J. Agr. Sci. Tech. (2017) Vol. 19: 1319-1332

Selectable Traits in Sorghum Genotypes for Tolerance to Salinity Stress

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ABSTRACT

Sorghum [*Sorghum bicolor* (L.) Moench] is moderately tolerant to salinity and it is important as a candidate crop for both fodder and grain in salt-affected areas. This pot experiment was conducted at Research Greenhouse of College of Agriculture, Shiraz University, Iran, to evaluate the relative effectiveness of biochemical traits and stress tolerance indices contributing to genotypic differences in salinity tolerance in 30 lines and 14 cultivars of sorghum. In addition, a new indicator, Storage Factor Index (SFI), was defined and used to quantify the Na^+ partitioning between shoot and root. Among the indices, stress tolerance index was found useful as a selection criterion. Furthermore, the tolerant genotypes had higher K^+/Na^+ ratio in shoot and root with greater SFI, indicating that most of Na^+ was stored in their roots. Although peroxidase and superoxide dismutase were enhanced under salinity conditions in both sensitive and tolerant genotypes, only Catalase (CAT) activity was found to be promoted in tolerant lines/cultivars. Proline accumulation did not appear to be related to salinity tolerance in sorghum lines/cultivars. Overall, our findings suggested that salinity tolerance in sorghum genotypes was not only associated with Na^+ exclusion from the shoot, but also with the enhancement of CAT activity.

Keywords: Catalase, Proline, Storage factor index, Stress tolerance index.

INTRODUCTION

Increased salinization is one of the major factors limiting plant growth and productivity, especially in arid and semi-arid areas (Barati *et al.,* 2017). Among crops, sorghum is one of the candidate crops for salt-affected areas, due to its high flexibility for extreme conditions (Kafi *et al.,* 2013) as well as high tolerance to drought and salinity stress (Bavei *et al.*, 2011). Several workers have reported that substantial genotypic variations have been recorded for sorghum cultivars under salinity conditions (Krishnamurthy *et al.,* 2007; Bavei *et al.*, 2011). However, some researchers have attributed the higher salinity tolerance in sorghum genotypes to a higher phytomass production (Krishnamurthy *et al.,* 2007). Generally, tolerance to salinity has been defined as the ability of a plant genotype to thrive under saline conditions, thereby minimizing yield loss (Arzani and Ashraf, 2016). Plants have developed various strategies including biochemical and bio-physical mechanisms to alleviate salinity stress on plant growth (Arzani and Ashraf, 2016). Proline can act as a compatible solute, osmoprotectant, and a protective agent for cytosolic enzymes and cellular organelles Furthermore, proline can be a carbon and nitrogen source, a membrane stabilizer and scavenger for free radicals (Verbruggen and Hermans, 2008). Although some workers have shown that salinity tolerance in many plant species is accompanied by proline accumulation (Arzani and Ashraf, 2016; Bazrafshan and Ehsanzadeh, 2016), some other researchers have indicated that proline

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accumulation has only been a reaction to salt stress, and salinity-sensitive cultivars accumulated higher amount of proline than salinity-tolerant genotypes, for instance, in sorghum (Lacerda *et al.,* 2003, 2005; Bavei *et al.,* 2011) and wheat (Houshmand *et al.,* 2005; Poustini *et al.,* 2007). To counteract salt-induced oxidative stress, plants commonly generate antioxidative enzymes such as SuperOxide Dismutase (SOD), Catalase (CAT) and Peroxidase (POD) (Pessarakli, 2011). It is generally demonstrated that tolerant cultivars have an enhanced or higher level of antioxidant activity compared to sensitive cultivars (Gupta and Huang, 2014), albeit it has also been suggested that, sometimes, the cultivar more sensitivity to salinity stress is associated with higher level of antioxidant activity (Tari *et al.,* 2013). It is widely accepted that selective uptake of K^+ over Na^+ is one of the most important mechanisms related to salinity tolerance (Bavei *et al.,* 2011; Shabala *et al.,* 2013; Tari *et al.,* 2013; Almodares *et al.,* 2014; Pandolfi *et al.,* 2016). Therefore, several workers suggested that greater K^+/Na^+ ratio and Na^+ exclusion could be used as a reliable criterion for screening and breeding salt tolerant cultivars (Netondo *et al.,* 2004; Krishnamurthy *et al.,* 2007; Zhu *et al.,* 2016). It seems that salinity-affected sorghum plants have high ability to restrict Na^+ and Cl^- translocation from the roots to the shoot (Tari *et al.,* 2013; Almodares *et al.,* 2014; Yan *et al.,* 2015). Although some researchers have already reported the physiological responses of sorghum cultivars to salinity, they normally have considered a few cultivars (Lacerda *et al.,* 2003, 2005; Netondo *et al.,* 2004; Bavei *et al.,* 2011). Those researchers who have compared many sorghum genotypes responses to salinity have not focused on all aspects of physiological attributes including proline accumulation, antioxidants, and ion distribution (Krishnamurthy *et al.,* 2007; Almodares *et al.,* 2014). Therefore, the main objective of the present investigation was to evaluate the possible physiological indices correlated to salinity tolerance in a large number of sorghum genotypes.

MATERIALS AND METHODS

Plant Materials and Growth Conditions

This experiment was carried out in a controlled environment (Research Greenhouse of College of Agriculture, Shiraz University, Iran) using 30 lines and 14 cultivars of sorghum (Table 1). The lines studied in this experiment were bred under different agro-climatic conditions in Iran and were released in 2013. The cultivars were commercially cultivated by sorghum growers and included: Jumbo, Nectar, Speed-feed, Sistan, Ghalami-herat, Pegah, Sepideh, KFS1, KFS2, KFS4, Broom corn, Sweet-sorghum, Kimia, and Moghan. Cultivars and lines of sorghum were obtained from the Seed and Plant Improvement Institute, Karaj, Iran. The pots were irrigated either with saline water (EC= 12 dS m^{-1}) or normal (EC= 2 dS m^{-1}) irrigation water. Plants were subjected to saline irrigation water (2:1 weight ratio of NaCl: $CaCl_2$) from seed emergence to harvesting time, representing farmers practice in most sorghum growing areas. To maintain consistency of salinity throughout the experiment, *EC* of pot drainage was also controlled by portable EC-meter (O'HAUS ST-300C-G). The average minimum and maximum temperature and relative humidity were 18 and 30°C, and 60%, respectively.

