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The Effects of Salt Stress on Growth Parameters and Carbohydrates Contents in Sweet Sorghum

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Abstract: In this study, two sweet sorghum cultivars (Soave and Sofra) were grown in nutrient solution containing 0, 30, 60 and 90 mM NaCl to evaluate the effects of salinity on growth parameters (shoot fresh weight, shoot dry weight, leaf area index and relative chlorophyll) and carbohydrate content (sucrose, glucose and fructose) in plant organs (leaf, shoot and root). The results showed that as salinity increased, the amount of the above growth parameters in plant parts of both Soave and Sofra decreased. However in all salinities, Soave had significantly higher shoot fresh weight and leaf area index than Sofra. As salinity increased, the amount of sucrose and glucose of both Soave and Sofra decreased while the amount of fructose increased. However, the sucrose content of Soave was higher than Sofra but its glucose and fructose content was lower than Sofra. Since salinity reduces shoot fresh weight and leaf area index, mainly in the salt sensitive plants. Also, salinity increases carbohydrate contents especially in the salt tolerance plants. Therefore, it seems that Soave is more salt tolerance than Sofra.

Key words: Sweet sorghum, growth parameters, carbohydrates soluble, salt tolerance

INTRODUCTION

Salinity is one of the major factors in reducing plant growth and productivity worldwide and affects about seven percent of the world's total land area (Flowers *et al*., 1997). Salt accumulation is mainly related to a dry climate, salt-rich parent materials of soil formation, insufficient drainage and saline groundwater or irrigation water. In Iran, according to the recently published soil map mild and moderate saline soils occupy approximately 25.5 million ha and strong saline soils cover about 8.5 million ha (Kehl, 2006). The major inhibitory effect of salinity on plant growth and yield has been attribute to: (1) osmotic effect (2) ion toxicity (3) nutritional imbalance leading to reduction in photosynthetic efficiency and other physiological disorders. Salinity greatly reduced sorghum growth and this effect was more pronounced at 250 mM than at 125 mM NaCl (Ibrahim, 2004). Sudhir and Murthy (2004) reviewed both multiple inhibitory effects of salt stress on photosynthesis and possible salt stress tolerance mechanisms in plants. Salinity reduced relative growth rates and increased soluble carbohydrates, especially in the leaves of salt sensitive genotype (Lacerda *et al*., 2005). In addition salt-stressed sorghum plants additionally accumulate organic solutes (El-Sayed *et al*., 1994), like proline, glycinabetaine, sugars, etc. (Lacerda *et al*., 2001). The total soluble sugar increased in sorghum sap with increasing salinity level (Ibrahim, 2004). Sucrose content of plant parts is an indicator of salt tolerance (Juan *et al*., 2005). The fructose level was always higher than that of the glucose and sucrose levels in response to various salinity treatments (Gill *et al*., 2001). Selection of salt tolerant cultivars is one of the most effective methods to increase the productivity of salinity in soils (Ali *et al*., 2004). By using these salt tolerant plants in breeding they produced programmed an improved plant having

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higher chlorophyll concentration, more leaf area, early and better yield potential etc. This genotype possessing the salt tolerance character will help in boosting up plants production in salt-affected soils (Ali *et al*., 2004). The presence of large genotypic variation for tolerance to salinity reported in sorghum (Krishnamurthy *et al*., 2007). Sorghum is characterized as moderately tolerant to salinity (Almodares and Sharif, 2005). Sorghum growth was significantly reduced at all salinity levels from 50 to 150 mM (El-Sayed *et al*., 1994). In this study, growth parameters and carbohydrate contents of two sweet sorghum cultivars were evaluated in response to salinity.

MATERIALS AND METHODS

Experimental Location, Plant Material and Experimental Design

This experiment was conducted at the Isfahan University in 2006. Seeds of two sweet sorghum [*Sorghum bicolor* (L.) Moench] cultivars were provided from Isfahan University Research Station in Iran. The experimental design was factorial with two factors: two cultivars (Soave and Sofra) and four salinity levels (0, 30, 60 and 90 mM NaCl) with five replications.

Plant Growth and Salt Treatment

Seeds of the above cultivars were imbibed for 24 h in distilled-water and transferred to the 500 mL square plastic containers that had vermiculite and Hoagland's solution. The exposed sand surfaces of pots were covered with aluminum foil to prevent growth of algae. The seedlings were grown under hydroponic conditions in a greenhouse: humidity was reached at 65% during the light period; photoperiod was 16 h light and 8 h dark; temperature was 26°C in light and 21°C in the dark at the base of the leaves (the growth zone). Each container had two plants. Hoagland's solution was added to the container every four days. Six week after germination, sodium chloride was added in Hoagland's solution to make saline treatments of 30, 60 and 90 mM. The control was irrigated with NaCl-free nutrient solution. To avoid osmotic shock, saline treatment was imposed incrementally; increasing the concentration by 30 mM every second day until the final concentration was reached. Pots were irrigated twice per day with nutrient solution and the excess was allowed to drain into collecting pans. It should be added that plants were grown under stress conditions for two weeks.

