

EVALUATION OF SUGAR BEET BREEDING POPULATIONS BASED MORPHO-PHYSIOLOGICAL CHARACTERS UNDER SALINITY STRESS

MEHDI TAGHIZADEGAN¹, MAHMOUD TOORCHI^{1*},
MOHAMMAD MOGHADAM VAHED¹ AND SAMAR KHAYAMIM²

¹Department of Plant Breeding and Biotechnology, Faculty of Agriculture, University of Tabriz, Tabriz, Iran;

²Sugar Beet Seed Institute, Agricultural Research, Education and Extension Organization, Karaj, Iran.

*Corresponding author's e-mail: Taghizadeh_meh@tabrizu.ac.ir

Abstract

Soil salinity is an important factor limiting crop productivity, especially in arid and semi-arid countries like Iran. Therefore, improving salt-tolerant varieties of crops such as sugar beet that could grow and produce acceptable yield in this stress condition is one of the most important objectives of plant breeding in Sugar Beet Seed Institute (SBSI) of Iran. The main objective of this study was to evaluate the different sugar beet genotypes in terms of salinity tolerance based on physiological and morphological traits in greenhouse conditions and comparing its results with field experiments. In this study, quantity and quality characters of 12 sugar beet (*Beta vulgaris* L.) advanced breeding populations were investigated under stress (EC = 16 ds/m) and non-stress conditions in a factorial experiment in the greenhouse and split plot experiment in the field at the experimental station of Sugar Beet Seed Institute (SBSI) in Mian-Doab, Iran. Several characteristics such as, root yield (RY), white sugar yield (WSY), sugar content (SC), leaf length (LL), leaf width (LW), petiole length (PL), root impurities (Na, K and N), relative water content (RWC), relative water loss (RWL), and proline content were determined. The analysis of variation showed significant differences among the breeding populations for most traits such as root and shoot fresh and dry weights, sugar yield, impurities, petiole length and proline. In general, salinity stress conditions increased proline, specific leaf weight, leaf sodium, root length and total dry weight in comparison with non-stress condition but other traits decreased in salinity stress condition in greenhouse. In meanwhile, salinity stress conditions increased sugar content and decreased other traits in the field as compared with the non-stress condition. Genotypes SC C2*S7, SC C2*S10 and SC C2*S11 were found to be superior to the other genotypes for root and white sugar yields and sugar content. Genotypes SC 261*S7 and 191 were had the least root yield in the stress condition.

Key words: Sugar beet, Salinity, Field, Greenhouse, Morphological traits, Proline salt.

Introduction

Soil salinity is a major environmental stress in agriculture worldwide, restricting plant growth and productivity of agricultural crops especially in arid, semi-arid and tropical regions through reducing nutrients uptake and increasing osmotic stress of plants (Merwad, 2016; Chunthaburee *et al.*, 2014; Mutlu & Bozcuk, 2007; Li *et al.*, 2010; Cha-Um & Kirdmanee, 2009). The inhibitory effect of salinity on plant growth and yield are associated with the low osmotic potential of the soil solution, ion toxicity, nutritional imbalance, specific ion effects, reduction in enzymatic, photosynthetic efficiency and other physiological disorders (Chauhan *et al.*, 2016; Wu *et al.*, 2013; Chaugool *et al.*, 2013; Hajiboland *et al.*, 2012). It is estimated that over 6 % of the world's total land area and 20% of the irrigated land area are affected by salinity (kazemeini *et al.*, 2018; Malik *et al.*, 2018; Aydinşakir *et al.*, 2013; Morales *et al.*, 2012). It is commonly accepted that most seed species *i.e.* pepper, corn, sugarcane, sugar beet, potato and cabbage cannot tolerate salinity higher than 10-20 % seawater, and many do not grow even at lower concentrations (Malik *et al.*, 2018; Wu1 *et al.*, 2017; Khorshid, 2016). Sugar beet (*Beta vulgaris* L.) is one of the most salt tolerant crops that is sensitive to salinity at germination and establishment stage (Kito *et al.*, 2017; Wu *et al.*, 2016; Khayamim *et al.*, 2014). It as an important commercial crop supplies approximately 35% of the world's sugar and is widely cultivated in arid and semi-arid regions of Iran (Jafarnia *et al.*, 2013). Sugar beet plant has a good ability in modifying its osmotic potential as a

response to salt stress (Khayamim *et al.*, 2014). Reduction of sugar beet root yield under salinity has yet to be clear. However, many investigations suggested that this reduction was caused by inhibition of photosynthesis or nutrient deficiency or by mineral toxicity (Esmaeili, 2011; Ober & Rajabi, 2010). Khorshidi *et al.*, (2014) studied the quantity and quality characters of 10 sugar beet breeding populations along with two control genotypes under low and severe salt stress and non-stress conditions. Results showed that root yield, sugar yield and K were reduced in salt stress conditions whereas sugar content (SC), Na, N and MS were increased by salt stress. The increased SC was result in osmotic adjustment in stress condition. The MSC2*8001-P.7 genotype was found to be superior to the others in the three environments. Khorshid & Rajabi (2014) concluded that the pollinator parent of the MSC2*8001-P.7 genotype was used as salt-tolerant parent for the subsequent sugar beet breeding programs. It is very important to screen sugar beet salt tolerant genotypes as fast as possible, so the relation of greenhouse and field experiment is very helpful in this case. In addition, SBSI is the only Asian sugar beet institute, which could produce salt tolerant varieties in this region so evaluation of its sugar beet population and genotypes is very important for improving and introducing a salt tolerant variety. The aims of the present study were to investigate the physiological and morphological traits in sugar beet in greenhouse conditions and compared with field experiments, and studying the effects of stress on the quality and quantity characters of sugar beet populations of different selection generations in stress and non-stress conditions.

