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EFFECT OF SOAKING WITH BREAD YEAST EXTRACT ON SORGHUM SEED GERMINATION UNDER SALT STRESS CONDITIONS

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Abstract

Salt stress negatively affects germination and seedling growth. Sorghum cultivars (Bohuth70, Inqath and Rabeh), seed soaking in dry yeast extract (3, 6 and 9 g l^{-1}) in addition to dry seeds and electrical conductivity (4, 10 and 16 dS m^{-1}) were studied. Traits of germination ratio at first and final counts, lengths of radicle and plumule, seedling dry weight and seedling vigour index were studied. The cultivar of Bohuth70 and concentration of yeast extract (9 g l^{-1}) were superior at all studied traits, while all traits values were reduced with increased saline stress. The combination (Bohuth70×9×4) was superior to most other treatments at first and final counts, radicle length and seedling dry weight, while superiority of plumule length and seedling vigour index belonged to the combination of (Rabeh×9×4). The genetic factor and seed soaking in yeast extract can enhance seed susceptibility to germinate with salt stress or not.

Key words: electrical conductivity, germination, *Saccharomyces cerevisiae*, sodium chloride.

Introduction

Increasing the concentration of soluble salts in the media of plant growth causes metabolic processes and weak plant growth or death (Zhu, 2007). Salt stress affects seed germination, seedling growth and subsequent growth stages due to the accumulation of soluble salts that limit the ability of seed or roots to absorb water with an entry of certain ions in an amount that does not match the cell's need to affect its vital processes (Tsakalidi and Barouchas, 2011). Salinity tolerance at the germination stage is an important factor because salinity is often found in the surface layer of soil (Majid and Gholamin, 2011). Some researchers have found that irrigation with relatively salty water reduces seed absorption of water and thus negatively affects processes related to nutrient uptake and embryo development (Al-Karaki, 2001; Miceli *et al.*, 2003). Ibrahim, (2014) found that the addition of NaCl at 0.5, 1.0 and 1.5% had reduced germination ratio at the first and final counts, length of both radicle and plumule and their fresh and dry weights. The types of stresses that the plant is exposed to in the presence of dissolved salts are osmotic potential, ionic toxicity, ionic imbalance, oxidative stress and stress due to hormonal imbalance (Zhu, 2002; Tester and Davenport, 2003; Ashraf, 2004). El-Naim *et al.,* (2012) found that the treatment of sorghum cultivars seed in high-concentration (16 dS m^{-1}) resulted in inhibition of germination ratio and seedling growth due to accumulation of salts in seed and then toxicity. Salt tolerance of sorghum cultivars might be due to their genetic variation. Indexes of germination and seedling vigor, lengths of shoot and root and seedling dry weight of forage sorghum cultivars were reduced significantly by salinity concentrations (Kandil *et al.*, 2012). Yeast is a microbial organism, a single cell, eukaryotic, multiplying by simple division or sprouting and produces oxycene, gerbillin and cytokine that an important to germination, growth and differentiation of plant tissues and their responsiveness to environmental conditions (Tartoura, 2001; El-Tohamy *et al.*, 2008). Bread dry yeast also produces many enzymes, contains vitamins and minerals such as P, K, Ca, Fe, Mn, Zn and other compounds, as well as amino acids such as lysine and methionine (Sacakli *et al.*, 2013). Fedotov *et al.,* (2017) studied the role of soil yeasts in seed germination and found that microorganisms multiply on surface of seeds that soaked in solution, also the endophytic microorganisms

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Table 1: Mean of squares (MS) according to analysis of variance for effect of cultivars, yeast and salt stress on traits studied in sorghum.

