



Comparison of some physiological responses to salinity and normal conditions in *Sugar Beet*

A.M. Khorshid^{1,3}, F.A. Moghadam^{2*}, I. Bernousi², S. Khayamim³ and A. Rajabi³

Department of Plant Breeding and Biotechnology,
Faculty of Agriculture, Urmia University, Urmia, Iran.

Received: 06-01-2018

Accepted: 04-06-2018

DOI: 10.18805/IJARE.A-320

ABSTRACT

This study was carried out in the Agricultural Research Center of West Azerbaijan, Iran in 2016. In this research, variations in different physiological and yield traits measurement of total dry weight, shoot fresh weight, shoot dry weight, root fresh weight, root dry weight, relative water content, relative water loss, root length, leaf area, root/shoot ratio, specific leaf weight, sodium content, potassium content and proline were investigated in normal and saline condition. The results indicated that in saline condition, total dry weight, root fresh weight, shoot dry weight, root/shoot ratio, specific leaf weight, root length, proline and Na content were increased and the other traits were decreased. Study of correlation of traits showed that most significant difference between the two conditions was observed for the root/shoot ratio, so that, this trait has negative significant relation with total dry weight, shoot fresh weight, shoot dry weight, root fresh weight, root dry weight, relative water content, leaf area, root length, specific leaf weight in saline condition, but in normal condition correlation is positive and significant only in the total dry weight, root fresh weight, shoot dry weight and root dry weight and was not significant in the other traits. Step-wise regression analysis for total dry weight as dependent variable revealed that in normal condition, root fresh weight, shoot fresh weight and Na content expound of 93.1% and in saline condition root fresh weight, root length, Na content and proline explicate of 81.3% of total variation exist in total dry weight. Therefore, it is suggested to consider different traits in breeding programs for normal and saline conditions.

Key words: *Beta vulgaris*, Physiological traits, Stepwise regression.

INTRODUCTION

Among abiotic stresses, salinity always limits the growth, distribution and production of plants. According to a recent estimate, 1128 million ha of global land is affected by salinity (Akhtar *et al.*, 2015). The main cause of salinity in Iran is the dry climate (low rainfall and high transpiration), high salinity stones, insufficient drainage, and lack of access to water and its quality (Kehl, 2006). In areas where evaporation is more than precipitation, salinity is a natural phenomenon. Areas with a lack of water and no natural drainage, salt accumulation causes soil salinity (Rains and Goyal, 2003). In saline regions, the average yield loss is estimated to be more than 50 % (FAO, 2000).

Generally, the plants are classified into two main groups according to the salinity tolerance mechanism: First, plants that reduce salt intake into their organs and this is done through selective absorption of the root cell, selective loading of xylem and the transfer of salt from xylem. Second, plants that reduce the accumulation of salt within the cytoplasm. Halophytes use both mechanisms to eliminate the effects of salinity (Munns, 2002). The tolerance of

halophytes to salinity stress is a set of physiological responses at three levels of cell, tissue and total plant. At the cellular level, ion distribution, osmolyte accumulation, enzyme reactions, osmotic adjustment, and genetic control are considered; at the tissue level, leaf thickness, salt exclusion, and stomatal conductance; and at the whole plant level, germination ability, vigor, and growth are taken into account (Seaman 2007). Changes in these parameters depend on the severity and duration of stress (Lakshmi *et al.*, 1996) and plant species (Dubey, 1994).

Information on physiological changes occurring during saline condition is lacking for *sugar beet*, which is relatively tolerant of saline environments. To confront with salinity, the use of cultivars is one of the most effective and economical ways in low salinity lands. On the other hand, the progress made in producing resistant varieties is relatively slow. Because there is limited knowledge about complex mechanisms that speak about salinity tolerance at the cellular level or the entire plant (Ashraf and Harris, 2004).

The aim of this study was to investigate of changes of physiological and yield traits in *sugar beet* genotypes in

*Corresponding author's e-mail: fayaz.amir@yahoo.com

¹Ph.D Student of Plant Breeding, Department of Plant Breeding and Biotechnology, Faculty of Agriculture, Urmia University, Urmia, Iran.