Material and Methods

Ten sugar beet (*Beta vulgaris* ssp. *vulgaris*) breeding populations and 2 control varieties (Table 1) were studied under stress and non stress conditions in the Greenhouse of West Azarbaijan Agricultural Research Center and at the research field of Sugar Beet Seed Institute (SBSI) in Mian-Doab. All the seeds were Iranian multigerm, diploid and open pollinators, provided from SBSI.

Greenhouse experiments: This experiment was conducted as a factorial based on a completely randomized design with three replications in 2013. The first factor included salinity levels with sodium chloride at 0 and 16 dS/m, and the second factor was sugar beet genotypes (Table 1). The cultivation of different genotypes was carried out in a pot containing perlite. Each genotype were planted in four pots and each pot having eight seeds. After crop emergence, four plants were kept. The first water was distilled water and the second water was added to the Hoagland solution (Table 2). Pots were irrigated by Hoagland solution (pH=5.5-5.7) with EC control of drainage. Crops were harvested after three months and leaf samples were taken from 4 to 7 leaf stage for measuring morphophysiological characters (Khayamim *et al.*, 2014).

Field experiments: Twelve sugar beet (*Beta vulgaris* ssp. *vulgaris*) breeding populations were compared under non-stress and stress conditions (Electrical conductivity= 22 dS/m) at during 2014. Seeds were cultivated manually on

50 cm rows. In both stress and non-stress conditions, land preparation operations fertilizing based on soil analysis, seed cultivation, weed and pest controls and wildfire were performed as usual. The harvest time was in November 2014. In order to determine the quantitative and qualitative yield of all plots, the experimental plots were harvested root number and weights were determined. Sugar beet root pulps were analyzed (Abdollahian *et al.*, 2005) for sugar content % (SC) by polarimetric method, sodium (Na) and potassium (K) by flamphotometry and alpha amino nitrogen(N) by Blue no method were measured with Beta-laser device.

Other traits such as sugar yield (SY) and white sugar (WSY) were calculated based on the above characteristics. In addition, molasses sugar (MS) was estimated using the Rhinefeld formula:

$$\% \text{ Alkalinity factor} = \frac{\text{K} + \text{Na}}{\alpha - \text{amino} - \text{N}}$$

$$\% \text{ MS} = 0.343 (\text{Na} + \text{K}) + 0.094 (\alpha - \text{amino}) - 0.31$$

$$\% \text{ White sugar content (W.S.C)} = \% \text{ S.C.} - \% \text{ MS}$$

$$\text{SY} = \text{RR} * \text{SC}$$

$$\% \text{ W.S.Y.} = \text{W.S.C.} * \text{RY}$$

$$\text{ECS} = \frac{\text{WSC}}{\text{SC}}$$

Table 1. List of genotypes tested in all two environmental conditions.

No.	breeding population	Genotype	Characteristic
1.	S-P.2	Multigerm (full sib)	Stress tolerance background
2.	S-P.3	Multigerm (full sib)	Stress tolerance background
3.	S-P.7	Multigerm (full sibe)	Stress tolerance background
4.	S-P.8	Multigerm (full sib)	Stress tolerance background
5.	SC C2*S7	Multigerm (hybrid)	Stress tolerance background
6.	SC C2*S10	Multi germ (hybrid)	Stress tolerance background
7.	SC C2*S11	Multi germ (hybrid)	Stress tolerance background
8.	SC 261*S2	Multi germ (hybrid)	Stress tolerance background
9.	SC 261*S7	Multi germ (hybrid)	Stress tolerance background
10.	8001	Multigerm	Base population
11.	7233-p.29* Ms C2	Multi germ (hybrid)	Tolerant check
12.	191	Multi germ	Susceptible check

Table 2. Compounds and their amount in Hoagland diet.

No.	Chemical name	Quantity in stoic solution (g/L)	Quantity in 100 liters (mL)
1.	NH ₄ H ₂ PO ₄	115	100
2.	KNO ₃	101	600
3.	Ca(NO ₃) ₂ .4 H ₂ O	236	400
4.	MgSO ₄ . 7 H ₂ O	246	200
5.	Fe-EDTA	5	150
6.	H ₃ BO ₃	0.38	
7.	ZnSO ₄ .7 H ₂ O	0.22	
8.	MnSO ₄ .4 H ₂ O	1.02	100
9.	CuSO ₄ .5 H ₂ O	0.08	
10.	(NH ₄) ₆ Mo ₇ O ₂₄ .4 H ₂ O	0.02	

Morphophysiological traits: Various physiological and morphological traits of sugar beet populations in both stress and non-stress conditions were studied.

