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

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**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

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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 flask, 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-ported 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.

\[
RWC = \left[ \frac{\text{fresh weight-dry weight}}{\text{total weight-dry weight}} \right] \times 100
\]

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:

\[
\text{RWL} = \left[ \frac{\text{fresh weight-wilt weight}}{\text{dry weight}} \right] \times \left[ \frac{\text{time to wilt}}{\text{time to dry}} \right] / 60
\]

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

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

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 DISCUSSION

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 abiotic 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 acommodation 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.
restriction of food transmission, reduce the proliferation and elongation of cells, thereby reducing the growth and development of leaf cells, reducing fresh and dry weight of leaves (Dadkhah and Griffiths, 2004).

Salinity and Leaf water content: RWC and RWL decreased in saline condition and this decrease was higher in the RWL (Table-1). Farkhondeh et al., (2012) showed that with increasing salt concentration, the RWC in sugar beet decreased. These results were conformed to the findings of Mouveni et al., (2004). In contrast, Jamil et al., 2007 reported that with increasing salinity there is no change in the RWC. Reduction in traits is due to the physiological dryness caused by salinity stress, the decrease in turgor pressure, and thus the limited water use efficiency for cell development processes. On the other hand, high salinity has prevented rooted development, which has reduced the capacity of water absorption by the plant.

Salinity and leaf ion concentration: Salinity significantly increased the amount of proline and Na content and significantly decreased K content (Table-1). Narendra et al., (2003) reported that salinity increases proline and Na content and in return, decreases K content in wheat leaves, proline and Na content increased by about 38% and 73% respectively but decline in K content was close to 19%. Increased Na content and proline have also been reported by Pakniyat and Armion (2007), Shehata (1989), Abbas et al., (2012) and Mekki and El-Gazzar (1999). They found that the salinity had a positive effect on Na concentration of sugar beet. The increase in Na accumulation under salt stress may be due to increased uptake, reduced the translocation or to disproportion changes in growth and uptake.

Proline acts as an osmotic regulator between the cytoplasm and the vacuole and, by performing osmotic regulation, protects the membrane against the reactive oxygen species and fixes the antioxidant enzymes (Huang et al., 2009). It also protects the structure of the cell through osmotic regulation (Malik et al., 2010) and is effective in accelerating the redox cell retrieval potential (Ashraf and Foolad, 2007). These makes proline act as a compatible substance and works to enhance the plant’s adaptation under stress condition.

Increasing the salt concentration in growth media resulted in reducing K uptake by sugar beet plants (Shehata et al., 2000) and in turn, K content in shoots (Reda et al., 1980). It has been frequently reported in the literature that K has an effect on the water uptake, turgor pressure and water relation associated with the stomatal opening (Mengel and Kirkby, 1980). Na can substitute for K in sugar beets (Cooke and Scott, 1993). These results are in agreement with those obtained by.

**Correlation and regression analysis:** For indirect traits such as physiological traits to be effective in screening
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** |
| N | 0.02 | 0.02 | 0 | 0.06 | 0.06 | -0.2 | 0.08 | 0.06 | 0.43 | 0.13 | 0.31 |
| S | -0.17 | -0.01 | -0.2 | -0.09 | 0.05 | 0.13 | 0.12 | 0.13 | -0.07 | -0.13 | 0.28 |
| **K** | N | -0.18 | -0.02 | 0.17 | -0.22 | -0.2 | -0.07 | 0.07 | -0.05 | 0.26 | 0.70 |
| S | -0.14 | -0.06 | -0.19 | -0.04 | 0.11 | -0.06 | -0.2 | 0.06 | -0.06 | 0.03 | -0.12 |
| **Na** | N | -0.25 | -0.16 | -0.27 | -0.18 | -0.2 | 0 | -0.09 | -0.14 | -0.14 | 0.1 |
| S | 0.36 | 0.32 | 0.37 | 0.27 | 0.13 | 0.24 | 0.33 | -0.06 | 0.24 | -0.27 | 0.05 |
| **R/S** | N | 0.56 | 0.26 | 0.52 | 0.65 | 0.6 | 0.11 | 0.05 | 0.03 | 0.16 |
| S | -0.54 | -0.82 | -0.43 | -0.48 | -0.72 | -0.49 | -0.27 | -0.38 | -0.69 |
| **SLW** | N | 0.19 | 0.16 | 0.28 | 0.26 | 0.18 | 0.43 | 0.13 | 0.05 | -0.31 |
| S | 0.19 | 0.28 | 0.23 | 0.24 | 0.25 | -0.22 | 0.24 | 0.23 | 0.24 | -0.22 |
| **RWC** | N | -0.01 | 0.002 | -0.01 | 0.03 | 0 | 0.16 | 0.25 | 0.16 | 0.24 | 0.25 | -0.22 |
| S | 0.27 | 0.46 | 0.22 | 0.29 | 0.34 | 0.07 | 0.08 | 0.03 | -0.13 | 0.11 |
| **RL** | N | 0.24 | 0.28 | 0.23 | 0.24 | 0.25 | -0.22 | 0.32 | 0.35 | 0.32 | 0.32 | 0.32 |
| S | 0.09 | 0.9 | 0.9 | 0.9 | 0.9 | 0.9 | 0.9 | 0.9 | 0.9 | 0.9 | 0.9 | 0.9 |
| **RFW** | N | 0.07 | 0.08 | 0.94 | 0.88 | 0.84 | 0.83 | 0.87 | 0.99 | 0.96 | 0.96 | 0.96 |
| S | 0.62 | 0.77 | 0.45 | 0.6 | 0.62 | 0.77 | 0.45 | 0.6 | 0.62 | 0.77 | 0.45 |
| **RDW** | N | 0.96 | 0.85 | 0.91 | 0.94 | 0.96 | 0.85 | 0.91 | 0.94 | 0.96 | 0.85 |
| S | 0.98 | 0.71 | 0.9 | 0.9 | 0.9 | 0.71 | 0.9 | 0.9 | 0.9 | 0.71 | 0.9 | 0.9 |
| **SFW** | N | 0.8 | 0.9 | 0.9 | 0.9 | 0.9 | 0.9 | 0.9 | 0.9 | 0.9 | 0.9 | 0.9 |
| S | 0.87 | 0.83 | 0.84 | 0.83 | 0.84 | 0.83 | 0.84 | 0.83 | 0.84 | 0.83 | 0.84 |

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

**Notes:** 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 lose, **RL:** root length, **LA:** Leaf Area, **R/S:** Root/Shoot ratio, **SLW:** Specific leaf weight, **Pro:** Prolin content, **Na:** Sodium content, **K:** Potassium content

**References:**

Ober et al., 2005. (Note: The reference should be properly formatted and linked if available.)

Ashraf and Harris, 2004. (Note: The reference should be properly formatted and linked if available.)
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.

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### 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          | R² Adjust  | SOV  | df  | MS          | R² 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  |            |            |
| Constant             | b±S₄ | R² Adjust | -0.729±0.35 |            | b±S₄ | R² Adjust | 0.577.9±223 | 0.813      |
| RFW                  | 1.172**±0.132 | 0.914 |            | RFW       | 1.36**±0.188 | 0.75 |            |
| SFW                  | 0.051**±0.018 | 0.926 |            | RL        | 0.047**±0.012 | 0.768 |            |
| Na                   | -0.0044±0.002 | 0.931 |            | Na        | 0.007**±0.002 | 0.788 |            |

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

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