Source of Variation	df	First count	Final count	Radicle length	Plumule length	Seedling dry weight	Seedling vigour index
C	2	880.44*	582.33*	26.813*	69.896*	0.071809*	1364779*
Y	3	916.85*	802.19*	187.548*	26.588*	0.004878*	3582236*
S	2	29732.11*	19760.33*	280.876*	159.778*	0.116791*	14517945*
C × Y	6	264.30*	53.74	6.100*	11.492*	0.008244*	86529*
C × S	4	390.44*	335.17*	20.241*	5.556*	0.005023*	340619*
Y × S	6	3230.19*	4752.19*	51.798*	4.633*	0.006559*	1527410*
C × Y × S	12	182.96*	198.57*	23.606*	9.200*	0.005636*	381789*
Residual	108	42.41	50.70	1.886	1.293	0.001493	26986
SE		6.5	7.1	1.4	1.1	0.04	164.3
CV		9.3	8.9	24.9	16.1	25.7	15.1

Cultivars (C), Yeast (Y), Salt stress (S), Standard error (SE) and Coefficient of variance (CV). * Significant at $P \leq 0.05$

inside the seed and the seeds themselves begin to develop when treated using a semi-dry method. Soil yeasts accelerated seeds development of wheat and barley (Fedotov *et al.*, 2017). This yeast composition varied between plant-stimulating hormones, activated materials of cellular division and formation of nucleic acids, nutrient compounds and metal elements, all of which could push an embryo to improve its ability to overcome the obstruction that caused by salt stress at high concentrations (Sacakli *et al.*, 2013). Abraheem *et al.*, (2016) found that soaking wheat seeds in yeast extract (8 g l^{-1}) gave the highest ratio of first and final germination, radicle length and seedling dry weight and yeast extract showed the ability to raise the values of germination and growth indicators of wheat seedlings at absence or presence of salt stress and up to 1.5% concentration of NaCl. Seed germination is a key to determine a good field establishment (Tanji, 2004). Hamza, (2006) tested viability and vigour seeds under the effect of cultivars; seed of "Inqath" cultivar was significantly superior in radical length, plumule length and seedling dry weight compare to Rabeh cultivar. Bafeel, (2014) mentioned that root growth was different among cultivars when treated with sea or normal water. The vitality and vigour of seed is an important indicator of their quality and their impact on field establishment and yield potential (Hamza *et al.*, 2008). Most cultivars of sorghum have low field emergence as common problem and cultivars or seed priming were effect on seed vitality and vigour traits significantly (Al-Baldawi and Hamza, 2017). Based on the above, the aim of the experiment is to increase the susceptibility of sorghum seed to germination and seedling growth under saline conditions by infusing it in yeast extract.

Table 2: Effect of cultivars on traits studied in sorghum.

Cultivars	First count (%)	Final count (%)	Radicle length (cm)	Plumule length (cm)	Seedling dry weight (mg)	Seedling vigour index
Bohuth70	75 a	84 a	6.4 a	7.9 a	0.192 a	1256 a
Inqath	66 c	78 b	5.3 b	5.7 b	0.116 c	918 c
Rabeh	69 b	79 b	4.9 b	7.6 a	0.143 b	1088 b

Means followed by the same letters didn't differ significantly at $P \leq 0.05$

Materials and Methods

A factorial laboratory experiment was carried out with three factors at the Seed Technology Laboratory, Department of Field Crops, College of Agricultural Engineering Sciences, University of Baghdad in 2017. The first factor was three cultivars of *Sorghum bicolor* L. (Bohuth70, Inqath and Rabeh). The second factor was seed soaking in three levels (3, 6 and 9 g l^{-1}) of dry yeast extract (*Saccharomyces cerevisiae*) as well as control treatment (dry seeds). The third factor was three levels of electrical conductivity (4, 10 and 16 dS m^{-1}) using NaCl salt. Complete randomized design with four repetitions was used. Nutrients, compounds and their ratio (%) in dry yeast of bread were N (1.2), P (0.13), K (1.2), Mn (0.013), Ca (0.04), Na (0.01), Mg (0.07), Zn (0.04), Cu (0.04), B (0.016), Mo (0.0003), total protein (5.3), carbohydrate (4.7), IAA (0.5) and GA3 (0.03).