Na⁺ and K⁺ concentrations were determined on dried leaf samples according to the method as described by Wang *et al.*, (2007, 2004). Ion analysis was performed using a flame photometer (2655-00, Cole-Parmer Instrument Co., Vernon Hills, USA).

Proline content was extracted and determined on fresh leaves according to the method as described by Bates *et al.*, (1973) using the ninhydrin reagent. Proline concentrations were estimated using an UV-Visible spectrophotometer (CARY 50 Probe, Varian Co., Palo Alto, USA) at 520 nm.

The relative water content (RWC) of leaf was determined according to the method of Smart & Bingham (1974) with some modifications. Leaf discs (1.5 cm²) were weighed to determine the fresh mass (FM), soaked in distilled water at 25°C for 4 h to determine the turgid mass (TM), then oven-dried at 100°C for 24 h to determine the dry mass (DM). Finally, RWC value was calculated using the following equation for each treatment:

$$\% \text{ RWC} = \frac{\text{FM} - \text{DM}}{\text{TM} - \text{DM}} \times 100$$

In this study, Ion leakage (IL) was used to evaluate membrane permeability in leaves which were cut out randomly, washed two times with distilled water in order to remove surface contaminants, and then placed individually in vials containing 10 mL of distilled water. The vials were incubated at room temperature (25°C) on a shaker (100 rpm) for 24 h and then measure EC of the solution (EC1). Then, the same vials with leaf samples were placed in an autoclave at 120°C for 2 h and the second measurement of electric conductivity (EC2) was done after cooling the solution to room temperature. The ion leakage was defined as EC1/EC2 and expressed in percents (Lutts *et al.*, 1995).

$$\text{EC} = \frac{\text{EC 1}}{\text{EC 2}} \times 100$$

For determining relative water loss (RWL), leaves from each treatment were weighed to determine the fresh mass (FM). Then, the leaves were wilted at 30°C for 2 h to determine the wilting mass (WM), then oven-dried at 100°C for 24 h to determine the dry mass (DM). Finally, following equation was used to calculate RWL:

$$\% \text{ RWL} = \frac{\text{FM} - \text{WM}}{\text{DM}} \times \frac{t1 - t2}{60}$$

Where FM is the leaves fresh weight, WM and DM are the wilting and dry weight of leaves, respectively. t1 and t2 are the time of measurement for wilting and dry weights, respectively.

Specific leaf weight (SLW) of samples was investigated at 30, 60 and 90 days. Specific Leaf Weight (SLW) was calculated, after drying samples using the following formulae:

$$\text{SLW} = \frac{\text{Leaf dry weight}}{\text{Total sampled leaf area}}$$

This incremental method assumes that the SLW of existing leaves remains unchanged, assessing the plant's canopy at each sampling as old, intermediate and new leaves.

After harvest, plants were washed with distilled water and separated into leaves and roots for fresh weight

measurement. After that, the leaves and the sliced roots were oven-dried at 80°C for 72 hours for dry weight measurement.

Data analysis

The data for all parameters were analyzed using the analysis of variance procedure of the Statistical Analysis System (SAS) software, SAS 9.1 (SAS Institute Inc., 2009). Means were compared using least significant difference test (LSD) at 5 % probability level.

Results and Discussion

Greenhouse experiments: In this growth stage, osmotic regulators and ions were affected by salinity stress more than photosynthetic traits. The effect of salinity on proline content, sodium, potassium, fresh and dry shoot weight, fresh and dry root weight, number of green leaves, petiole length and relative leaf water content were significant at 1% probability level (Table 2). Salt stress increased the amount of leaf proline by about 4 times, while decreasing potassium content by about 12 percent. Also, with the increase in the salinity stress, the sodium content of the leaf was increased about 2 times while the relative water content of the leaves was decreased about 18 percent (in Table 3 available in Electronic Supplementary Material). Among these osmolites, proline responds faster and better to stress. This parameter can be used as a suitable criterion for evaluation of stress tolerance in sugar beet genotypes. Salinity affects the two processes of irrigation and ion relations in the plant, and at the first few minutes of stress, a plant is faced to osmotic stress. However, if the plant is exposed to salinity stress for a long time, it will suffer from ionic stress after osmotic stress. The results showed that genotypes SC 261*S7 and 191 stored the least amount of sodium, potassium and proline in the leaves. This indicates that these genotypes naturally and genetically inhibit the absorption of these elements in the leaf and possibly retain more sodium in their root canola. Because of salinity stress, the amount of leaf sodium in most genotypes was about 1.5 times higher, but the amount of sodium of these genotypes increased by only 50 and 70 percent. In addition, with the increase in the salinity stress, the amount of potassium in sugar beet 7233-p.29* Ms C2 genotype was more than other genotypes. The reduced potassium levels in sensitive cultivars indicate that stopping the growth of the airways due to metabolic changes may lead to an ionic imbalance or ionic toxicity in the plant root. In addition, increased salinity stress reduced aerial and respiratory organs, leaf area, RWC, RWL, and R/S ratio. According to Table 4 (available in Electronic Supplementary Material), by increasing salinity, the total dry weight and shoot, root length and leaf area index are increased. The reason for the increase of these traits can be because the plant saves all the material produced in the leaf in order to withstand the

