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Salinity Stress Effect on Proline and Chlorophyll Rate in Four Beet Cultivars

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ABSTRACT

Sensitivity to soil salinity during beet seed germination is among the main tackles for plant deployment in fields. To study this, a research was conducted in greenhouse in a factorial experiment based on randomized complete blocks design with three replications. The first factor included stress levels and the second factor included genotypes. To apply salinity stress sodium chloride 16 dS/m was used. Seeds were planted in plastic pots in greenhouse conditions and plants irrigation was done by Hoagland nutrient solution. The proline and chlorophyll of shoots were measured by the end of growth stage. Results suggested that genotype No. 4 with a mean of 19.25 had the highest rate of chlorophyll and genotype No. 1 with mean of 13.25 had the lowest chlorophyll rate. Also, genotype No. 3 with a mean of 5.86 had the highest proline rate. Results indicated that salinity stress application could decrease chlorophyll rate while it increases proline rate. Decrease in plant chlorophyll decreases the photosynthetic activity. Increase in proline along with increase in salinity level specifies the osmotic balance maintenance in low water potential. Results generally showed that increase in proline production as an osmotic regulatory mechanism in high salinity levels which decreases the seedling growth.

Keywords: salinity stress, chlorophyll, proline, beet

INTRODUCTION

Increase in the world population has added to the significance of considering the reduction of freshwater resources, agricultural lands salinization and the feasibility of tolerant plants in unfavorable environmental condition. Seed germination and primary seeding growth are among the most sensitive stages to environmental stresses among most crops [1]. World and Iran saline lands are expanding due to the excessive agricultural activities [2]. Hence, the potential production of crops in this condition is not possible. To cope with this issue, identifying and selecting more tolerant cultivars seems to be of significance [3]. Salinity stress does not only affect one growth stage, but it could affect the plant differently considering the stress intensity, stress intensity type, plant tolerance, various growth stages, tissue type and plant organ (development) [4]. Various researches have been conducted assessing beet resistance towards salinity in vitro and also germination and complete growth in vivo, so far [5,6,7]. Beet is high resistive towards salinity at cellular level and in complete plant form. Therefore, cellular tolerance and complete plant conditions are completely compatible [8].

Presence of any type of salt in the plant growth environment leads to increase in osmotic pressure and water stress. However, salts' toxicity is different. Although chloride sodium is known as a low toxicity salt, it is among the most

common salts and as a result one of the most problematic salts [9]. Increase in salinity level increases the osmotic regulator amount (proline) which could result in plant tolerance towards environmental stress [10]. Two paths could be effective in producing proline. One is to use glutamate and the other is to use ornithine as leading paths in plants [11]. The effect of increase in producing proline on resisting drought and salinity stresses is controversial and in addition to increase in proline synthesis, decrease in proline catabolism could be related to its accumulation in low water potential [12].

MATERIALS AND METHODS

This research was conducted in Ardabil IAU laboratory, in 2012. Materials used in this research were provided from Beet Seed Modification and Preparation Institute in Karaj. Genotypes used in this research are presented in table 1. To study this, the research was conducted in a factorial experiment based on randomized complete blocks design with three replications. The first factor included stress levels and the second factor included genotypes. To apply salinity stress sodium chloride 16 dS/m was used. For sampling, leaves were used and they were put in aluminum layers. They were immediately frozen by liquid nitrogen. After pounding them, they were put in freezer at -20 °C. Then the sample was died and pounded in a pounder for 48 hours at 75 °C. The sample was transformed into white ash at 550 °C in the oven during 5 hours. The following laboratory measurements were conducted as the followings. To record the chlorophyll rate, sampling was done after 50 days of stress from 4 to 7 leaf leaves and during the sampling, plants were at 8 to 10 leaf stage. Sampling was done on one stage growth leaves, for their growth changes are slow. This was conducted manually by manual chlorophyll meter machine.

Leaves proline was measured by Bates et al [13]. modified method. 0.5 gr of leaf sample which was covered in aluminum sheets and were put in liquid nitrogen at -80 °C, was pounded in pounder and homogenized by sulfosalicylic acid (3 percent) Homogenized solutions were centrifuged for 20 minutes at 4 °C in 5000 rmp, by refrigerated centrifuge KUBOTA 6900 (Made in Japan). Subsequently, Whatman filter paper No. 2 was used for filtering the centrifuged samples. 2 ml glacial acetic acid and 2 ml ninhydrin reagent were added to 2 ml of derived supernatant. Reaction solution was boiled at 100 °C (thermae) for one hour. Subsequently, the solution was put in ice container for 30 minutes. 4 ml toluene was added to the solution and vertexed for 20 seconds. During turbulence, chromophore containing toluene was separated and absorption around 520 Nm was recorded in spectrophotometer, comparing to the control solution containing toluene. Proline density was determined by drawing the standard curve. To reset the spectrophotometer, control solution which contained all materials except leaf sample was used, using the proline standards which are provided by the same method, standard curve was calculated. Finally, the leaf proline was calculated based on concentration and sample weight was calculated based on mg in sample wet weight. Statistical calculations were conducted by SPSS-16 and MSTAT-C software. Diagrams and tables were drawn by Word and Excel software.