²Department of Plant Breeding and Biotechnology, Faculty of Agriculture, Urmia University, Urmia, Iran.

³Sugar Beet Seed Institute, Agricultural Research, Education and Extension Organization (AREEO). Karaj, Iran.

saline and normal conditions and determine the dependence relationship between yield traits and other yield and physiological traits as well as identify the best selection criteria for genetic improvement of these traits via indirect and direct selection.

MATERIALS AND METHODS

This experiment was conducted to evaluate various physiologic and morphologic attributes in salinity conditions and their relationship with salt tolerance at the Agricultural Research Center of West Azerbaijan, Iran, 2016. The experiment was a factorial based on randomized complete block design with 3 replications. The first factor included salinity levels with sodium chloride at 0 (control) and 16 ds/m, and the second factor was 45 *sugar beet* genotypes. Different genotypes were cultured in pots containing washed perlite. Each genotype was planted in 4 pots and in each pot, eight seeds. After plant deployment, 4 seedlings were kept and the rest were removed. The first water was distilled water and second water with hoagland diet. Irrigation continued weekly adjusted with controlling EC drainage, until the end of the growing period, which lasted about three months (12-10 Leaf). The electrical conductivity and acidity of the saline solution were controlled by EC meter and pH meter, respectively. At all of the experiment, sampling from leaves four to seven took place.

The studied traits in this research are: total dry weight-TDW (gr), shoot fresh weight-SFW (gr), shoot dry weight-SDW (gr), root fresh weight-RFW (gr), root dry weight-RDW (gr), relative water content-RWC (%), relative water lose-RWL (%), root length-RL (cm), leaf area- LA (cm²), root/shoot ratio-R/S, specific leaf weigh-SLW (gr/cm²), sodium-Na content (mg/g), potassium- K content (mg/g) and proline (mg/g). Leaf Area meter (DELTA-T, Co. Durham, UK) was used to determine the leaf area in cm². The amount of proline in the ninhydrin method was measured by the proposed method of Bates *et al.* (1973). Fresh samples of leaves were taken at harvest from each pot to determine the Na and K contents in leaves. Na and K content were measured according to AOAC (2000), by taking leaf samples (200 g) that oven dried at 70 °C for 48 h and made into fine powder by mortar. 0.5 g dried sample of leaves was placed in crucibles in an electric furnace at 500°C to obtain the ash. The ash was put into 50 ml Volumetric flasks, then adding 5 ml of 2N HCl, mixed with boiling distilled water and filtered by Whatman paper No. 2. The Na and K contents were measured using flame photometer and re-reported as mg g⁻¹ of dry weight.

The relative water content was measured by Morant-Manceau *et al.*, (2004) and slightly modified using the equation below.

$$RWC = \frac{[(\text{fresh weight} - \text{dry weight}) / (\text{total weight} - \text{dry weight})] \times 100}{}$$

The amount of relative water loss, calculated in grams of water lost from leaf dry weight, was calculated in 8 hours, by Yang (1991) by the following equation:

$$RWL = \frac{[(\text{fresh weight} - \text{wilt weight}) / \text{dry weight}] \times [(\text{time to wilt} - \text{time to dry}) / 60]}{}$$

The amount of specific leaf weight was calculated by Barrs and Weatherly (1962) using the following equation:

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

Statistical analysis (variance, correlation and stepwise regression analysis) was performed using SAS program (Version 6.12, SAS Institute Inc., Cary, USA) and the means compared using the LSD test at p=0.05.

RESULTS AND DISCUSION

The results of the analysis of variance and the mean comparisons of conditions for studied traits are shown in Table 1 (Comparisons of mean of genotypes have not been shown).

Salinity and growth factor: Plant growth was measured as TDW, SFW, SDW, RFW, RDW, RL, LA, R/S and SLW. All growth characters were significantly varied in saline condition. So that, TDW, RFW, SDW, RL, R/S and SLW were increased and SFW, RDW and LA were decreased (Table-1). In *sugar beet*, plant growth, leaf area, root and shoot dry weight decreased significantly with increasing salt concentration (Jamil *et al.*, 2007). The effect of salinity on SDW was higher than RDW, so that under this condition, SDW increased, but RDW decreased, In return, the SFW decreased but the RFW increased, so salinity prevented shoot growth but increased root growth.