stress tolerance. Also, with comparing the percentage of dry matter of the plant under these conditions, salinity stress at this stage increased dry matter content in most genotypes, except for sensitive genotype SC 261*S7 and S-P.3. In other words, in the genotype is sensitive from the very early stages of growth and the amount of airborne function decreases. In tolerant genotypes, at this stage of growth (8 leaf), by decreasing the leaf area, osmoregulation is done which leads to increased organ function, dry matter and growth stimulation. Therefore, it can be concluded that stress in the early stages of growth can stimulate the growth of sugar beet. Meanwhile, it has been reported that mild stresses can also stimulate sugar beet growth. In terms of interaction of genotypes at different levels of salinity, the amount of leaf proline, total dry matter, fresh and dry weight of root, leaf area and potassium level were significant at 1% level (in Table 3 available in Electronic Supplementary Material). In the stage of sugar beet deposition, salinity stress reduced the amount of leaf proline by about 4 times and the leaf area by about 2.5 times respectively (in Table 5 available in Electronic Supplementary Material). This indicates that with the development of stress in the plant and the development of the growth stage, more amounts of leaf area are reduced to the stress. Sodium not only influences potassium absorption based on the ionic strength of the soil, but also the selective action of the root membrane. In other words, plants tolerate more potassium. Meanwhile, it is observed that the relative water content and the water lost by the plant are not affected by salinity. Therefore, in the osmotic stress step, salinity decreases the plant growth and the effect of ion toxicity on the plant is higher. The decrease in chickpea (Sohrabi *et al.*, 2008) and sugar beet growth due to salinity stress are related to the ionic effect. Because the increase in sodium accumulation results in a loss of ionic balance and osmotic regulation, and can cause toxic effects on the plant. In addition, the osmotic regulation in shoalaceous plants is due to the accumulation of sodium and chloride ions. Also, a plant such as sugar beet does the osmotic regulation with sodium accumulation and its distribution to the vaccine, which stimulates the activity of ATPase and increases the protonic force associated with the antipores of sodium hydrogen. However, in crops such as, rice, corn, sugar beet and cotton and *Chenopodium glaucum*, reduced growth and germination with osmotic stress are associated.

Field results (quantitative and qualitative root yield):

There were significant differences between different genotypes regarding root yield, sugar yield, white sugar yield, extraction coefficient(ECS) sodium, nitrogen and molasses sugar content at 1% probability level and for other traits were not statistically significant (in Table 5 available in Electronic Supplementary Material). There were no statistically significant interaction between

genotype and environment for root, sugar and white sugar yields, relative water content (RWC) and relative water loss (RWL). This indicated that all genotypes had the same reaction for each of these traits in each environment, but there were significant differences for sodium, nitrogen, molasses and ECS (sugar extraction coefficient) at 1% probability level and for potassium at 5%. This indicates the response of the treatments to the studied environmental conditions. That means the environment has some significant effects on some masses, and the rest is less likely to occur, with a sharp increase in salinity, root yield, sugar, and white sugar yields decreased by about 100 percent. While this decrease was lower for other studied traits. Salinity increased root sodium, resulting in an increase in the percentage of molasses sugar and the withdrawal of sugar from the process. Comparison of traits under stress and non-stress conditions showed that under stress condition, the white sugar yield, potassium, RWC and RWL were decreased and the traits of sugar, N and sodium content of the root were increased (Table 6). Reducing root yield indicates that stress can be effective in reducing root yield and, with regard to stress-free environments, RWC reduction is indicative of uneven water storage in the masses. In addition, salt stress has increased nitrogen, sodium, and molasses sugar content. Salt conditions have increased sugar content relative to other conditions. High sugar is common in salt stress conditions. Because the plant will be able to cope with the phenomenon of salinity in this way, thus regulating its osmotic pressure against the destructive effects of salinity. Meanwhile, sugar content was not significant in salt stress tests, since stress on all treatments affected an equal amount and could not show a definite response. In order to compare the mean of the studied traits in stress and non-stress conditions, the meanings for each trait were compared to the total masses in Table 7 at the same time. Table 8 shows the results of the mean comparison for the genotypes examined in stressed and non-stressed conditions. The highest root yield, sugar content and white sugar yield were obtained from the treatments of 11.11, 15.12 and 11.3 t ha⁻¹, and the lowest was 9, 34.3, 6.6 and 6 t ha⁻¹, respectively.

Alpha amini nitrogen and molasses sugar percentage: All impurities in the root include sodium, potassium and alpha amino nitrogen inside the molasses sugar content, and there is a high correlation between these traits. The accumulation of these elements disrupts the sugar extraction process from sugar beet. The results showed that the lowest levels of harmful nitrogen related to genotypes SC C2*S11 and SC 261*S2 and the percentage of molasses sugar belonged to genotypes S-P.8 and SC 261*S2 of this experiment. Other researchers have reported reduced yields of white sugar due to environmental stresses, including drought and salinity.

