratio and radicle length (0.656 and 0.456), respectively, (Table 5).

Table 4. Plumule length (cm) by the effect of cultivars, priming and salt stress in sorghum

Seed priming	Cultivars	Salt stress (dS.m^{-1}) 0	6	9	12	Seed priming × Cultivars
Primed seed	Buhoth70	13.3	10.2	8.4	7.6	9.9
	Inqath	8	6.7	5.6	3.4	5.9
	Rabeh	8.6	7.8	5.8	5.5	6.9
Unprimed seed	Buhoth70	10.3	7.5	6.5	5.4	7.4
	Inqath	5.2	4.6	4.3	2.6	4.2
	Rabeh	7	6.5	5.1	3.7	5.6
LSD 5%			NS			0.6
						Seed priming
Seed priming × Salt stress	Primed seed	10	8.2	6.6	5.5	7.6
	Unprimed seed	7.5	6.2	5.3	3.9	5.7
LSD 5%			NS			0.3
						Cultivars
Cultivars × Salt stress	Buhoth70	11.8	8.9	7.4	6.5	8.6
	Inqath	6.6	5.7	4.9	3	5.1
	Rabeh	7.8	7.1	5.4	4.6	6.2
LSD 5%			0.8			0.4
Salt stress		8.7	7.2	5.9	4.7	
LSD 5%			0.5			

[NS] Non-significant at $p<0.05$

Table 5. Simple correlation coefficient values among the studied traits in sorghum under the influence of cultivars, priming and salt stress in sorghum

Studied traits	Germination ratio	Radicle length	Plumule length	Dry seedling weight
Radicle length	0.639**			
Plumule length	0.656**	0.456**		
Seedling dry weight	0.577**	0.418**	0.648**	
Dry seedling weight	0.839**	0.730**	0.918**	0.664**

** Significant at $p<0.01$

3.4 Seedling dry weight (mg)

The cultivar of Buhoth70 was superior compared to others, and Rabeh cultivar gave the lowest mean (Table 6). The reason for the superiority of Buhoth70 cultivar in dry seedling weight maybe returns to its superiority in the percentage of germination (Table 2). This indicates that the seeds produced seedlings have a vigor which should have the ability to make new materials efficiently and quickly to transfer it to the developing embryonic axis, resulting in increased accumulation of dry matter [26]. The treatment of primed seed was significantly superior compared to the unprimed (Table 6), maybe because of the gibberellin effect on chlorophyll ratio, which means improvement of plant growth through increased accumulation of dry matter. Ma and Subedi [27] found that dry seedling weight decreased significantly with the increase in salt stress levels. The treatment of the 6 dS.m^{-1} gave the highest mean without significantly different from the control treatment, while the lowest mean treated with the 12 dS.m^{-1} (Table

6). The reason may be due to the toxic effect of sodium chloride, disruption of dry matter accumulation and the decrease in biological activity, resulting in reduced dry weight as well as lower lengths of radicle and plumule. The results of the binary combinations of cultivars and salt stress showed that the cultivars differed in their ability to tolerate salt stress at the same stress level and gave the highest mean without significant difference with the two combinations (primed seed × 6) and (primed seed × 9), while the lowest mean was due to the combination (unprimed seed × 12) (Table 6). A highly significant correlation found between seedling dry weight and each one of germination ratio and lengths of radicle and plumule (0.577, 0.418, and 0.648), respectively, (Table 5). It seems that this trait affected by plumule length more than radicle length, and this supported by the higher correlation value with plumule length compared to radicle length (Table 6). The combination (Buhoth70 × 0) significantly exceeded most other combinations and gave the highest mean without differing significantly with the combination (Buhoth70 x 6), while the lowest belonged to (Rabeh × 9) (Table 6). The results of combinations between priming and salt stress showed that priming had improved seed performance to tolerate salt stress compared to the unprimed at the same stress level (Table 6). The combination (primed seed × 0) significantly exceeded others and gave the highest mean without significant differences with the two combinations (primed seed × 6) and (primed seed × 9), while the lowest mean was due to the combination (unprimed seed × 12) (Table 6). A highly significant correlation found between seedling dry weight and each one of germination ratio and lengths of radicle and plumule (0.577, 0.418, and 0.648), respectively, (Table 5). It seems that this trait is affected by plumule length more than radicle length, and this supported by the higher correlation value with plumule length compared to radicle length.