The pots (19 cm diameter, 18 cm height, containing 5 kg soil) were filled with 2:1 ratio of field soil and sand and were watered (+leaching fraction requirement) to reach the field capacity level. The physicochemical properties of the soil used for experimentation are given in Table 2. The water needed for keeping the soil moisture at field capacity level was determined by daily weighing of the pots. Ten seeds of each sorghum line/cultivar were sown at a depth of 2-3 cm and, after emergence; the seedlings were thinned to 5. Forty-days old plants (at vegetative stage) were harvested and divided into root and shoot, dried in aerated oven at 72°C for 2 days and the dry weights were recorded. Forty-days shoot dry weight of salt treated plants versus

Table 1. Mean values of shoot dry weight, tolerance indices and proline accumulation in 44 sorghum lines/cultivars.

Cultivars/Lines	Shoot dry weight (g plant^{-1})				Tolerance Indices			Proline (µM g^{-1} FW)			
	Control	Salinized	Changes		SSI[c]	STI[d]	GMP[e]	Control	Salinized	Changes	
			%[a]	S[b]						%	S
KDFGS1	1.55	1.32	-15	ns	0.27	0.28	1.43	3.53	4.86	38	ns
KDFGS2	1.82	1.03	-43	**	0.80	0.26	1.37	2.81	5.16	84	**
KDFGS3	1.69	0.82	-51	**	0.95	0.19	1.18	3.15	5.66	80	**
KDFGS4	2.05	0.93	-55	**	1.01	0.26	1.38	3.42	5.42	58	*
KDFGS5	1.45	0.64	-56	**	1.03	0.13	0.96	3.78	5.85	55	*
KDFGS6	1.88	0.95	-49	**	0.92	0.25	1.34	2.44	5.31	118	**
KDFGS7	1.85	0.78	-58	**	1.07	0.20	1.20	2.61	5.95	128	**
KDFGS8	1.71	1.02	-40	ns	0.75	0.24	1.32	3.12	5.25	68	**
KDFGS9	1.92	0.75	-61	**	1.13	0.20	1.20	2.4	5.94	148	**
KDFGS10	3.33	0.8	-76	**	1.41	0.37	1.63	1.98	5.89	197	**
KDFGS11	1.83	0.81	-56	**	1.03	0.20	1.22	2.7	5.81	115	**
KDFGS12	1.76	0.93	-47	ns	0.87	0.23	1.28	3.03	5.38	78	**
KDFGS13	1.98	0.63	-68	**	1.26	0.17	1.12	2.2	5.74	161	**
KDFGS14	1.87	0.69	-63	**	1.17	0.18	1.14	2.54	5.84	130	**
KDFGS15	4.41	2.43	-45	*	0.83	1.48	3.27	1.44	4.15	188	**
KDFGS16	1.81	1.03	-43	*	0.80	0.26	1.37	2.89	5.11	77	**
KDFGS17	1.84	1.06	-42	ns	0.78	0.27	1.40	2.64	5.06	92	**
KDFGS18	3.81	0.9	-76	**	1.41	0.47	1.85	1.92	5.45	184	**
KDFGS19	3.92	2.13	-46	**	0.85	1.15	2.89	1.91	4.31	126	**
KDFGS20	1.64	0.89	-46	**	0.85	0.20	1.21	3.27	5.47	67	**
KDFGS21	1.86	0.65	-65	**	1.20	0.17	1.10	2.59	5.86	126	**
KDFGS22	1.96	0.94	-52	**	0.96	0.25	1.36	2.33	5.33	129	**
KDFGS23	4.85	0.86	-82	**	1.52	0.58	2.04	1.5	5.71	281	**
KDFGS24	1.79	1.00	-44	**	0.82	0.25	1.34	2.92	5.29	81	**
KDFGS25	1.95	0.75	-62	**	1.14	0.20	1.21	2.39	5.92	148	**
KDFGS26	4.23	0.84	-80	**	1.48	0.49	1.88	1.41	5.89	318	**
KDFGS27	1.44	0.85	-41	ns	0.76	0.17	1.11	3.85	5.63	46	*
KDFGS28	1.53	0.88	-42	*	0.79	0.19	1.16	3.57	5.51	54	*
KDFGS29	2.52	1.23	-51	**	0.95	0.43	1.76	2.00	4.54	127	**
KDFGS30	1.07	0.61	-43	ns	0.80	0.09	0.81	3.87	6.03	56	**
Jumbo	4.71	2.63	-44	ns	0.82	1.71	3.52	1.24	3.01	143	**
Nectar	4.77	0.79	-83	**	1.54	0.52	1.94	1.21	6.08	402	**
Speed feed	4.59	2.6	-43	*	0.80	1.65	3.45	1.52	3.80	150	**
Sistan	4.28	2.34	-45	*	0.84	1.38	3.16	1.77	3.86	118	**
Ghalami Herat	4.73	2.69	-43	*	0.80	1.76	3.57	1.20	3.63	203	**
Pegah	4.52	2.55	-44	*	0.81	1.59	3.39	1.30	4.09	215	**
Sepideh	3.18	0.74	-77	**	1.42	0.32	1.53	1.98	5.79	192	**
KFS1	2.06	0.87	-58	**	1.07	0.25	1.34	2.14	5.56	160	**
KFS2	4.36	2.49	-43	**	0.79	1.50	3.29	1.54	4.24	175	**
KFS4	1.6	0.88	-45	**	0.83	0.19	1.19	2.64	5.5	108	*
Broom corn	1.91	1.11	-42	ns	0.78	0.29	1.46	2.43	4.95	104	*
Sweet sorghum	4.11	2.37	-42	**	0.78	1.34	3.12	1.65	4.04	145	**
Kimia	2.09	0.83	-60	**	1.12	0.24	1.32	2.01	5.57	177	**
Moghan	4.68	2.54	-46	**	0.85	1.64	3.45	1.22	3.1	154	**
Mean	2.70	1.24	-54	**	-	-	-	2.36	5.14	117	**
LSD (0.05)	0.49	0.45	-	-	-	-	-	1.02	1.57	-	-

[a] Percentage of changes upon salinity stress; [b] Significance level; [c] Stress susceptibility index; [d] Stress tolerance index, [e] Geometric mean productivity. [ns] Non-significant; * Significant at 0.05 probability level, ** Significant at 0.01 probability level.