Table 3. Analysis of variance for the traits measured in stress and non-stress conditions under greenhouse conditions.

DF	TDW	SFW	SDW	RFW	RDW	RWC	RWL	GREEN	WILT	TOTL	RL	LA	PETL	LL	LW	R/S	SLW	Na	K	PRLN	
REP	2	0.71	19.65	0.39	0.27	0.08	377.91	0.03	0.60	0.83	0.77	7.38	374	6.48	8.05	2.44	0.03	436	114	120	71.26
A	1	2.71**	170**	4.42**	3.03**	0.21**	2873.03**	0.33**	4.85**	0.78 ^{ns}	1.74 ^{ns}	111**	4779**	211**	78.03**	58.6**	1.21**	746**	11644**	827**	9453**
B	11	0.68**	11.7**	0.43**	0.15*	0.06**	413.12**	0.03 ^{ns}	3.02**	0.94*	6.33**	12.7*	52.74 ^{ns}	24.5**	1.84 ^{ns}	0.29 ^{ns}	0.02**	39.9 ^{ns}	164.9 ⁺	153**	52.84**
A*B	11	0.49 ⁺	5.60 ⁺	0.31**	0.18**	0.04**	107.31	0.04 ^{ns}	0.64 ^{ns}	0.29 ^{ns}	1.40 ^{ns}	5.69 ^{ns}	35.81 ^{ns}	3.26 ^{ns}	1.13 ^{ns}	0.50 ^{ns}	0.01**	42.8 ^{ns}	127 ^{ns}	229**	35.04**
Error	46	0.10	3.04	0.07	0.01	143.02	0.00	0.6	0.38	1.30	6.34	59.26	3.91	1.72	0.59	0.001	37.96	88.28	9.35	13.19	
CV	14.48	26.56	25.31	21.38	24.86	17.88	57.20	12.11	14.50	12.85	17.49	26.15	20.67	17.84	19.37	20.66	43.30	18.95	5.55	20.01	

ns, *, ** and +: are non-significant and significant at 10%, 5% and 1%, respectively

Table 4. Means comparison of the traits measured in stressed and non-stressed conditions for greenhouse conditions.

	TDW	SFW	SDW	RFW	RDW	RWC	RWL	GREEN	WILT	TOTL	RL	LA	PETL	LL	LW	R/S	SLW	Na	K	PRLN
Non-stressed	1.1 ^b	6.4 ^a	0.8 ^b	0.6 ^b	0.3 ^a	73.3 ^a	0.17 ^a	6.1 ^b	2.6 ^a	8.7	13.1 ^b	29.7 ^a	11.3 ^a	8.4 ^a	4.9	0.1 ^a	10.8 ^b	36.7 ^b	58.6 ^a	6.8 ^b
Stressed	1.5 ^a	3.2 ^b	1.3 ^a	1.0 ^a	0.2 ^b	60.5 ^b	0.03 ^b	6.6 ^a	2.4 ^b	9.0	15.6 ^a	12.9 ^b	7.8 ^b	6.3 ^b	3.0	0.4 ^b	17.8 ^a	62.4 ^a	51.5 ^b	29.5 ^a

Table 5. Means comparison of the different genotypes in stressed and non-stressed conditions for greenhouse conditions.