The seed were sterilized with the solution of sodium hypochlorite (1%) for 30 seconds and then washed with distilled water and dried with paper towels. The yeast extract was digested by dissolving 0.5 g of sucrose in 1 liter of warm distilled water (30°C). The mixture was stirred to ensure sucrose was dissolved and dry yeast was added in the above weights to obtain the required concentrations. These solutions were incubated at 30°C incubator for 4 hours and then filtered to obtain the required solutions and then seed were soaked in

Table 3: Effect of yeast on traits studied in sorghum.

Yeast (g l^{-1})	First count (%)	Final count (%)	Radicle length (cm)	Plumule length (cm)	Seedling dry weight (mg)	Seedling vigour index
Dry seed	64 c	78 b	3.9 c	6.8 b	0.136 b	864 c
3	68 b	77 b	4.9 b	6.2 c	0.149 ab	970 b
6	73 a	79 b	4.4 bc	7.1 b	0.152 ab	961 b
9	75 a	87 a	8.9 a	8.2 a	0.164 a	1555 a
Means followed by the same letters didn't differ significantly at $P \leq 0.05$						

Table 4: Effect of cultivars on traits studied in sorghum.

Salt stress (ds m^{-1})	First count (%)	Final count (%)	Radicle length (cm)	Plumule length (cm)	Seedling dry weight (mg)	Seedling vigour index
4	92 a	99 a	8.3 a	9.1 a	0.207 a	1714 a
10	75 b	84 b	3.8 b	6.6 b	0.114 b	864 b
16	43 c	59 c	4.5 c	5.5 c	0.131 c	684 c
Means followed by the same letters didn't differ significantly at $P \leq 0.05$						

for 24 hours with keeping in incubation in 25°C and then seed were air dried for 24 hours at room temperature. 200 pure seed (50 seed per replicate) were placed in paper towels alternately and drew and placed in the germinator under 25°C and humidity 80% for 10 days and then studied the following traits: Germination ratio at the first and final counts (%) in the standard laboratory germination test; These tests were carried out after 4 and 10 days of laying seed in a germinator, respectively and calculated by dividing the number of normal seedlings by the total number of seed expressed as a ratio (ISTA, 2013). Lengths of radicle and plumule (cm) and seedling dry weight (mg); Randomly, 10 normal seedlings were taken at the end of standard laboratory germination test and measuring the lengths of radicle after the separation of the point of contact with the seed and plumule after separation from the point of contact with the mesocotyl and then placed in paper bags perforated in an oven at 80°C for 24 hours and then weighed (Hampton, TeKrony, 1995). Seedling vigour index was calculated according to the equation proposed by Abdul-Baki and Anderso, (1973); seedling vigour index = germination ratio at final count × (radicle length + plumule length). Data were collected and analyzed statistically according to the analysis of variance as complete randomized design. The least significant difference was used to compare means at 5% probability level (Steel and Torrie, 1980). Statistical program (Genstat) to analyze data was used.

Results and Discussion

The variance analysis results (Table 1) showed significant differences in all traits which studied under the effect of cultivars, yeast, salt stress and their combinations, except for the effect of the combination between cultivars and yeast. The highest effect of the independent factors on the traits of first and final counts, radicle length and seedling vigour index were due to the effect of salt stress followed by the effect of yeast and then the effect of cultivars. The highest effect of the independent factors on the plumule length and seedlings dry weight were due to the effect of salt stress followed by the effect of cultivars and then the effect of yeast.

The cultivar of Bohuth70 and the concentration of yeast extract (9 g l^{-1}) were superior at all studied traits, while Inqath cultivar and dry seed gave the lowest values for those traits (Tables 2 and 3). The values of all traits

Table 5: Effect of cultivars × yeast on traits studied in sorghum.