Measurement of Growth Parameters and Carbohydrates Content

Fourteen days after salt application, shoot fresh weight, leaf area index and relative chlorophyll were measured. Shoot dry weight was obtained after drying at 75°C for 72 h. leaf area index was measured according to Welles and Norman (1991). Relative chlorophyll was determined by Chlorophyll-meter using Norikane *et al*. (2003) method. Carbohydrates were extracted using a technique described by Lacerda *et al*. (2005). In this extract, soluble carbohydrates (sucrose, glucose and fructose) were determined by High Performance Liquid Chromatography (HPLC). The HPLC separation was accomplished by using an aminopropyl column (4.6×250 mm) with a mobile phase of 80% acetonitrile/20% water (Biermann and Mcginnis, 1989).

Statistical Analysis

Statistical analyses were performed using Statistical Analysis System (SAS) computer program. The means were compared according to Duncan multiple range test.

RESULTS AND DISCUSSION

Effects of Salinity on the Growth Parameters

The effect of cultivars and salt concentrations for shoot fresh weight and leaf area index was significant at 5% level. The results indicated that Soave had significantly higher shoot fresh weight

Table 1: Mean comparisons* between sweet sorghum cultivars for measured characteristics

Cultivars	FW (mg)	DW (mg)	LAI (mm^2)	R. Chl. (%)
Soave	576.17a	60.22	25.75a	25.07
Sofra	431.30b	48.75	17.19b	24.61

FW: Shoot fresh weight; DW: Shoot dry weight; LAI: Leaf Area Index; R. Chl.: Relative chlorophyll *: Values within each column followed by the same letter are not significantly different at 5% level, using Duncan multiple range test

Table 2: Mean comparisons* among salt concentrations on measured characteristics in sweet sorghum

Salt concentrations (mM)	FW (mg)	DW (mg)	LAI (mm^2)	R. Chl (%)
Control	750.75a	76.35a	32.07a	30.41a
30	537.85b	61.88b	24.76ab	28.15a
60	402.35c	43.77c	17.09bc	24.20ab
90	323.00c	35.95c	11.99c	16.61b

FW: Shoot fresh weight; DW: shoot dry weight; LAI: Leaf Area Index; R. Chl.: Relative chlorophyll *: Values within each column followed by the same letter are not significantly different at 5% level, using Duncan multiple range test

(576.17 mg) and leaf area index (25.75 mm^2) than Sofra (431.30 mg and 17.19 mm^2, respectively). The growth parameter (shoot fresh weight and leaf area index) differences between the two cultivars may be related to their genetic diversity (Table 1). Krishnamurthy *et al.* (2007) reported that the differences between sorghum cultivars were related to their genetic differences which are similar to our results. On the other hand, it seems that high fresh weight of Soave is due to high photosynthesis, which is as a result of leaf area index. Netondo *et al.* (2004) reported that whole plant photosynthesis is restricted by leaf area expansion. They also reported in sorghum, reduction in fresh and dry weight of plant tissues reflect the increased in metabolic energy cost and reduced carbon gain, which my be associated to salt adaptation.

The effects of salt concentrations on growth parameters (shoot fresh and shoot dry weight, leaf area index and chlorophyll content) were significant at 5 percent level. The results showed that as salinity increases, the amount of the above parameters decreased. These reductions of growth parameters were not significant at 60 and 90 mM NaCl (Table 2). Netondo *et al.* (2004) reported that in sorghum, salinity reduced leaf area about 86%. Also, Ali *et al.* (2004) indicated that in sorghum, the reduction in leaf area, yield and yield components under saline conditions were also due to reduced growth as a result of decreased water uptake, toxicity of sodium and chloride in the shoot cell as well as reduced photosynthesis. In addition, Netondo *et al.* (2004) reported that in sorghum, chlorophyll percentage decreased with the increase in salinity and photosynthetic activity decreases when plants are grown under saline conditions leading to reduced growth and productivity. Their results indicate that salinity affected photosynthesis per unit leaf area indirectly through stomatal closure and to a smaller extent through direct interference with the photosynthetic apparatus. Also, it was reported that salinity decreases whole plant photosynthesis by restricting leaf area expansion. The interaction between cultivars and salt concentrations were not significant. Thereby, it seems that shoot fresh weight and leaf area index can not be discriminated between salt-tolerance and salt-sensitive cultivar in sorghum.