RDW at saline condition decreased by compared to normal condition (Table-1). This might be due to the type of *sugar beet* root (storage root) and also water deficiency caused by concentration of salt in the growth medium. Abdollahian-Noghabi (1999) found that shoot/root ratio of *Beta vulgaris* increased under drought stress condition.

Most plants, when subjected to salinity, can adjust the osmotic pressure to reduce the tungsten pressure. Following the occurrence of transient water deficiencies, and in order to balance the amount of water abstraction from the leaf surface (transpiration) and the rate of water supply from the root, plants increase the production of abscisic acid by closing the stomata and the closure of stomata limits access to CO₂ (Ashraf and McNeilly, 2004) and ultimately reduced ecological yield.

This inconsistent with the result of previous research, which showed that salinity decreased leaf area due to a combination of a decrease in cell number and cell size (De-Herralde *et al.*, 1998). Witkowski and Lamont (1991) reported that plants might reduce water loss by reducing their evaporation surface. Therefore, leaves tend to be smaller and thicker in saline conditions. Decrease in the production of photosynthetic materials due to the closure of stomata

genotypes (yield is a direct trait), the genetic correlation of these attributes with yield should be high and have high heritability. The selection methods based on them should be widely applied (Ober *et al.*, 2005). However, the use of morphological and physiological traits related to yield, which have good heritability, can be effective in selecting tolerant genotypes. Due to the complexity of salinity tolerance in the plant and the fact that the study is done inter or intracellular level, cannot use a specific trait as an effective factor in selecting the tolerant genotype. Also, selection based on several molecular or morphological traits is much more effective than selection based on an attribute. The inheritance complexity of yield traits under stress conditions limits the

effectiveness of selection based on these traits (Ashraf and Harris, 2004).

In saline condition, RWC has positive and significant correlation with SFW, RDW and LA and negative relation with R/S, but in normal condition, there was no significant relationship between this trait and the other traits (Table-2). As the same way, in saline condition RWL has positive and significant relation with SWL and Na content, but these correlations are not significant in normal condition. RL in two conditions has positive and significant correlation with SFW and RDW but in saline condition has more correlation with TDW. Proline has negative and significant correlation with LA in normal condition but in saline

Table 2: Pearson's correlations between pairs of studied traits in *beta vulgaris* in normal and saline condition

		TDW	SFW	SDW	RFW	RDW	RWC	RWL	RL	LA	R/S	SLW	Pro	Na
SFW	S	0.8**												
	N	0.9**												
SDW	S	0.98**	0.71**											
	N	0.99**	0.9*											
RFW	S	0.87**	0.83**	0.84**										
	N	0.96**	0.88**	0.94**										
RDW	S	0.62**	0.77**	0.45**	0.6**									
	N	0.96**	0.85**	0.91**	0.94**									
RWC	S	0.27	0.46**	0.22	0.29	0.34*								
	N	-0.01	0.002	-0.01	0.03	0								
RWL	S	0.16	0.25	0.16	0.24	0.08	0.03							
	N	0.24	0.28	0.23	0.24	0.25	-0.22							
RL	S	0.42**	0.32*	0.35*	0.32*	0.5**	0.08	-0.08						
	N	0.29	0.32*	0.23	0.21	0.37*	-0.13	0.11						
LA	S	0.66**	0.82**	0.57**	0.64**	0.72**	0.43**	0.11	0.31*					
	N	0.37*	0.34*	0.36*	0.29	0.35*	0.19	0.18	0.19					
R/S	S	-0.54**	-0.82**	-0.43**	-0.48**	-0.72**	-0.49**	-0.27	-0.38*	-0.69**				
	N	0.56**	0.26	0.52**	0.65**	0.6**	0.11	0.05	0.03	0.16				
SLW	S	0.19	0.33*	0.16	0.28	0.26	0.18	0.43**	0.13	0.05	-0.31*			
	N	0.02	0.02	0	0.06	0.06	-0.2	0.08	0.06	-0.43*	0.01			
Pro	S	-0.17	-0.01	-0.2	-0.09	0.05	0.13	0.12	0.13	-0.07	-0.13	0.28		
	N	-0.04	-0.04	-0.03	-0.1	-0.06	-0.13	0.06	0.16	-0.34*	-0.1	0.12		
Na	S	0.36*	0.32*	0.37*	0.27	0.13	0.24	0.33*	-0.06	0.24	-0.27	0.05	0.21	
	N	-0.25	-0.16	-0.27	-0.18	-0.2	0	-0.09	-0.14	-0.14	-0.12	0.1	-0.01	
K	S	-0.14	-0.06	-0.19	-0.04	0.11	-0.06	-0.2	0.06	-0.06	0.03	-0.12	0.15	-0.32*
	N	-0.18	-0.2	-0.17	-0.22	-0.2	-0.07	0.07	-0.05	-0.26	-0.07	0.01	0.37*	0.133