Cultivars	Yeast (mg l^{-1})	First count (%)	Final count (%)	Radicle length (cm)	Plumule length (cm)	Seedling dry weight (mg)	Seedling vigour index
Bohuth70	Dry seed	73 ab	80 a	4.1 efg	8.7 a	0.198 ab	1071 c
	3	77 a	82 a	5.9 d	6.8 cd	0.182 b	1127 c
	6	72 ab	81 a	5.0 def	8.4 ab	0.170 bc	1133 c
	9	76 a	93 a	10.4 a	7.7 bc	0.220 a	1691 a
Inqath	Dry seed	57 d	77 a	3.4 g	4.4 f	0.098 e	602 g
	3	62 cd	75 a	5.1 de	5.3 ef	0.118 de	912 de
	6	70 b	76 a	4.0 fg	5.4 e	0.104 e	738 f
	9	75 ab	82 a	8.8 b	7.7 bc	0.144 cd	1421 b
Rabeh	Dry seed	62 cd	77 a	4.1 efg	7.2 cd	0.112 e	918 de
	3	64 c	74 a	3.7 g	6.4 d	0.148 cd	869 ef
	6	76 a	80 a	4.3 efg	7.6 bc	0.181 b	1010 cd
	9	75 a	86 a	7.5 c	9.3 a	0.129 de	1553 b
Means followed by the same letters didn't differ significantly at $P \leq 0.05$							

Table 6: Effect of cultivars × salt stress on traits studied in sorghum.

Cultivars	Salt stress (ds m⁻¹)	First count (%)	Final count (%)	Radicle length (cm)	Plumule length (cm)	Seedling dry weight (mg)	Seedling vigour index
Bohuth70	4	96 a	99 a	10.1 a	9.8 a	0.245 a	1976 a
	10	74 c	85 b	4.6 cd	7.2 b	0.151 c	997 d
	16	54 d	69 c	4.3 de	6.7 b	0.181 b	794 fg
Inqath	4	88 b	98 a	7.1 b	7.1 b	0.192 b	1379 c
	10	74 c	83 b	3.3 f	5.5 c	0.078 e	698 gh
	16	36 e	53 d	5.6 c	4.4 d	0.078 e	679 hj
Rabeh	4	92 ab	99 a	7.7 b	10.3 a	0.182 b	1786 b
	10	77 c	84 b	3.5 ef	7.1 b	0.113 d	897 de
	16	40 e	55 d	3.5 ef	5.5 c	0.132 cd	579 j
Means followed by the same letters didn't differ significantly at P ≤ 0.05							

were reduced with increased saline stress and a significant difference at each level studied was found (Table 4).

The underlying capability of the Bohuth70 cultivar seems to be high; and this has begun to be evident when the Bohuth70 cultivar was superior in comparison with the Inqath and Rabeh cultivars in all traits studied (Table 2). This is reinforces the fact that refers to the nature of the genetic structure and its inherent potential which effects on the variance of behavior nature and the cumulative growth of any crop. This is consistent with the results of Hamza, (2006) and Bafeel, (2014).

Soaking seeds with extract of dry bread yeast led to has improve their ability to germinate and seedling growth (Table 3) because of the benefits that related to stimulating and accelerating the metabolites that occur in the seed during the germination process; as well as dry bread yeast extract contains many compounds which contribute directly and indirectly to the growth and development of the embryo and this is consistent with what mentioned by Tartoura, (2001); El-Tohamy *et al.,* (2008) and Sacakli *et al.,* (2013).

Salt stress is still a real impediment to the ability of seeds to germinate and to form a normal seedling capable of self-reliance. This is due to the role of salts in discouraging metabolic processes that occur during germination as well as discouraging subsequent processes that promote growth and create a normal seedling. This is illustrated by the results in table 4, as all the traits studied significantly have been reduced, with saline stress being increased at each level. This is consistent with what mentioned by Zhu, (2007), Tsakalidi and Barouchas, (2011) and Ibrahim, (2014).

The combination (70 × 9) was superior to the rest of the parameters; first count, radicle length, seedling dry weight and seedling vigour index and the combination (Rabeh × 9) was superior at plumule length, while there was no significant difference at the final count (Table 5).