	TDW	SFW	SDW	RFW	RDW	RWC	RWL	GRL	WILT	TOTL	RL	LA	PETL	LL	LW	R/S	SLW	Na	K	PRLN
1.	1.93	7.59	1.44	1.16	0.49	56.9	0.13	8	3	11	16.1	26.2	12.2	8.4	4.1	0.190	16.2	54.7	58.8	21.2
	A	A	BA	A	A	D	A	A	BA	A	A	A	A	A	A	CED	BA	BA	BA	BA
2.	1.53	6.13	1.15	0.92	0.38	60.4	0.12	7	3	10	15.6	23.5	12.4	7.8	3.9	0.183	16.1	52.0	52.2	19.6
	BAC	BA	BC	BA	BA	DC	DC	B	BA	BA	BA	BA	A	BA	BA	CED	BA	BAC	BAC	BAC
3.	1.66	5.28	1.49	0.80	0.17	60.6	0.12	6	2	9	15.6	23.7	11.6	8.0	4.0	0.192	13.3	51.9	54.0	17.1
	BA	BC	A	BC	DE	DC	DC	BCD	BDC	BDC	BA	BA	BA	BA	BA	CED	BA	BAC	DC	BDC
4.	1.23	3.95	1.05	0.93	0.18	62.2	0.09	6	2	8	13.7	20.0	10.9	7.3	3.9	0.284	13.0	51.4	61.7	22.9
	EDC	BCD	DC	BA	DE	BDC	BDC	D	D	D	BAC	BA	BDAC	BA	B	B	B	BAC	A	A
5.	1.17	4.58	0.92	0.80	0.25	74.5	0.09	6	2	8	16.2	19.6	8.6	6.6	3.8	0.240	11.1	55.5	58.5	16.1
	EDC	BC	DC	BC	DC	BAC	BAC	CD	BDC	D	A	BA	FDEG	B	CB	B	B	A	BA	DC
6.	0.89	3.23	0.73	0.63	0.16	80.7	0.08	6	2	8	13.0	18.6	6.5	6.8	3.7	0.235	12.3	53.9	45.6	14.4
	EF	CD	ED	BC	DE	A	A	CD	BDAC	D	BC	BA	G	BA	CBD	B	B	BAC	F	D
7.	1.40	4.69	1.09	0.78	0.31	75.5	0.07	6	3	9	16.1	23.6	8.5	7.9	4.1	0.168	13.8	49.6	55.3	14.8
	BDC	BC	C	BC	BC	BA	BA	CD	BAC	BDC	A	BA	FEG	BA	E	E	BA	BAC	BDC	D
8.	1.15	4.54	0.93	0.73	0.22	60.9	0.1	7	3	10	13.9	23.7	9.3	7.3	0.4	0.234	12.8	43.0	49.3	21.3
	EDC	BC	DC	BC	DCE	DC	DC	BC	BA	BAC	BAC	BA	BDEC	BA	CBD	B	B	DC	EF	BA
9.	1.25	5.52	1.02	0.89	0.23	62.5	0.08	7	3	10	13.4	18.8	8.9	7.1	3.6	0.243	15.1	38.5	52.0	17.6
	EDC	BA	DC	BA	DCE	BDC	BDC	BA	A	A	BAC	BA	FDEC	BA	CB	BA	BA	D	ED	BDC
10.	1.08	4.05	0.90	0.76	0.18	64.2	0.14	6	2	8	11.7	20.6	6.8	6.8	4.1	0.215	12.6	51.9	61.8	21.3
	EDF	BCD	DC	BC	DE	BDC	BDC	CD	BDC	D	C	BA	FG	BA	CED	B	B	BAC	A	BA
11.	1.43	5.47	1.17	0.84	0.26	64.3	0.12	6	2	8	13.6	21.5	11.0	7.3	4.2	0.171	21.7	44.0	57.9	15.6
	BDC	BA	BAC	BC	DC	BDC	BDC	BCD	DC	DC	BAC	BA	BAC	BA	ED	A	A	BDC	BAC	DC
12.	0.68	2.20	0.55	0.56	0.13	79.8	0.1	6	2	8	13.9	15.7	8.0	6.9	3.7	0.357	13.4	48.4	53.2	15.9
	F	D	E	C	E	A	A	D	BDC	D	BAC	B	FEG	BA	A	A	BA	BDAC	ED	DC

Table 6. Analysis of variance for the traits measured in stress and non-stress conditions under field conditions.

	DF	RY	SY	WSY	SC	Na	K	N	ECS	MS	RWC	RWL
rep	2	19 ^{NS}	0.20 ^{NS}	1.24 ^{NS}	0.86 ^{NS}	4.03 ^{NS}	1.38 ^{NS}	9.25 ^{**}	29.1 ^{NS}	1.2 ^{NS}	520.5 ^{NS}	12.36 ^{NS}
A	1	38937 ^{**}	1174 [*]	616 [*]	218 ^{**}	44.79 ⁺	34.20 [*]	13.42 ^{NS}	261 ⁺	0.4 ^{NS}	1016 ^{NS}	0.45 ^{NS}
rep*A	2	333	22.67	16.38	0.66	4.06	1.11	6.17	24.85	0.53	205.43	29.08
B	11	456 ^{**}	17.75 ^{**}	9.41 ^{**}	3.33 ^{NS}	12.78 ^{**}	1.05 ^{NS}	4.98 ^{**}	47.3 ^{**}	2.67 ^{**}	125 ^{NS}	64.68 ⁺
A*B	11	61 ^{NS}	2.14 ^{NS}	2.75 ^{NS}	5.16 [*]	12.18 ^{**}	1.91 [*]	3.54 ^{**}	54.1 ^{**}	2.63 ^{**}	80.5 ^{NS}	66.09 ⁺
Error	11	40	3.10	2.40	2.62	2.54	0.81	0.92	17.02	0.60	93.91	37.39
CV	11	14.36	16.39	7.41	28.09	13.61	25.72	5.30	18.17	11.84	27.87	

Table 7. Means comparison of the traits measured in stressed and non-stressed conditions for field conditions.

	RY	SY	WSY	SC	NA	K	N	ECS	MS	RWC	RWL
non-stressed	81.4	16.3	12.4	20.1	4.88	7.29	3.29	75.9	4.17	85.6	6.32
	A	A	A	B		A					
Stress	34.9	8.22	6.53	26.6	6.46	5.91	4.16	79.7	4.32	78.1	6.16
	B	B	B	A		B					

Table 8. Means comparison of the different genotypes in stressed and non-stressed conditions for field conditions.