Table 6. Dry seedling weight (mg) by the effect of cultivars, priming and salt stress in sorghum

Seed priming	Cultivars	Salt stress ($dS.m^{-1}$)				Seed priming × Cultivars
		0	6	9	12	
Primed seed	Buhoth70	0.118	0.112	0.1	0.095	0.106
	Inqath	0.073	0.081	0.084	0.082	0.08
	Rabeh	0.079	0.073	0.064	0.065	0.07
Unprimed seed	Buhoth70	0.102	0.1	0.072	0.065	0.085
	Inqath	0.067	0.076	0.06	0.055	0.065
	Rabeh	0.055	0.064	0.042	0.051	0.053
LSD 5%		NS				NS
						Seed priming
Seed priming × Salt stress	Primed seed	0.09	0.089	0.082	0.081	0.085
	Unprimed seed	0.075	0.08	0.058	0.057	0.067
LSD 5%		0.009				0.004
						Cultivars
Cultivars × Salt stress	Buhoth70	0.11	0.106	0.086	0.08	0.095
	Inqath	0.07	0.079	0.072	0.068	0.072
	Rabeh	0.067	0.069	0.053	0.058	0.062
LSD 5%		0.011				0.005
Salt stress		0.082	0.084	0.07	0.069	
LSD 5%		0.006				

NS Non-significant at $p<0.05$

3.5 Seedling vigor index

Buhoth70 cultivar was superior compared to others, and Rabeh cultivar had the lowest mean (Table 7). The treatment of primed seed was significantly superior compared to the unprimed (Table 7). The mean of seedling vigor index decreased significantly with increased saline stress levels, with the control treatment giving the highest mean, while the lowest mean returned to the 12 $dS.m^{-1}$ (Table 7). There was an inverse linear relationship between saline stress levels and means of seedling vigor index. This is due to the same results trends seedling growth such as germination ratio, lengths of radicle and plumule (Table 2,3 and 4). The results of the binary combinations of cultivars and salt stress showed that the cultivars differed in their ability to tolerate salt stress at the same stress level (Table 7). The combination (Buhoth70 x 0) significantly surpassed the rest combinations and gave the highest mean, while the lowest mean belonged to the combination (Buhoth70 x 12) (Table 7). The results of the binary combinations between priming and salt stress showed that priming had improved seed performance to tolerate salt stress compared to the unprimed at the same stress level (Table 7). The combination (primed seed × 0) significantly exceeded the rest combinations and gave the highest mean, while the lowest mean was due to the combination (unprimed seed x 12) (Table 7). There was a significant and positive correlation between seedling vigor index and each one of germination ratio, radicle and plumule lengths and seedling dry weight (0.839, 0.730, 0.918 and 0.664), respectively, (Table 5). The highest value was with plumule length, which means its essential criteria and represents division activity, elongation, growth that reflects the seedling vigor index.

Table 7. Seedling vigor index by the effect of cultivars, priming and salt stress in sorghum

Seed priming	Cultivars	Salt stress ($dS.m^{-1}$) 0	6	9	12	Seed priming × Cultivars
Primed seed	Buhoth70	1,424	1,123	876	623	1,011
	Inqath	1,118	933	603	444	775
	Rabeh	1,018	797	556	557	732
Unprimed seed	Buhoth70	1,025	777	629	383	703
	Inqath	688	494	344	200	431
	Rabeh	617	538	383	289	457
LSD 5%			NS			NS
						Seed priming
Seed priming × Salt stress	Primed seed	1,187	951	678	541	839
	Unprimed seed	777	603	452	290	531
LSD 5%			125			36
						Cultivars
Cultivars × Salt stress	Buhoth70	1,225	950	753	503	857
	Inqath	903	714	473	322	603
	Rabeh	818	667	470	423	595
LSD 5%			88.2			44.1
Salt stress		982	777	565	416	
LSD 5%			50.9			

[NS] Non-significant at $p<0.05$

4. Conclusions

It can conclude that the variance between cultivars to tolerate salt stress belonged to their potential ability. The increase of salt stress during germination stage led to decrease germination rate. Seed priming is an effective technique to improve seed performance under salt stress. Therefore, it can recommend priming sorghum seed before planting under salt conditions.

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