Table 2. Some physicochemical properties of the experimental soil.

EC (dS m^{-1})	pH	Organic matter (%)	Nitrogen (%)	Phosphorus (mg kg^{-1})	Potassium (mg kg^{-1})	Texture
0.6	7.09	1.12	0.15	17	420	Silty loam

the control ones as shoot dry weight ratio were used to evaluate the salinity tolerance of sorghum lines/cultivars (Krishnamurthy *et al*., 2007).

Stress Indices

Three stress indices including Geometric Mean Productivity (GMP) (Fernandez, 1992); Stress Susceptibility Index (SSI) (Fischer and Maurer, 1978), and Stress Tolerance Index (STI) (Fernandez, 1992) were applied to evaluate the genotypic performance of lines/cultivars under saline conditions.

Antioxidant Enzymes and Proline Content

The last fully expanded leaves were cut to measure oxidative damage status by measuring the activity of some antioxidant enzymes including SuperOxide Dismutase (SOD) (EC 1.15.1.1), Peroxidase (POD) (1.11.1.7) and Catalase (CAT) (1.11.1.6). SOD, POD and CAT were determined using Beauchamp and Fridovich (1971), Chance and Maehly (1995) and Dhindsa *et al.* (1981) methods, respectively. Proline level was measured according to Bates *et al.* (1973) method.

Ion Distribution

The dried shoots (stem+leaves) and roots were used to measure Na^+ and K^+ concentrations by 410-Corning flame photometer. The samples were ashed by Oven (Paragon) at 600°C for 4 hours. Na and K contents were measured using 2N chloride acid extract (Horneck and Hanson, 1998).

To quantify Na^+ ion partitioning between root and shoot, we used Storage Factor Index (SFI) calculated as: *SFI= RI/TAI* (Pirasteh-Anosheh and Emam, 2016).

Where, *RI* and *TAI* are root Na^+ accumulation and total amount of Na^+ absorbed, respectively. Indeed, the Storage Factor Index (SFI) refers to the proportion of any ion (e.g. Na^+ or Cl^-) which remains in the root cells. A zero value of *SFI* means that almost all absorbed ions are transported to the shoot; whereas a value of 1 means all absorbed ions are stored in the root (Pirasteh-Anosheh and Emam, 2016). More Na^+ accumulation in roots and lower transportation to the shoot is considered as a mechanism for higher salinity tolerance in plants (Shabala *et al.*, 2013; Almodares *et al.*, 2014); thus, higher *SFI* could be used as an index for higher salinity tolerance potential.

Statistical Analysis

A Completely Randomized Design (CRD) with factorial treatments (30 lines and 14 cultivars and two salinity levels) with three replications was used. Analysis of variance was carried out using SAS (Statistical Analysis System) (SAS release 9.2, 2002) and the means were compared using the Least Significant Difference (LSD) test at *P*= 0.05. The Pearson correlation coefficients between phytomass production (under both conditions) and tolerance indices were determined.

RESULTS AND DISCUSSION

Stress Indices

Shoot dry weight, tolerance indices and proline level of genotypes are showed in

Table 1. There was substantial variation among genotypes with respect to shoot dry weight under both control and stress conditions (Table 1). Although the higher shoot dry weight under normal conditions was obtained by KDFGS23 line and Nectar cultivar (Table 1), they showed remarkably lower shoot dry weight under saline conditions (82 and 83 percent reduction, respectively). Similarly, lines number 10, 13, 14, 21, 26 and Sepideh and Kimia cultivars showed similar pattern (Table 1) indicating high sensitivity to salt stress. Interestingly, SSI index also verified these findings, where these lines/cultivars had higher SSI index (Table 1). For example, the highest value of SSI index was observed in lines number 23 (1.52), 26 (1.48) and Nectar cultivar (1.54) (Table 1). These differences have normally been attributed to the genetic potential capability of each genotype (Krishnamurthy *et al.,* 2007). Under saline conditions, Ghalami-herat, Jumbo, Speed-feed, Pegah, Moghan, KFS2, KDFGS15, Sweet sorghum, and Sistan produced higher shoot dry weight (Table 1). Indeed, these genotypes also had higher shoot dry weight under normal conditions and showed higher value for *STI* (higher than 1) and *GMP* (Table 1). Furthermore, highly significant positive correlations were found between *GMP* and *STI* (Table 3). In fact, indices *GMP* and *STI* were equally able to identify genotypes. Also, the correlation coefficients revealed that SSI was negatively correlated with shoot dry weight ($r= -0.43^{**}$) under saline conditions and had no correlation with shoot dry weight under normal conditions (Table 3). These findings have also been reported by Sio-Se Mardeh *et al.* (2006) who showed negative correlation between *SSI* and yield under stress conditions.

Similarly, Ali *et al.* (2013) found that SSI index could not capture genotypes with both high yield potential as well as stress tolerance. Our findings is also confirmed by the results of Porch (2006) and Ali *et al.* (2013) who found better performance and more effectiveness for *STI* compared to *SSI* to distinguish higher yielding genotypes across different environments. On the other hand, *STI* index considers genotypes with high yield potential and stress tolerance (Fernandez, 1993).

As a different face of the coin, Stress Susceptibility Index (SSI) can introduce genotypes with more stable yield under stress conditions (Fischer and Maurer, 1978). Indeed, the lower values for Stress Susceptibility Index (SSI) shows lower difference in the yield between the stress and normal conditions, which means more stability in yield.

Proline Content

A significant accumulation of proline occurred in all lines/cultivars as salinity stress was imposed (Table 1); however, the genotypes differed significantly in proline accumulation (Table 1). The accumulation of proline was higher in Nectar cultivar (402%) and lines number 10 (197%), 23 (281%) and 26 (381%) which were found to be sensitive genotypes based on tolerance indices. In contrast, salinity tolerant genotypes such as Ghalami-herat (203%),

Table 3. Correlation coefficients for tolerance indices and shoot dry weight.