Seed response of each cultivar was varied after primed with dry bread yeast extract. In general, that response was positive compared to the response of dry seed of each cultivar for most traits studied, as well as when the cultivars were compared at each level of dry

Table 7: Effect of yeast × salt stress on traits studied in sorghum.

Yeast (mg l⁻¹)	Salt stress (ds m⁻¹)	First count (%)	Final count (%)	Radicle length (cm)	Plumule length (cm)	Seedling dry weight (mg)	Seedling vigour index
Dry seed	4	83 c	99 a	5.1 cd	8.4 b	0.187 b	1343 d
	10	47 e	90 cd	3.2 f	6.2 cd	0.110 ef	839 e
	16	63 d	44 g	3.3 f	5.7 d	0.111 ef	409 f
3	4	88 b	99 a	9.5 b	8.4 b	0.220 a	1776 b
	10	86 bc	93 bc	3.4 ef	6.4 cd	0.104 ef	902 e
	16	30 g	39 g	1.8 g	3.8 e	0.123 def	231 g
6	4	98 a	100 a	5.8 c	9.0 b	0.232 a	1478 c
	10	81 c	84 d	4.1 def	6.8 c	0.093 f	910 e
	16	39 f	53 f	3.3 f	5.6 c	0.131 cde	494 f
9	4	99 a	96 ab	12.7 a	10.6 a	0.187 b	2258 a
	10	86 bc	67 e	4.5 de	7.1 c	0.149 cd	805 e
	16	40 f	98 ab	9.5 b	7.0 c	0.157 bc	1602 c
Means followed by the same letters didn't differ significantly at P ≤ 0.05							

Table 8: Effect of cultivars × yeast × salt stress on germination ratio at first and final count (%) and radicle length (cm) in sorghum.

Cultivars	Yeast ($g\ l^{-1}$)	First count (%) Salt stress ($dS\ m^{-1}$)			Final count (%) Salt stress ($dS\ m^{-1}$)			Radicle length (cm) Salt stress ($dS\ m^{-1}$)		
		4	10	16	4	10	16	4	10	16
Bohuth70	Dry seed	85	63	72	100	84	56	5.5	3.4	3.5
	3	100	82	48	100	94	53	12.0	3.5	2.3
	6	100	74	43	100	79	65	6.0	4.2	4.9
	9	99	78	51	97	82	100	17.0	7.5	6.6
Inqath	Dry seed	79	36	57	100	95	35	4.2	2.5	3.5
	3	80	88	19	99	90	36	10.6	3.0	1.7
	6	94	81	35	99	84	45	4.8	4.1	2.9
	9	100	92	32	92	61	94	8.6	3.5	14.3
Rabeh	Dry seed	84	42	59	98	91	41	5.7	3.7	3.0
	3	84	87	22	99	94	28	5.9	3.8	1.5
	6	100	89	40	100	90	49	6.7	4.1	2.1
	9	99	89	38	100	59	100	12.5	2.5	7.5
LSD (5%)		9			10			1.9		

Least significant difference at $P \leq 0.05$ (LSD 5%), Not significant (ns)

bread yeast extract (Table 5). This reinforces the reality of the active influence between genetic structure and other factors influencing it directly and indirectly. The superiority of Bohuth70 cultivar were clearly through high positive response compared to another cultivars regardless if their seeds were dry or primed. This corresponds to what mentioned by Fedotov *et al.,* (2017)

The combination (70 × 4) was superior to the rest at all studied traits, while the lowest values for the same traits were due to the combination (Inqath × 16) (Table 6).

Table 9: Effect of cultivars × yeast × salt stress on plumule length (cm), seedling dry weight (mg) and seedling vigour index in sorghum.