Effects of Salinity on the Carbohydrates Contents

The results showed that shoot, leaf and root had the highest sucrose (52.66%), glucose (44.98%) and fructose levels (24.94%), respectively. Shoot sucrose content in sweet sorghum is more important than glucose and fructose because of its use in food industry (Fig. 1).

The distribution of sucrose, glucose and fructose contents were different for plant organs and cultivars. Among plant organs and cultivars root, shoot and leaf of Sofra had the highest sucrose (57.15%), fructose (29.08%) and glucose levels (54.85%), respectively. In shoot, Soave had lower sucrose and higher glucose than Sofra (Table 3). On the other hand in leaf, Soave had higher sucrose and lower glucose than Sofra. Hare *et al.* (1998) reported that the distribution and amount of

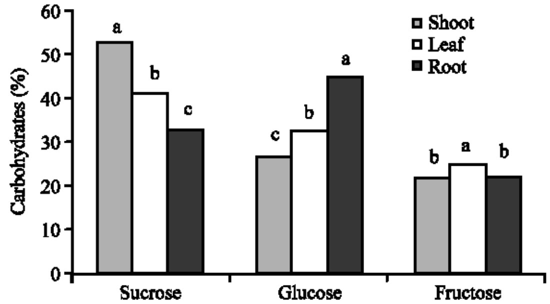

Fig. 1: Mean comparisons* among sweet sorghum organs for sucrose, glucose and fructose. *: Values within each treatment followed by the same letter are not significantly different at 5% level, using Duncan multiple range test

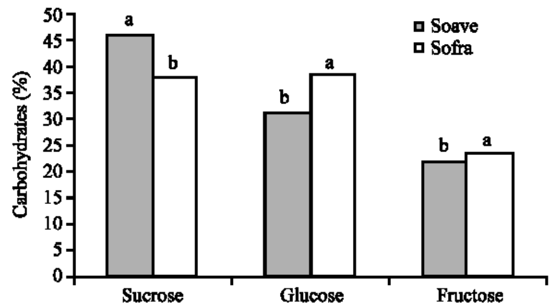

Fig. 2: Mean comparisons* between sweet sorghum cultivars for soluble carbohydrate. *: Values within each treatment followed by the same letter are not significantly different at 5% level, using Duncan multiple range test

Table 3: Interaction between plant organs and sweet sorghum cultivars on soluble carbohydrate

Treatments	Sucrose (%)	Glucose (%)	Fructose (%)
t_1v_1	42.16c	28.84e	27.16a
t_2v_1	40.11e	37.00b	22.71b
t_3v_1	40.49d	35.12c	24.32b
t_1v_2	25.54f	54.85a	20.15c
t_2v_2	57.15a	29.56d	14.05d
t_3v_2	48.17b	23.81f	29.08a

t_1: Leaves; t_2: roots; t_3: shoots and v_1: Soave; v_2: Sofra; *: Values within each column followed by the same letter are not significantly different at 5% level, using Duncan multiple range test

carbohydrate present within the cells vary widely among plant species and cultivars. Therefore, it seems that in Soave, sucrose in the leaf did not translocate to shoot or it was break-up to glucose and fructose.

Soave had higher sucrose and lower glucose and fructose levels than Sofra. It seems that Soave could be more salt tolerant than Sofra (Fig. 2). In this relation, Juan *et al*. (2005) reported that the most salt-resistant in tomato cultivars are characterized by greater amount of sucrose. Their results reveal the validity and effectiveness of certain nutritional and biochemical indicators of salt stress, such as the K^+/Na^+ ratio and sucrose, indicating the importance of lipid per-oxidation as the determinant physiological process in the selection of salinity-tolerant tomato plants.

As salt concentration increased, sucrose content of Soave increased while it decreased in Sofra (Table 4). In this relation, the accumulation of soluble sugars in plants has been widely reported as a

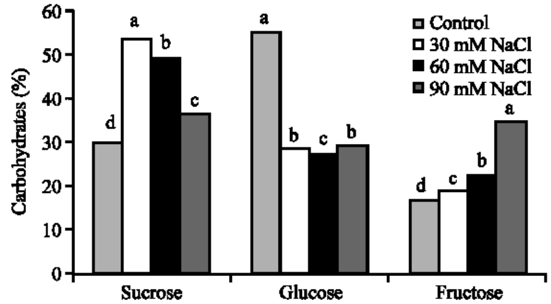

Fig. 3: Mean comparisons* among salt concentrations for soluble carbohydrate. *: Values within each treatment followed by the same letter are not significantly different at 5% level, using Duncan multiple range test

Table 4: Interaction between salt concentrations and cultivars on soluble carbohydrate