	RY	SY	WSY	SC	Na	K	N	ECS	MS	RWC	RWL
1.	55	12	9.3	23	4.3	6.6	3.5	81	3.8	89	3.2
	CD	B	BA		DE		BDC	A	DC		
2.	63	12	9.1	21	6	6.7	4.6	76	4.5	84	5.6
	B	B	B		DCE		BA	DEC	BC		
3.	60	13	10	22	6	6.8	4.9	77	4.5	85	3.4
	CBD	BA	BA		DC		A	BDAC	BC		
4.	61	12	9.8	20	4.5	6.1	2.9	80	3.6	82	5.8
	CBD	B	BA		DE		D	BA	D		
5.	71	15	11	22	6.5	6.1	3	75	4.3	87	5.3
	A	A	A		BC		D	DE	DC		
6.	63	13	10	21	5.2	6.4	3.1	79	3.9	79	8.5
	B	BA	BA		DCE		D	BDAC	DC		
7.	62	13	9.9	22	4.8	6.8	2.6	78	3.9	83	15
	CB	BA	BA		DCE		D	BDAC	DC		
8.	58	12	9.3	22	4.2	6.3	2.7	80	3.5	76	6
	CBD	B	BA		E		D	BAC	D		
9.	34	7.6	6	23	5.3	6.9	4.3	80	4.3	85	5.3
	E	C	C		DCE		BAC	BAC	DC		
10.	55	12	8.8	22	8.5	7.6	4.7	72	5.6	79	4.6
	CD	B	B		A		A	E	A		
11.	61	13	10	23	8.2	6.8	5	76	5.3	73	4.1
	CBD	BA	BA		BA		A	BDEC	BA		
12.	55	12	9.4	22	4.6	6.4	3.4	81	3.8	80	8
	D	B	BA		DE		DC	A	DC		

Conclusion

This study showed that root yield, sugar yield and K were reduced in salt stress conditions whereas SC, Na, N and MS were increased by salt stress. Genotypes SC C2*S7, SC C2*S10 and SC C2*S11 were found to be superior to the other genotypes for root and white sugar yields and sugar content. Genotypes SC 261*S7 and 191 had the least root yield in the stress condition. Meanwhile, the results showed that the lowest levels of harmful nitrogen related to

genotypes SC C2*S11 and SC 261*S2 and the percentage of molasses sugar belonged to genotypes S-P.8 and SC 261*S2 of this experiment.

Acknowledgements

The authors gratefully acknowledge the Tabriz University, Tabriz, the research station of Sugar Beet Seed Institute (SBSI) in Mian-Doab, as well as West Azarbaijan Agricultural Research Center for providing facilities for this work.