	Yp [a]	Ys [b]	SSI [c]	STI [d]	GMP [e]
Yp	1				
Ys	0.73^{**}	1			
SSI	0.23^{ns}	-0.43^{**}	1		
STI	0.85^{**}	0.97^{**}	-0.23^{ns}	1	
GMP	0.89^{**}	0.96^{**}	-0.18^{ns}	0.99^{**}	1

[a] Shoot dry weight in non-stress conditions; [b] Shoot dry weight in stress conditions; [c] Stress Susceptibility Index; [d] Stress Tolerance Index, [e] Geometric Mean Productivity. ** Significant at 1% probability level, [ns] Non-significant.

Jumbo (143%), Speed-feed (150%), Pegah (215%), Moghan (177%), KFS2 (175%), KDFGS15 (188%), Sweet sorghum (145%) and Sistan (118%) had lower proline accumulation under saline conditions (Table 1). It was also found that shoot dry weight ratio i.e. shoot dry weight under salinity/shoot dry weight under control was negatively correlated with proline accumulation under saline conditions ($r= -0.52^{**}$) (data not shown). These findings are supported by Lacerda *et al.* (2003, 2005) who indicated that cultivars of sorghum which were classified as salinity-sensitive accumulated higher levels of proline. Although proline accumulation is a common response to wide range of stress conditions which contributes substantially to the cytoplasmic osmotic adjustment, yet, the relationship between proline accumulation and salinity tolerance is not proved (Verbruggen and Hermans, 2008; Munns and Tester, 2008; Yan *et al.*, 2015). For example, though several researchers have already found that salinity tolerance is normally associated with proline accumulation (Arias-Baldrich *et al.*, 2015; Bazrafshan and Ehsanzadeh, 2016), some studies have demonstrated that salt-sensitive cultivars exhibited greater accumulation of proline (Lacerda *et al.*, 2003, 2005; Tavakoli *et al.*, 2016). Regarding the positive correlation between Na^+ shoot concentration and proline accumulation ($r= 0.78^{**}$) (data not shown), it seems that proline accumulation for sensitive genotypes is considered as a mechanism to survive under stress conditions (Poustini *et al.*, 2007). Also, it appears that proline accumulation is only a result of salt injury rather than an adaption and acclimation to salinity conditions. On the other hand, accumulation of one mole of proline usually needs 41 mole of ATP consumption (Munns and Tester, 2008), and this process usually occurs at the expense of plant growth. So, proline accumulation might be associated with the reduction in plant growth. Therefore, our findings add more evidence supporting the idea that there might be no correlation between proline accumulation and salinity tolerance.

Responses of Antioxidant Enzymes

Salinity stress stimulated SOD and POD activities in all lines/cultivars (Table 4). There were remarkable variations among genotypes regarding antioxidant activities (Table 4). Under saline conditions, greater extent and more significant enhancing effect on antioxidant activity was observed in tolerant genotypes including Ghalami-herat, Jumbo, Speed-feed, Pegah, Moghan, KFS2, KDFGS15, Sweet sorghum, and Sistan (Table 4). Based on the results of the present investigation, tolerant genotypes had greater $O_2•-$ scavenging ability compared to susceptible ones. It has been reported that POD can contribute to different processes including lignification, oxidation of phenolics, regulation of cell elongation and detoxification (Chaparzadeh *et al.*, 2004; Seckin *et al.*, 2010). SuperOxide Dismutase (SOD), as a key enzyme can cope with oxidative stress by rapidly converting $O_2•-$ into H_2O_2 and O_2 to maintain normal physiological processes in plants (Hu *et al.*, 2012). CAT activity was enhanced in salt-tolerant lines/cultivars (Table 4). Interestingly, in contrast to POD and SOD, consistent trend was not observed for CAT activity in sensitive genotypes (Table 4). For example, CAT activity was decreased in some genotypes including lines number 13, 18, 21, 26 and Sepideh and KFS1 cultivars (Table 4). Also, the changes of CAT activity was not significant in Nectar, Sepideh and Kimia cultivars (known as sensitive genotype) (Table 4). Our results are in agreement with previous reports by Hu *et al.* (2012) and Lee *et al.* (2013) who found that under salinity stress CAT activity decreased in sensitive genotypes. Additionally, Xue and Liu (2008) showed no significant increases of CAT activity in leaves of salt-sensitive cultivars. Chaparzadeh *et al.* (2004) indicated that the changes in CAT activity were dependent on the plant species,

Table 4. Mean values of POD, SOD and CAT activity in 44 sorghum lines/cultivars.