Table 1- Name of Genotypes used in This Research

NO	Genotype
1	1-30881-88
2	2-31268-89
3	3-31290
5	4-7233-P29

RESULTS AND DISCUSSION

ANOVA results on studied traits (Table 2) suggested that there is a significant difference found between salinity stress levels on both studied traits at 1percent. so that, in both chlorophyll trait and proline using sodium chloride 16 dS/m decreased chlorophyll and proline. As it could be observed in Table 3, sodium chloride led into 23.84 percent decrease in chlorophyll and 22.95 percent increase in proline in plant. Various adjustments are applied to preserve turgescence in plants affected by salinity. Proline is the most effective osmotic regulator substance in plants affected by salinity [14]. Results indicated that there is a significant difference between genotypes on both traits at 5percent and 1percent. However, the interaction between these two traits was not significant (Table 2). Considering the genotypes mean comparison on chlorophyll meter (Figure 1), genotype No. 4 with a mean of 19.28 had the highest value and formed class A along with genotypes No. 2 and No. 3. Genotype No. 1 with a mean of 13.74 had the lowest value. Also, genotype No. 3 with a mean of 5.86 had the highest proline value and formed class A along with genotypes No. 1 and No. 2. Genotype No. 4 with a mean of 3.66 had the lowest proline (Figure 2).

It could be concluded that increase in proline production as an osmotic regulatory mechanism could lead to decrease in plant growth. This could imply a high cost for preserving plants to provide a better growth environment in plants affected by salinity.

Table 2- Studied Traits ANOVA

Source of Variations	df	Mean Square	
		Chlorophyll	Proline
rep		12.088	2.543
Stress Levels		118.37**	10.062**
Genotype		31.352*	5.298**
S * G		8.915ns	0.824ns
Error		7.252	0.648
CV (%)		16.41	16.13
* and ** Significantly at p < 0.05 and < 0.01, respectively and ns No significant difference			

Table 3- Mean Comparison Table for Various Salinity levels on Studied Traits

Stress Levels	Characters	
	Chlorophyll	Proline
water (control)	18.63	4.35
Sodium chloride 16 dS m	14.19	5.64
Reduction Percent	23.84 %	-22.95 %

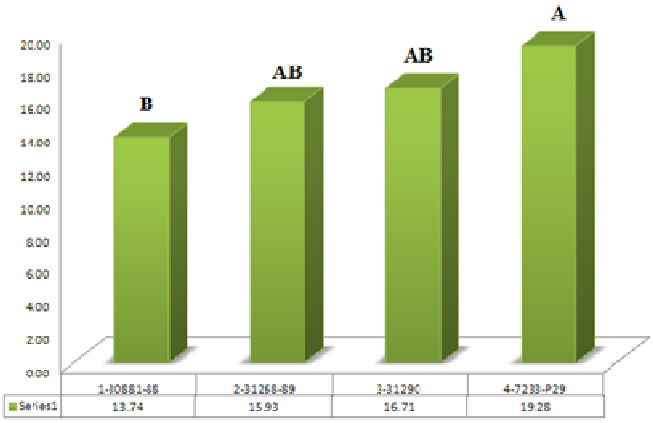

Figure 1- Mean Comparison on Studied Genotypes for Chlorophyll Meter

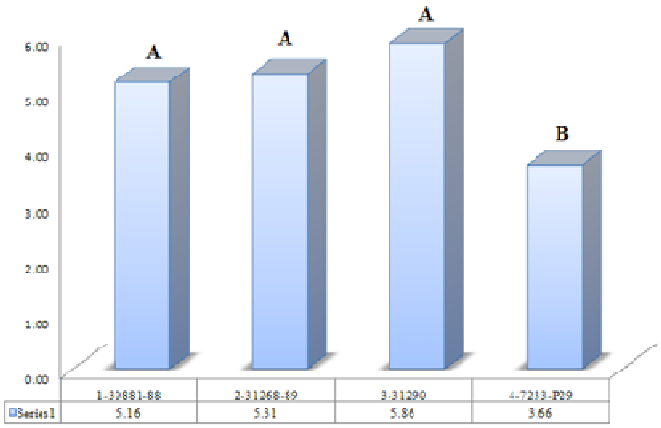

Figure 2- Mean Comparison on Studied Genotypes for Proline

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