*,**: Significant at the 5% and 1% levels respectively. N: Normal condition, S: Saline condition TDW: Total dry weight, SFW: Shoot fresh weight, SDW: Shoot dry weight, RFW: Root fresh weight, RDW: Root dry weight, RWC: Relative water content, RWL: Relative water loss, RL: root length, LA: Leaf Area, R/S: Root/Shoot ratio, SLW: Specific leaf weight, Pro: Proline content, Na: Sodium Content, K: Potassium content

Table 3: The results of stepwise regression analysis in which 12 out of 14 studied traits were selected for normal and saline condition.

Normal Condition				Salinity Condition			
SOV	df	MS	R ² Adjust	SOV	df	MS	R ² Adjust
Regression	3	1.732**	0.931	Regression	4	0.841**	0.813
Residual	41	0.009		Residual	40	0.017	
Total	44			Total	44		
	b±Sa		R ² Adjust		b±Sa		R ² Adjust
Constant	0.299*±0.122			Constant	-0.729*±0.35		
RFW	1.172**±0.132	0.914		RFW	1.36**±0.188	0.75	
SFW	0.051**±0.018	0.926		RL	0.04**±0.012	0.768	
Na	-0.004*±0.002	0.931		Na	0.007**±0.002	0.788	
				Prolin	-577.9**±223	0.813	

*, **: Significant at the 5% and 1% level s respectively.

SFW: Shoot fresh weight, RFW: Root fresh weight, RL: root length, , Pro: Prolin content, Na: Sodium Content,

condition no significant correlation is observed. In saline condition, Na content has positive significant relation with TDW, SFW, SDW and RWL and negative relation with K content, but in normal condition relations was not significant. The most significant difference between the two conditions was observed for the first R/S ratio, so that, this trait has negative significant relation with TDW, SFW, SDW, RFW, RDW, RWC, LA, RL, SLW in saline condition, but in normal condition correlation is positive and significant only in the TDW, RFW, SDW and RDW and is not significant in the other traits.

If the selection of genotypes is based on specific indices at the level of the whole plant, it will be more appropriate and reliable. Application of reliable traits for screening of genotypes can be effective in breeding process and production of resistant varieties (Ashraf and Harris, 2004). Step-wise regression analysis for TDW as dependent variable (Table 3) revealed that in normal condition, RFW, SFW and Na accounted for 93.1% of variation exist in TDW. Amongst, RFW and SFW accounted for 92.6% of total variation designated importance of these traits to explain variation of TDW. In return, in saline condition traits RFW, RL, Na and Pro accounted 81.3% of variation and amongst them, RFW, RL and Na content accounted of 78.8% of total

variation determined importance of these attributes to display variation of TDW. Na has negative and positive effect on TDW under normal and saline conditions, respectively. On the other hand, the effect of proline is also negative in saline condition.