Cultivars/ Lines	POD[a] (Units min^{-1} mg^{-1} protein)				SOD[b] (Units min^{-1} mg^{-1} protein)				CAT[c] (Units min^{-1} mg^{-1} protein)			
	Control	Salinized	Changes		Control	Salinized	Changes		Control	Salinized	Changes	
			%[d]	S[e]			%	S			%	S
KDFGS1	3.31	6.19	87	**	7.10	18.38	159	**	3.61	14.57	304	**
KDFGS2	3.20	5.92	85	**	7.50	15.17	102	**	3.12	13.58	335	**
KDFGS3	2.93	6.2	112	**	9.06	19.83	119	**	4.35	7.82	57	**
KDFGS4	3.83	6.25	63	**	6.67	14.25	114	**	5.5	7.53	37	ns
KDFGS5	2.72	5.71	110	**	6.58	15.78	140	**	4.28	10.79	152	**
KDFGS6	3.29	5.84	78	**	7.17	16.18	126	**	6.86	10.85	58	*
KDFGS7	3.36	5.98	78	**	6.58	19.64	198	**	5.10	7.66	50	**
KDFGS8	2.59	4.27	65	**	7.89	18.39	133	**	6.97	13.04	87	**
KDFGS9	3.58	7.14	99	**	9.10	20.04	120	**	3.17	4.89	45	*
KDFGS10	4.69	6.03	29	**	10.33	17.29	67	**	5.01	6.53	30	*
KDFGS11	3.54	6.16	74	**	7.58	16.78	121	**	4.67	5.12	10	ns
KDFGS12	3.09	6.68	116	**	6.17	18.94	207	**	4.78	10.69	124	**
KDFGS13	3.77	7.66	103	**	7.76	16.42	112	**	6.24	13.48	-12	ns
KDFGS14	3.47	7.10	105	**	8.17	21.29	161	**	5.67	6.69	18	**
KDFGS15	4.20	9.31	122	**	13.11	24.56	87	**	5.17	14.25	176	**
KDFGS16	3.14	6.64	111	**	8.67	20.59	137	**	5.87	13.94	137	**
KDFGS17	2.80	5.7	104	**	8.91	22.62	154	**	4.57	10.05	120	**
KDFGS18	4.52	7.74	71	**	11.61	19.74	70	**	6.06	5.51	-9	ns
KDFGS19	4.56	8.94	96	**	10.75	25.79	140	**	4.41	16.60	276	**
KDFGS20	2.90	5.28	82	**	7.68	19.86	159	**	4.53	10.51	132	**
KDFGS21	3.42	5.6	64	**	8.55	18.91	121	**	5.36	4.46	-17	ns
KDFGS22	3.70	6.85	85	**	6.54	18.32	180	**	4.70	8.93	90	**
KDFGS23	3.98	5.8	46	*	12.11	19.24	59	**	4.79	5.55	16	ns
KDFGS24	3.10	6.44	108	**	9.28	21.76	134	**	5.12	12.04	135	**
KDFGS25	3.62	6.12	69	**	9.93	18.31	84	**	3.78	4.51	19	ns
KDFGS26	4.74	7.24	53	**	7.89	16.21	105	**	6.21	5.83	-6	ns
KDFGS27	2.88	4.75	65	**	5.54	14.46	161	**	6.26	14.04	124	**
KDFGS28	2.76	4.93	79	**	7.78	18.63	139	**	4.40	12.05	174	**
KDFGS29	3.98	8.27	108	**	9.58	23.17	142	**	5.14	7.81	52	**
KDFGS30	3.01	5.83	94	**	6.27	17.26	175	**	5.53	10.95	98	**
Jumbo	4.09	9.75	138	**	12.23	24.97	104	**	4.88	9.97	104	**
Nectar	4.05	6.85	69	**	12.37	18.69	51	*	4.69	5.27	12	ns
Speed feed	4.76	9.22	94	**	13.25	25.51	93	**	6.29	14.62	132	**
Sistan	4.31	9.11	111	**	12.75	25.88	103	**	4.42	10.48	137	**
Ghalami Herat	4.28	8.82	106	**	13.52	27.18	101	**	5.93	13.73	131	**
Pegah	4.14	9.43	128	**	13.38	28.09	110	**	4.58	11.51	151	**
Sepideh	4.57	7.92	73	**	10.38	18.61	79	**	4.26	4.10	-4	ns
KFS1	3.86	5.38	39	**	9.75	20.12	106	**	5.65	5.03	-11	ns
KFS2	4.51	8.64	92	**	11.95	21.94	84	**	4.22	11.44	195	**
KFS4	2.68	4.58	71	*	9.83	17.29	76	**	4.67	11.24	141	**
Broom corn	3.55	5.88	66	**	9.33	19.10	105	**	4.36	10.11	132	**
Sweet sorghum	4.80	9.1	90	**	13.26	24.11	82	**	4.23	15.61	269	**
Kimia	4.52	7.83	73	**	7.88	16.95	115	**	3.98	4.37	10	ns
Moghan	4.1	8.16	99	**	11.17	27.61	147	**	5.91	16.53	180	**
Mean	3.70	6.89	86	**	9.38	20.08	114	**	4.98	9.87	98	**
LSD (0.05)	0.74	0.88	-	-	1.39	1.48	-	-	0.53	0.83	-	-

[a] Peroxidase; [b] Superoxide dismutase; [c] Catalase; [d] Percentage of changes upon salinity stress, [e] Significance level. [ns] Non-significant; * Significant at 0.05 probability level, ** Significant at 0.01 probability level.

growth stage at which the stress is imposed, as well as the duration and intensity of the stress. It is also found that, in some cases, the variation of CAT activity can be different even between two cultivars of the same species. Unlike the POD and SOD, CAT activity was significantly and positively associated with shoot dry weight ratio ($r= 0.72^{**}$) (data not shown) under saline conditions. Therefore, it can be concluded that, at least in the sorghum genotypes studied in the present experiment, CAT activity may be one of the most important mechanism involved in tolerance to salinity. In other words, the current study showed that increased CAT activity in tolerant genotypes along with significant enhancing of SOD and POD activities have played a crucial protective role against the oxidative stress caused by salt stress. These results are in agreement with Noreen and Ashraf (2009) who reported that only CAT activity was a reliable marker for recognizing salt-tolerant pea cultivars.

Ion Distribution

A significant increase in Na^+ concentration of shoot and root occurred in response to salinity stress (Tables 5 and 6). The magnitude of such increase in Na^+ concentration differed among genotypes (Tables 5 and 6). For example, Na^+ content in sensitive genotypes such as lines 10, 13, 14, 23, 26 and Nectar cultivar was significantly greater than tolerant genotypes including Ghalami-herat, Jumbo, Speed-feed, Pegah, Moghan, KFS2, KDFGS15, Sweet sorghum, and Sistan (Table 5 and 6). In addition, it was found that Na^+ concentration in root (ranged from 1.03 to 3.15 and from 2.97 to 7.02 for the control and saline treatments, respectively) was higher than shoot (ranged from 0.2 to 0.75 and from 1 to 3.95 for the control and saline treatments, respectively) (Tables 5 and 6). Shoot dry weight ratio had negative correlation with shoot ($r= -0.65^{**}$) and root ($r= -0.58^{**}$) (data not shown) Na^+ concentration under saline conditions. Control of Na^+ transport from root to the shoot has been reported as an efficient mechanism for salinity tolerance in sorghum (Krishnamurthy *et al.*, 2007; Bavei *et al.*, 2011; Almodares *et al.*, 2014; Yan *et al.*, 2015).