Treatments	Sucrose (%)	Glucose (%)	Fructose (%)
v_1c_1	33.53f	35.85b	37.70b
v_1c_2	47.88e	34.71c	18.75d
v_1c_3	49.81c	32.97d	14.14e
v_1c_4	55.18b	21.15f	23.79c
$v_2\ c_1$	25.35g	74.67a	12.17f
$v_2\ c_2$	59.40a	22.07e	18.43d
$v_2\ c_3$	48.85d	21.07f	29.97b
$v_2\ c_4$	18.17h	36.40b	45.38a

v_1: Soave; v_2: Sofra and c_1: concentration of control (0 mM NaCl); c_2: 30 mM NaCl; c_3: 60 mM NaCl; c_4: 90 mM NaCl. *: Values within each column followed by the same letter are not significantly different at 5% level, using Duncan multiple range test

response to salinity (Gill *et al*., 2001; Murakeozy *et al*., 2003; Juan *et al*., 2005). Perez-Alfocea *et al*. (1996) reported that in tomato plants after 90 days of salt treatment, the sucrose concentration in leaves increased with moderate salinity. Therefore, it seems that Soave is more salt tolerant than Sofra.

Sucrose content was highest at 30 mM NaCl (53.64%) and then decreased with higher salt concentrations. As salt concentrations increased, glucose content decreased while fructose content increased (Fig. 3). Glucose and fructose contents were highest at control (55.26%) and at 90 mM NaCl (34.59%), respectively. Gill *et al*. (2001) reported similar results indicating that fructose level was always higher than glucose and sucrose in response to various salinity treatments.

The highest sucrose, glucose and fructose levels were obtained in shoot at 30 mM NaCl (71.00%), leaf at control (68.53%) and roots at 90 mM NaCl (35.46%), respectively (Table 5). These results are in agreement with Hartzendorf and Rolletschek (2001) who reported that in plant of *Phragmites australis,* the contents of sucrose, glucose and fructose were highest in shoot, leaf and root at 1.5% salinity. Also, Oliver *et al*. (1998) reported that carbohydrate metabolism is very sensitive to changes in plant status, showing alterations in fructose, glucose and sucrose concentrations when salt stress intensity was more pronounced. In 30 mM NaCl, leaf and shoot had the highest sucrose and the lowest glucose and fructose. As salt concentration increased, sucrose decreased while glucose and fructose increased. Invertase cleaves sucrose into the two monosaccharides (Scholes *et al*., 1996). Thereby, it seems that sucrose reduction is due to the increase of invertase activity as is reported by Muscolo *et al*. (2003) indicating increase of invertase activity in plants grown in salt stress condition. It seems that increase of invertase activity in leaves of plants treated with highest salt concentrations indicates a major request of hexoses, substrates necessary to respiratory processes. In plants exposed to the highest salt concentration a low activity of GK (glucose kinase), GPI (Glucose Phosphate Isomerase) and PK (pyruvate kinase), the three enzymes with an important role in the regulation of

Table 5: Interaction between plant organs and salt concentrations for soluble carbohydrate

Treatments	Sucrose (%)	Glucose (%)	Fructose (%)
t_1c_1	16.92k	68.53a	14.73f
t_1c_2	44.52h	29.70e	26.42c
t_1c_3	52.17c	16.77i	26.30c
t_1c_4	50.92d	16.67i	32.30b
t_2 c_1	16.99k	69.26a	14.83f
t_2 c_2	45.40f	33.08d	21.46d
t_2 c_3	45.11g	37.72c	17.16ef
t_2 c_4	25.54j	39.88b	35.46a
t_3 c_1	54.41b	27.99f	19.71de
t_3 c_2	71.00a	22.40h	7.88g
t_3 c_3	50.70e	26.57g	22.67d
t_3 c_4	34.55i	29.77e	36.00a

t_1: leaves; t_2: roots; t_3: shoots and c_1: concentration of control (0 mM NaCl); c_2: 30 mM NaCl; c_3: 60 mM NaCl; c_4 : 90 mM NaCl. *: Values within each column followed by the same letter are not significantly different at 5% level, using Duncan multiple range test

glycolysis was observed (Podestà and Plaxton, 1994). The lower activity of these glycolytic enzymes causes the accumulation of hexoses, which helps to explain the lower biomass production and also the increase of invertase activity in plants grown in salt stress condition (Muscolo *et al*., 2003). In general, salt stress increases soluble carbohydrates in plants (Lacerda *et al*., 2005). Since at 90 mM NaCl, Sucrose, glucose and fructose of Soave in the root, shoot and leaf were higher than Sofra, therefore Soave is more tolerant to salt than Sofra. In conclusion, selection of salt tolerance cultivars is one of the most effective methods to increase the productivity of salty soil. Also sucrose content of plant parts is an indicator of salt tolerance. Thereby, because at 90 mM NaCl sucrose content in the root, shoot and leaf of Soave was higher than Sofra, so to increase productivity of salty land, it is recommended to plant Soave than Sofra.

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