References

- Abdollahain, N.M., R. Sheikholeshami and B. Babae. 2005. Technological quantitative and qualitative expression and explanation of sugar beet. *Sugar Beet*, 21: 101-104. (Persian with English abstract).
- Aydiñşakir, K., S. Erdal and M. Pamukçu. 2013. The effects of different salt concentrations on germination and seedling parameters of silage corn (*Zea mays* L.) Varieties. *Anadolu J. Agr. Sci.*, 28(2): 94-100.
- Bates, L.S., R.P. Waldren and I.D. Teare. 1973. Rapid determination of free proline for water stress studies. *Plant & Soil*, 39: 205-207.
- Chaugool, J., H. Naito and Sh. Kasuga, H. Ehara. 2013. Comparison of young seedling growth and sodium distribution among sorghum plants under salt stress. *Plant Prod. Sci.*, 16(3): 261-270.
- Chauhan, R.R., A. Singh and P.K. Singh. 2016. Effect of salt stress on Na⁺ and K⁺ uptake at seedling stage in sorghum cultivars. *Int. Res. J. Biol. Sci.*, 5(10): 28-34.
- Cha-Um, S. and Ch. Kirdmanee. 2009. Effect of salt stress on proline accumulation, photosynthetic ability and growth characters in two maize cultivars. *Pak. J. Bot.*, 41(1): 87-98.
- Chunthaburee, S., J. Sanitchon, W. Pattanagul and P. Theerakulpisut. 2014. Alleviation of Salt Stress in Seedlings of Black Glutinous Rice by Seed Priming with Spermidine and Gibberellic Acid. *Not. Bot. Hort. Agrobo.*, 42(2): 405-413.
- Esmacili, M.A. 2011. Evaluation of the effects of water stress and different levels of nitrogen on sugar beet (*Beta vulgaris*). *Int. J. Biol.*, 3: 89-93.
- Hajiboland, R., N. Ebrahimi and Ch. Poschenrieder. 2012. Bound putrescine, a distinctive player under salt stress in the natrophilic sugar beet in contrast to glycophyte tobacco. *J. Sci., Islamic Republic Iran.*, 23(2): 105-114.
- Jafarnia, B., R. Ghorbani, A. Zare Feizabady and A.R. Ghaemi. 2013. Impact of crop density and soil fertilization on sugar beet. *Intl. J. Agri. Crop Sci.*, 5(24): 2991-2999.
- Kazemeini, S.A., H. Pirasteh-Anosheh, A. Basirat and N.A. Akram. 2018. Salinity tolerance threshold of berseem clover (*Trifolium alexandrinum*) at different growth stages. *Pak. J. Bot.*, 50(5): 1675-1680.
- Khayamim, S., R. Tavakkol Afshari, S. Sadeghian, K. Poustini, F. Rouzbeh and Z. Abbasi. 2014. Seed Germination, plant establishment, and yield of sugar beet genotypes under salinity stress. *J. Agr. Sci. Tech.*, 16: 779-790.
- Khorshid, A. 2016. Biplot analysis of salinity tolerance indices in sugar beet breeding lines. *Adv. Plants Agri. Res.*, 5(2): 1-6.
- Khorshid, A. and A. Rajabi. 2014. Investigation on quantity and quality characters of sugar beet advanced breeding populations in drought and salinity stress and non-stress conditions. *Int. J. Agri. Crop Sci.*, 7(9): 532-536.
- Khorshidi, A., M. Niazkhani, I. Bernousi and A. Rajabi. 2014. Improving salinity tolerance in an open-pollinated sugar beet population. *Int. J. Farm & Alli Sci.*, 3(6): 696-701.
- Kito, K., T. Yamamori, C. Theerawitaya, S. Cha-um, M. Fukaya, V. Rai and T. Takabe. 2017. Differential effects of excess potassium and sodium on plant growth and betaine accumulation in sugar beet. *J. Agri. Aspect.*, 1-7.
- Li, R., F. Shi and K. Fukuda. 2010. Interactive effects of salt and alkali stresses on seed germination, germination recovery, and seedling growth of a halophyte *Spartina alterniflora* (Poaceae). *S. Afr. J. Bot.*, 76: 380-387.
- Lutts, S., J.M. Kinet and J. Bouharmont. 1995. Changes in plant response to NaCl during development of rice (*Oryza sativa* L.) varieties differing in salinity resistance. *J. Exp. Bot.*, 46: 1843-1852.
- Malik, A., A.S. Shakir, M.J. Khan, Naveedullah, M. Latif, M. Ajmal and S. Ahmad. 2018. Effects of different mulching techniques on sugar beet performance under semi-arid subtropical climatic conditions. *Pak J. Bot.*, 50(3): 1219-1224.
- Malik, A., A.S. Shakir, Naveedullah, M.J. Khan, M. Latif, M. Shahzad Khattak and T.A. Khan. 2018. Interactive effect of deficit irrigation and mulching on sugar beet productivity in Pakistan. *Pak. J. Bot.*, 50(5): 1775-1783.
- Merwad, M.A.A. 2015. Effect of potassium fertilisation and salicylic acid on yield, quality and nutrient uptake of sugar beet (*Beta vulgaris* L.) grown in saline soil. *MJSS.*, 19: 95-105.
- Morales, S.G., L.I. Trejo-Téllez, F.C. Gómez Merino, C. Caldana, D. Espinosa-Victoria and B.E. Herrera Cabrera. 2012. Growth, photosynthetic activity, and potassium and sodium concentration in rice plants under salt stress. *Acta Scientiarum. Agronomy*, 34: 317-324.
- Mutlu, F. and S. Bozcuk. 2007. Salinity-induced changes of free and bound polyamine levels in sunflower (*Helianthus annuus* L.) roots differing in salt tolerance. *Pak. J. Bot.*, 39(4): 1097-1102.
- Ober, S.E. and A. Rajabi. 2010. Abiotic stress in sugar beet. *Sugar Tech.*, 12(3-4):294-298.
- Smart, R.E. and G.E. Bingham. 1974. Rapid estimates of relative water content. *Plant Physiol.*, 53: 258-260.
- Sohrabi, Y., Gh. Heidari and B. Esmailpour. 2008. Effect of salinity on growth and yield of desi and kabuli chickpea cultivars. *Pak. J. Biol. Sci.*, 11(4): 664-667.
- Wang, S.M., C.G. Wan, Y.R. Wang, H. Chen, Z.Y. Zhou and H. Fu. 2004. The characteristics of Na⁺, K⁺ and free proline distribution in several droughtresistant plants of the Alxa Desert. *China. J. Arid Environ.*, 56: 525-539.
- Wang, S.M., J.L. Zhang and T.J. Flowers. 2007. Low affinity Na⁺ uptake in the halophyte Suaeda maritima. *Plant Physiol.*, 145: 559-571.
- Wu, G.Q., N. Liang, R.J. Feng and J.J. Zhang. 2013. Evaluation of salinity tolerance in seedlings of sugar beet (*Beta vulgaris* L.) cultivars using proline, soluble sugars and cation accumulation criteria. *Acta Physiol Plant.*, 35: 2665-2674.
- Wu, G.Q., R.J. Feng and Q.Z. Shui. 2016. Effect of osmotic stress on growth and osmolytes accumulation in sugar beet (*Beta vulgaris* L.) plants. *Plant Soil Environ.*, 62: 189-194.
- Wu1, G.Q., R.J. Feng, S.J. Li and Y.Y. Du. 2017. Exogenous application of proline alleviates salt-induced toxicity in sainfoin seedlings. *J. Ani. & Plant Sci.*, 27(1): 246-251.

(Received for publication 15 November 2017)