CONCLUSION

In recent years, various agronomic traits have been considered regarding tolerance to salinity and since for screening genotypes, physiological and morphological traits are less affected by environmental factors. The result indicated that in saline condition, TDW, RFW, SDW, RL, R/S, SLW, proline and Na content were increased and SFW, RDW, LA and K content were decreased. On the other hand, RFW, SFW and Na content in normal condition and RFW, RL, Na content and proline in saline condition have the greatest impact on TDW. Na Content has negative and positive effect on TDW under normal and saline conditions, respectively. Therefore, it is suggested to consider different traits in breeding programs for normal and saline conditions.

ACKNOWLEDGEMENT

This work was supported by 1-Sugar Beet Seed Institute, Karaj, Iran. 2-The Center of Agriculture and Natural Resource of West-Azarbaijan Urmia, Iran. 3-Agricultural Research, Education and Extension organization (AREEo).

REFERENCES

- Abbas F., Mohanna A., Al-Lahham Gh. and AL-Jbawi E., (2012). Osmotic adjustment in *sugar beet* plant under salinity stress. *J. Sugar beet*, **28**(1):37- 43.
- Abdollahian-Noghabi M., (1999). Ecophysiology of *sugar beet* Cultivars and weed species subjected to water deficiency stress. Ph.D. Dissertation, University of Reading, Reading.
- Akhtar S.S., Andersen M.N., Naveed M., Zahir Z.A. and Liu F. (2015). Interactive effect of biochar and plant growth-promoting bacterial endophytes on ameliorating salinity stress in maize. *Functional Plant Bio.*, **42**(8):770-781.
- A.O.A.C., (2000). Association of Official Analytical Chemistry Official Methods of Analysis. 17th. Ed, Washington, DC USA. **2**(44):1- 43.
- Ashraf M. and Foolad M.R., (2007). Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Env. Exp. Bot.*, **59**:206-216.
- Ashraf M. and Harris P.J.C., (2004). Potential biochemical indicators of salinity tolerance in plants. *Plant Sci.*, **166**:3-16.
- Ashraf M. and McNeilly T. (2004). Salinity tolerance in *Brassica* oilseeds. *Crit. Rev. Plant Sci.*, **23**:157-174.
- Barrs H.D. and Weatherly P.E., (1962). A re-examination of the relative turgidity technique for estimating water deficit in leaves. *Australian J. Bio. Sci.*, **15**:413-428.
- Bates I.S., Waldern R.P. and Teare I.D., (1973). Rapid determination of free proline for water stress studies. *Plant and Soil*, **39**:205-207.