We also used another index, namely, storage factor, to show the proportion of Na^+ ion which has been left in the root cells. Our results showed that in both sensitive and resistant cultivars, there was a general trend of storage factor reduction under saline condition. However, the lower reduction of storage factor under salinity stress was observed in tolerant cultivars such as Ghalami-herat (8%), Jumbo (11%), Pegah (8%), Speed-feed (10%), Moghan (14%), Sweet-sorghum (12%), Sistan (13%), KDFGS15 (14%) and KFS2 (14%) (Figure 1), while greater reduction in storage factor index under saline conditions has been recorded in sensitive genotypes including Nectar (17%), Sepideh (17%), KDFGS23 (24%) and KDFGS26 (27%) (Figure 2).

Shoot dry weight under salinity stress was positively related to storage factor index ($r= 0.52^{**}$) (data not shown) indicating that the lower rate of Na^+ transfer from the root to the shoot is associated with salt tolerant plants (Arzani and Ashraf, 2016). In contrast to Na^+ ion, salinity significantly reduced K^+ content in shoot and root of all genotypes, particularly in sensitive genotypes (Tables 5 and 6). For example, K^+ concentration of shoot and root in line 23, known as sensitive line, decreased 60 and 78%, respectively, whereas, the reduction in K^+ content in Jumbo, as a tolerant cultivar, were 28 and 48%, respectively (Tables 5 and 6). Similar to Na^+ concentration, K^+/Na^+ ratio significantly decreased under salinity stress. The overall mean of K^+/Na^+ ratio in shoot and root under saline conditions were seven and five folds lower than the control, respectively (Tables 5 and 6). Shoot dry weight ratio was positively correlated to shoot ($r= 0.62^{**}$) and root ($r= 0.58^{**}$) (data not shown) K^+/Na^+ ratio under saline conditions. Regarding the close negative

Table 5. Mean values of Na^+, K^+ and K^+/Na^+ ratio in shoot in 44 sorghum lines/cultivars.

Cultivars/ Lines	Shoot [Na] (mg g^{-1} DM)				Shoot [K] (mg g^{-1} DM)				Shoot [K/Na]			
	Control	Salinized	Changes		Control	Salinized	Changes		Control	Salinized	Changes	
			%[a]	S[b]			%	S			%	S
KDFGS1	0.65	1.35	108	*	5.48	4.58	-16	ns	8.43	3.39	-60	**
KDFGS2	0.53	1.47	177	**	6.63	5.16	-22	**	12.51	3.51	-72	**
KDFGS3	0.61	2.33	282	**	6.05	5.16	-15	**	9.92	2.21	-78	**
KDFGS4	0.56	1.76	214	**	7.37	4.80	-35	**	13.16	2.73	-79	**
KDFGS5	0.68	3.41	401	**	5.76	3.10	-46	**	8.47	0.91	-89	**
KDFGS6	0.43	1.74	305	**	7.14	4.93	-31	*	16.60	2.83	-83	**
KDFGS7	0.47	2.65	464	**	6.98	3.95	-43	**	14.85	1.49	-90	**
KDFGS8	0.59	1.72	192	**	6.1	5.12	-16	ns	10.34	2.98	-71	**
KDFGS9	0.41	2.68	554	**	7.17	3.65	-49	**	17.49	1.36	-92	**
KDFGS10	0.32	2.59	709	**	8.01	4.00	-50	**	25.03	1.54	-94	**
KDFGS11	0.51	2.34	359	**	6.66	4.10	-38	**	13.06	1.75	-87	**
KDFGS12	0.57	1.84	223	**	6.34	4.74	-25	*	11.12	2.58	-77	**
KDFGS13	0.39	3.83	882	**	7.29	3.07	-58	**	18.69	0.80	-96	**
KDFGS14	0.44	2.98	577	**	7.09	3.16	-55	**	16.11	1.06	-93	**
KDFGS15	0.34	1.31	285	**	8.14	5.67	-30	**	23.94	4.33	-82	**
KDFGS16	0.56	1.71	205	**	6.53	5.13	-21	ns	11.66	3.00	-74	**
KDFGS17	0.48	1.46	204	**	6.85	5.19	-24	ns	14.27	3.55	-75	**
KDFGS18	0.32	1.85	478	**	8.04	4.71	-41	**	25.13	2.55	-90	**
KDFGS19	0.35	1.35	286	**	7.78	5.59	-28	**	22.23	4.14	-81	**
KDFGS20	0.62	1.91	208	**	5.99	4.67	-22	ns	9.66	2.45	-75	**
KDFGS21	0.45	3.24	620	**	7.07	3.12	-56	**	15.71	0.96	-94	**
KDFGS22	0.40	1.75	338	**	7.21	4.92	-32	**	18.03	2.81	-84	**
KDFGS23	0.2	3.24	1520	**	8.33	3.33	-60	**	41.65	1.03	-98	**
KDFGS24	0.53	1.74	228	**	6.5	5.09	-22	ns	12.26	2.93	-76	**
KDFGS25	0.40	2.88	620	**	7.19	3.42	-52	**	17.98	1.19	-93	**
KDFGS26	0.29	3.67	1166	**	8.07	3.12	-61	**	27.83	0.85	-97	**
KDFGS27	0.71	2.24	215	**	5.22	3.42	-34	*	7.35	1.53	-79	**
KDFGS28	0.64	1.97	208	**	5.86	4.62	-21	ns	9.16	2.35	-74	**
KDFGS29	0.32	1.41	341	**	7.69	5.28	-31	**	24.03	3.74	-84	**
KDFGS30	0.75	3.92	423	**	5.09	3.04	-40	**	6.79	0.78	-89	**
Jumbo	0.26	1.15	342	**	8.26	5.91	-28	*	31.77	5.14	-84	**
Nectar	0.23	2.62	1039	**	8.30	3.22	-61	**	36.09	1.23	-97	**
Speed feed	0.28	1.12	300	**	8.2	6.25	-24	ns	29.29	5.58	-81	**
Sistan	0.31	1.17	277	**	8.12	5.99	-26	*	26.19	5.12	-80	**
Ghalami Herat	0.26	1.00	285	**	8.27	6.41	-22	ns	31.81	6.41	-80	**
Pegah	0.29	1.03	255	**	8.17	6.32	-23	ns	28.17	6.14	-78	**
Sepideh	0.35	2.94	740	**	7.75	3.40	-56	**	22.14	1.16	-95	**
KFS1	0.35	2.12	506	**	7.39	4.57	-38	**	21.11	2.16	-90	**
KFS2	0.29	1.32	355	**	8.1	5.83	-28	*	27.93	4.42	-84	**
KFS4	0.63	2.06	227	**	5.89	4.61	-22	ns	9.35	2.24	-76	**
Broom corn	0.42	1.44	243	**	7.17	5.25	-27	*	17.07	3.65	-79	**
Sweet sorghum	0.33	1.29	291	**	7.98	5.54	-31	**	24.18	4.29	-82	**
Kimia	0.38	2.33	513	**	7.43	4.32	-42	**	19.55	1.85	-91	**
Moghan	0.26	1.30	400	**	8.26	5.96	-28	*	31.77	4.58	-86	**
Mean	0.43	2.07	376	**	7.15	4.62	-35	*	19.08	2.75	-86	**
LSD (0.05)	0.29	0.21	-	-	0.77	0.56	-	-	4.14	0.61	-	-