Cultivars	Yeast ($g\ l^{-1}$)	Plumule length (cm) Salt stress ($dS\ m^{-1}$)			Seedling dry weight (mg) Salt stress ($dS\ m^{-1}$)			Seedling vigour index Salt stress ($dS\ m^{-1}$)		
		4	10	16	4	10	16	4	10	16
Bohuth70	Dry seed	11.6	7.1	7.3	0.271	0.155	0.168	1708	889	617
	3	8.4	6.5	5.5	0.220	0.173	0.152	2036	937	410
	6	11.1	7.6	6.5	0.228	0.117	0.164	1712	931	757
	9	8.3	7.5	7.3	0.262	0.158	0.240	2449	1232	1393
Inqath	Dry seed	5.5	3.8	3.8	0.156	0.068	0.071	968	598	240
	3	8.3	5.2	2.4	0.253	0.019	0.084	1869	724	144
	6	5.9	5.9	4.4	0.197	0.058	0.059	1067	814	334
	9	8.9	7.3	7.0	0.164	0.167	0.100	1612	655	1998
Rabeh	Dry seed	8.2	7.6	6.0	0.134	0.108	0.093	1354	1030	371
	3	8.5	7.4	3.4	0.188	0.122	0.135	1423	1046	140
	6	9.9	6.9	6.0	0.271	0.103	0.170	1656	984	391
	9	14.6	6.5	6.7	0.136	0.120	0.131	2713	530	1415
LSD (5%)		1.6			0.054			230		

Least significant difference at $P \leq 0.05$ (LSD 5%), Not significant (ns)

The behavior of the cultivars was similar and the highest values of the traits studied were related with the lowest level of salt stress and vice versa (Table 6). This is due to the negative effect of high salinity accumulation which causes ionic toxicity due to ionic and hormonal imbalance and this is consistent with Zhu, (2002); Tester and Davenport, (2003); Ashraf, (2004) and El-naim *et al.,* (2012). The performance of the Bohuth70 cultivar was under salt stress better than the other two (Table 6), possibly because of a variation in the genetic structure that leads to variation at saline stress tolerance and this is in line with Kandil *et al.,* (2012).

The combination (9 × 4) was superior to the rest at first count, lengths of radicle and plumule and seedling vigour index. Also, the combination (6 × 4) was superior at final count without significantly different with the combination (9 × 4), while the lowest values for the same traits were due to the combination (3 × 16) (Table 7).

The behavior of dry or primed seeds was similar under salt stress levels. All traits that studied were enhanced after seed priming treatment with yeast regardless under high or low level of salt stress (Table 7). This is mean seed priming with yeast has a role to improve salt stress tolerance. This is maybe due to the positive effect of yeast composition during seed germination process. The positive effect of high level of yeast under low salt stress was better than that under high level of salt stress (Table 7), possibly because of salt stress negatively effect on microorganism activity too under high levels. This is in line with Fedotov *et al.*, (2017); Sacakli *et al.,* (2013); Abraheem *et al.*, (2016).

The combination (Bohuth70 × 9 × 4) was superior to most other treatments at first and final counts, radicle length and seedling dry weight without any significant differences with some other treatments. As for superior traits of plumule length and seedling vigour index was belong to the combination of (Rabeh × 9 × 4) (Table 8).

The results in table 8 showed the positive response of the cultivars to the high levels of seed priming by yeast compared to dry seeds under the low level of salt stress at the first count and these differences have disappeared at the final count, as there is insignificant difference between all cultivars under all priming levels including dry seeds at low salt stress level. However, these differences were evident in other traits such as radicle length, seedling dry weight, plumule length and seedling vigour index (Tables 8 and 9). This variation is due to the interaction between study factors (cultivars × yeast × salt stress) resulting from the difference in the amount and trend of the response to the genetic structure under the influence of several environmental variables such as microbiology and salt stress.

Conclusions

It's clear that salinity causes ionic imbalance, leading to reduce metabolic activity, cells division and expansion. Salt stress effects on seed germination and seedling growth which are known to be more sensitive stags for most crops and seed priming maybe reduce these negative effect in addition to genotypes. Therefore, it can be concluded that the genetic factor and seed soaking in yeast extract can enhance seed susceptibility to germinate with salt stress or not.

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