- Cooke D.A. and Scott R.K., (1993). The *sugar beet* crop. Chapman and Hall London, pp. 262-265. Dadkhah A., and Griffiths G., (2004). Stomatal and nonstomatal components to inhibition of photosynthesis in leaves of *sugar beet* plants under salt stress. *Iranian Agric. Res.*, **23**:35-50.
- De Herralde F., Biel C., Save R., Morales M.A., Torrecillas A. and Alarcon J.J. (1998). Effect of water and salt stresses on the growth, gas exchange and water relations in *Argyranthemum Coronopiflium* plants. *Crop Sci.*, **139**:9-17.
- Dubey R.S., (1994). Protein synthesis by plants under stressful conditions. In: Handbook of Plant and Crop Stress, [Pessaraki, M. (ED)] Marcel Dekker, New York, pp: 277-299.
- FAO AGL. (2000). Land and plant nutrition management service: Global network on integrated soil management for sustainable use of salt affected soils. <http://www.fao.org/ag/agl/agll/spush>.
- Farkhondeh R., Nabizadeh E. and Jalilnezhad N., (2012). Effect of salinity stress on proline content, membrane stability and water relations in two *sugar beet* cultivars. *Int. J. Agric. Sci.*, **2**(5):385-392.
- Huang Y., Bie Z., Liu Z., Zhen A. and Wang. W., (2009). Protective role of proline against salt stress is partially related to the improvement of water status and peroxidase enzyme activity in cucumber. *Soil Sci. and Plant Nut.*, **55**:698-704.
- Jamil M., Shafiqand R. and Rha E.S., (2007). Salinity effect on plant growth, PSII photochemistry and chlorophyll content in *sugar beet* (*Beta vulgaris* L.) and cabbage (*Brassica oleraceacapitata* L.). *Pakistan J. Bot.*, **39**(3):753-760.
- Kehl M., (2006). Saline soils of Iran with example from the alluvial plain of Korbal, Zagros Mountains. Proceeding of the international conference. Soil and Desertification- Integrated research for the sustainable management of soils in dry lands. Germant.www.desertnet.de/proceedings/start.htm
- Lakshmi A., Ramanjulu S., Veeranjanyulu K. and Sudhakar C., (1996). Effect of NaCl on photosynthesis parameters in two cultivars of mulberry. *Photosynthetica*, **32**(2):285-289.
- Malik A.A., Li W.G., Lou L.N., Weng J.H. and Chen J. F., (2010). Biochemical/physiological characterization and evaluation of in vitro salt tolerance in cucumber. *African J. Biotech.*, **9**(22):3284-3292.
- Mekki, B.B. and El-Gazzar M.M., (1999). Response of root yield and quality of *sugar beet* (*Beta vulgaris*, L.) to irrigation with saline water and foliar potassium fertilization. *Annals agric. Sci. Ain Shams Univ., Cairo*, **44**(1):213-225.
- Mengel K. and Kirkby E.A., (1980). Potassuim in crop production. *Adv. Agron.*, **33**:59-110.
- Moaveni P., Ranji Z. and Noor-Mohammadi G.H., (2004). Study of some physiological parameters and organic composition for salt tolerant and sensitive genotypes of *sugar beet*. *Iranian J. of Crop Sci.*, **6**(1):12-24.
- Munns R. (2002). Comparative physiology of salt and water stress. *Plant, Cell and Env.*, **25**:239-250.
- Morant-Manceau A., Pardir E. and Tremblin G., (2004). Osmotic adjustment, gas exchange and chlorophyll fluorescence of a hexaploid triticale and its parental species under salt stress, *J. Plant Physio.*, **161**:25-33.
- Narendra K., Roy, Ashwani, Srivastava K., Sharma S.G. and Singh A.K., (2003). Influence of Salinity on sodium, potassium and proline content in wheat (*Triticum aestivum* L.) leaves and its mitigation through presoaking treatments. *Indian. Jan J. Agric. Res.*, **37** (2):128 - 131.
- Ober E.S., Bloa M.L., Clark C.J.A., Royal A., Jaggard K.W. and Pidgon J.D., (2005). Evaluation of physiological traits as indirect selection criteria for drought tolerance in *sugar beet*. *Field Crops Res.*, **91**:231-249.
- Pakniyat H. and Armion M., (2007). Sodium and praline accumulation as osmoregulators in tolerance of *sugar beet* genotypes to salinity. *Pakistan J. Bot.*, **10**(22):4081-4086.
- Rains D.W. and Goyal S.S., (2003). Strategies for managing crop production in saline environments: An overview In: [Goyal S.S., Sharma S.K. and Rains D.W.,] Crop Production in Saline Environments. *The Food Products Press*. 1-10.
- Reda K.A., Shalaby A.A., KishkH. T. and Hegazi A.M., (1980). Some effects of potassium on growth yield and chemical composition of beet irrigated with saline water containing different levels of boron. *Ain Shams Univ., Fac. Agric., Res. Bull.* **12337**: 16 pp.
- Seaman J., (2007). Mechanisms of salt tolerance in halophytes: Can crop plant resistance to salinity be improved. APS 402 Dissertation. Candidate no: 000124971. 1-11.
- Shehata M.M., (1989). Physiological studies on the tolerance of some *sugar beet* varieties to salinity. Ph.D. Thesis, Fac. Agric., Cairo Univ., Egypt.
- Shehata M.M., Shohair A. A. and MostafaS.N., (2000).The effect of soil moisture level on four *sugar beet* varieties. *Egypt. J. Agric. Res.*, **78**(3):1141-1160.
- Yang R.C., Jana S. and Clarke J.M., (1991). Phenotypic diversity and associations of some potentially droughtresponsive characters of durum wheat. *Crop Sci.*, **31**:1484-1491.
- Witkowski E.T.F. and Lamont B.B., (1991). Leaf specific mass confounds leaf density and thickness. *Oecologia*, **88**:486- 490.