[a] Percentage of changes upon salinity stress, [b] Significance level. [ns] Non-significant; * Significant at 0.05 probability level, ** Significant at 0.01 probability level.

Table 6. Mean values of Na^+, K^+ and K^+/Na^+ ratio in root in 44 sorghum lines/cultivars.

Cultivars/ Lines	Root [Na] (mg g^{-1} DM)				Root [K] (mg g^{-1} DM)				Root [K/Na]			
	Control	Salinized	Changes		Control	Salinized	Changes		Control	Salinized	Changes	
			%[a]	S[b]			%	S			%	S
KDFGS1	2.41	4.19	74	**	3.14	2.75	-12	ns	1.30	0.66	-50	**
KDFGS2	2.19	4.09	87	**	3.97	2.62	-34	*	1.81	0.64	-65	**
KDFGS3	2.44	5.69	133	**	3.65	2.01	-45	**	1.50	0.35	-76	**
KDFGS4	1.71	4.51	164	*	4.40	2.46	-44	**	2.57	0.55	-79	**
KDFGS5	2.55	6.63	160	**	3.07	1.14	-63	*	1.20	0.17	-86	**
KDFGS6	1.91	4.19	119	**	4.23	2.54	-40	**	2.21	0.61	-73	**
KDFGS7	2.12	5.87	177	**	4.18	1.95	-53	**	1.97	0.33	-83	**
KDFGS8	2.41	4.15	72	**	3.78	2.59	-31	**	1.57	0.62	-60	**
KDFGS9	1.85	5.89	218	**	4.28	1.73	-60	**	2.31	0.29	-87	**
KDFGS10	1.47	5.78	293	**	5.40	1.98	-63	**	3.67	0.34	-91	**
KDFGS11	2.18	5.75	164	**	4.13	1.99	-52	**	1.89	0.35	-82	**
KDFGS12	2.36	4.60	95	**	3.84	2.44	-36	**	1.63	0.53	-67	**
KDFGS13	1.72	6.90	301	**	4.32	1.1	-75	**	2.51	0.16	-94	**
KDFGS14	2.03	6.37	214	**	4.20	1.42	-66	**	2.07	0.22	-89	**
KDFGS15	1.58	3.21	103	**	5.92	2.84	-52	**	3.75	0.88	-76	**
KDFGS16	2.21	4.12	86	*	3.95	2.60	-34	**	1.79	0.63	-65	**
KDFGS17	2.13	3.93	85	**	4.14	2.66	-36	**	1.94	0.68	-65	**
KDFGS18	1.42	4.71	232	**	5.74	2.40	-58	**	4.04	0.51	-87	**
KDFGS19	1.63	3.89	139	**	5.68	2.78	-51	**	3.48	0.71	-79	**
KDFGS20	2.47	5.12	107	**	3.58	2.28	-36	*	1.45	0.45	-69	**
KDFGS21	2.09	6.46	209	**	4.19	1.41	-66	**	2.00	0.22	-89	**
KDFGS22	1.73	4.39	154	**	4.30	2.53	-41	**	2.49	0.58	-77	**
KDFGS23	1.03	5.69	452	**	6.33	1.37	-78	**	6.15	0.24	-96	**
KDFGS24	2.27	4.16	83	**	3.94	2.56	-35	*	1.74	0.62	-65	**
KDFGS25	1.84	6.19	236	**	4.29	1.63	-62	**	2.33	0.26	-89	**
KDFGS26	1.39	5.59	302	**	5.79	1.82	-69	**	4.17	0.33	-92	**
KDFGS27	2.62	5.3	102	**	2.91	2.21	-24	ns	1.11	0.42	-62	**
KDFGS28	2.50	4.98	99	*	3.49	2.28	-35	**	1.40	0.46	-67	**
KDFGS29	1.66	3.27	97	**	4.39	2.70	-38	**	2.64	0.83	-69	**
KDFGS30	3.15	7.02	123	**	2.88	1.00	-65	**	0.91	0.14	-84	**
Jumbo	1.17	3.11	166	*	6.18	3.20	-48	**	5.28	1.03	-81	**
Nectar	1.10	5.80	427	**	6.31	1.55	-75	**	5.74	0.27	-95	**
Speed feed	1.23	3.06	149	**	6.09	3.10	-49	**	4.95	1.01	-80	**
Sistan	1.60	3.18	99	*	5.49	2.79	-49	**	3.43	0.88	-74	**
Ghalami Herat	1.13	2.97	163	**	6.25	3.33	-47	**	5.53	1.12	-80	**
Pegah	1.24	3.01	143	**	6.01	2.92	-51	**	4.85	0.97	-80	**
Sepideh	1.62	6.28	288	**	4.42	1.45	-67	**	2.73	0.23	-92	**
KFS1	1.79	5.22	192	**	4.41	2.24	-49	**	2.46	0.43	-83	**
KFS2	1.31	3.16	141	**	5.84	2.88	-51	**	4.46	0.91	-80	**
KFS4	2.48	5.12	106	**	3.52	2.27	-36	**	1.42	0.44	-69	**
Broom corn	1.86	3.92	111	**	4.26	2.69	-37	**	2.29	0.69	-70	**
Sweet sorghum	1.51	3.29	118	*	5.57	2.82	-49	**	3.69	0.86	-77	**
Kimia	1.70	5.39	217	**	4.45	2.17	-51	**	2.62	0.40	-85	**
Moghan	1.19	3.12	162	**	6.12	2.91	-52	**	5.14	0.93	-82	**
Mean	1.86	4.75	155	**	4.61	2.27	-51	**	2.82	0.54	-81	**
LSD (0.05)	0.65	1.34	-	-	0.58	0.48	-	-	0.88	0.16	-	-

[a] Percentage of changes upon salinity stress, [b] Significance level. [ns] Non-significant; * Significant at 0.05 probability level, ** Significant at 0.01 probability level.

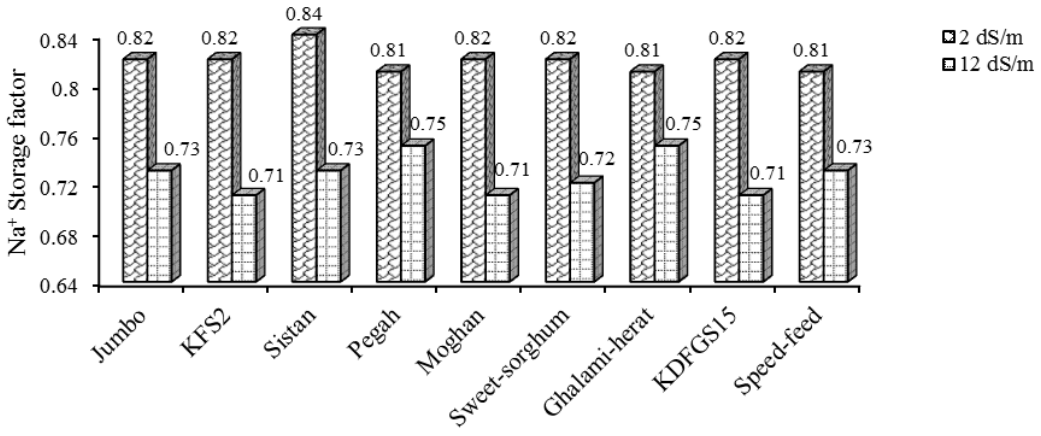

Figure1. Na^+ storage factor for tolerant genotypes.

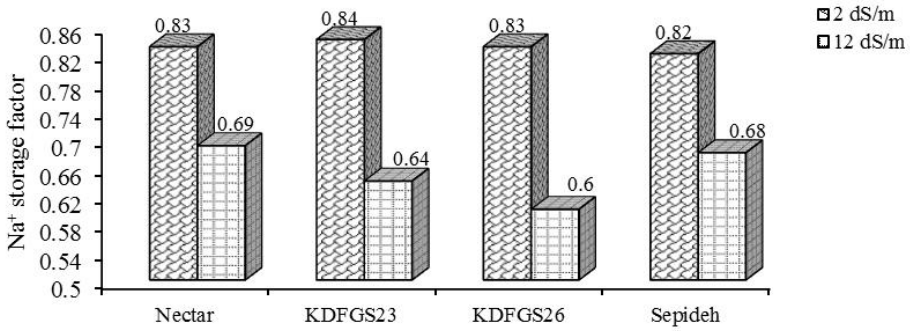

Figure2. Na^+ storage factor for sensitive genotypes.

relationship between shoot dry weight ratio and Na^+ content under salinity stress, it appeared that Na^+ content could be used as a reliable selection criterion to screen the tolerant genotypes.

CONCLUSIONS

It was concluded that more accumulation of Na^+ in roots and selective uptake of K^+ versus Na^+, i.e. higher *SFI*, was an effective mechanism for salt tolerance in sorghum lines/cultivars.

Furthermore, among antioxidant enzymes, CAT activity was highly dependent on plant genotypes and strongly correlated with salinity tolerance. Proline concentration increased in all genotypes as a general response to salinity stress, therefore, it did not appear to be a suitable criterion for selection of tolerant lines/cultivars.

ACKNOWLEDGEMENTS

This research was supported by Shiraz University. The authors also thank Prof. A. R. Sepaskhah (from Shiraz University) and Dr. S. A. Tabatabaei (from Agricultural and Natural Resources Research Center of Yazd, Iran) for their critical review and help with completing this work.

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ویژگی‌های قابل انتخاب در تحمل ژنوتیپ‌های سورگوم به تنش شوری

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چکیده

سورگوم [*Sorghum bicolor* (L.) Moench] با درجه تحمل نسبی شوری و اهمیت بالا در تولید دانه و علوفه به عنوان یکی از مهم‌ترین گیاهان در مناطقی که با مشکل شوری مواجه هستند، مورد توجه است. این آزمایش در گلخانه تحقیقاتی دانشکده کشاورزی دانشگاه شیراز، ایران به منظور بررسی اهمیت نسبی شاخص‌های بیوشیمیایی و شاخص‌های تحمل مرتبط با تحمل به تنش شوری در ۳۰ لاین و ۱۴ رقم سورگوم انجام شد. بعلاوه، شاخص جدیدی بنام ذخیره‌سازی یون (SFI) برای تعیین سهم نسبی ریشه و شاخساره در تجمع سدیم تعریف و مورد استفاده قرار گرفته است. در بین شاخص‌ها، شاخص تحمل به تنش به عنوان بهترین معیار مشخص شد. علاوه بر این، ژنوتیپ‌های متحمل بیشترین نسبت پتاسیم به سدیم را در شاخساره و ریشه و بالاترین میزان (STI) را به خود اختصاص دادند؛ که این حاکی از ذخیره ی بخش عمده ی سدیم در ریشه ها بود. اگرچه فعالیت آنزیم های آنزیم‌های پراکسیداز و سوپراکسید دیسموتاز در شرایط تنش شوری در تمامی ژنوتیپ های حساس و متحمل افزایش یافت، لیکن، فعالیت آنزیم کاتالاز در شرایط تنش شوری فقط در ژنوتیپ‌های متحمل افزایش یافت. به نظر می‌رسد تجمع پرولین ارتباطی با تحمل به تنش شوری در ژنوتیپ های سورگوم نداشت. در کل، نتایج این پژوهش نشان داد که تحمل به تنش شوری در سورگوم نه تنها با تجمع کمتر یون سدیم در شاخساره، بلکه با افزایش فعالیت آنزیم کاتالاز همراه است.