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Effect of salinity stress levels on amount of proline of three factors of rape seed

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ABSTRACT

Rape seed (Brassica napus L.) is the third important oil plant in the world. The sensitivity to salinity at the germination stage is known as the main obstacle of desirable establishment of the plant in the fields. To this aim an experiment in a greenhouse in a random factorial block design was done. The experiment was repeated three times. The first stress factor was in the five levels (1- Control, 2- 50 mM, 3- 75 mM, 4- 100 mM and 5- 150 mM) and the second genotypes factor included (1: Talaye 2: Okoti and 3: Tasiv). At the end of the growth stage, the Proline of air organs was measured. Results showed that the amount of proline of Okoti genotype with the average of 0.7367 gets the maximum value. The results showed that salinity stress increases the proline. The increase of proline with salinity increase shows the importance of the osmotic balance in low water potential condition. In general the results of the experiments showed that increase in production of proline - as an osmotic regulatory mechanism for survival in high salinity levels- can lead to reduced growth of the seedling.

Keywords: salinity stress, chlorophyll, proline, Rape seed

INTRODUCTION

Rape seed (*Brassica napus* L.) is one of the important oilseeds in Iran and the world. And its ability of different varieties to germinate and grow at low temperatures has led to the development of its cultivation in cold regions of the world. It has more than 40 percent oil and Konjale and is full of protein and is known as one of the richest seeds of oil in the world in recent decades. The cultivation of it has been increased from 8.2 million hectares in 1970 to more than 30.2 million hectares in 2007 worldwide (FAO, 2007).

The plants are dealing with a variety of biological and non-biological stresses and tensions, and among all non-biological stresses, the salinity is very damaging to the plants and sharply reduces the plants productivity (Hameed et al, 2008). The increase in world population, the reduction of fresh water and salinity of lands has made it inevitable to investigate the probability of producing plants resistant to inappropriate environmental situations. In many crops, seed germination and seedling early growth stages are from the most sensitive stages to environmental stresses (Cook 1979). Saline lands in the world due to indiscriminate activities and irregular agriculture are continuously expanding and growing (Haghnia, 1371).

Thus, the potential production of agricultural products is not possible in these conditions. To deal with this problem is necessary to identify and select the most tolerant cultivars (Hall, 2001). The salinity effects plant not only on its growth stage, but due to the stress type, intensity of stress, the resistance of plants and plant tissues and developmental stages (evolution) is different (Mass and Hoffman, 1997). Presence of any kinds of salt in growth environment increases osmotic pressure and water stress but salts toxicity are different in the process. Despite of the fact that sodium chloride is known as a salt with less toxic; however, it is one of the most common types of salts and consequently it is considered one of the most harmful salts (Bliss et al. 1984).

The salinity, by increasing the production of free radicals, changes metabolic processes and enzyme activities (Hameed et al, 2008). In order to neutralize the toxicity of active oxygen species, there are effective defenses antioxidants in plant cells that prevent the formation of free radicals, or remove them. Of this antioxidant we can mention super oxide dismutase, proxitaz and catalase (Blokhina et al, 2003). Some experiments showed that the amount of proline under salt stress is influenced by plant hormones (Yusef et al, 2008). It is found that one way of plant adaptation to environmental stresses such as salinity is proline accumulation (Hameed et al, 2008), which is considered as a protective esmolit (Jain et al 2001).

Changing the amount of proline is one of the most common phenomena have been reported which is induced by salt stress and is involved in stress tolerance mechanisms (Lutts et al, 1999). Proline reduces the increase of oxygenize robisco activity under salt stress (Srivastava et al, 2008). With increase of levels of salinity, the amount of osmosic regulators is added which can cause the plant tolerance to environmental stresses (Gzik, 1996). Two ways may be effective in proline making process in plants, one of which is making use of glutamate and the other is using ornitine (Delauney and Verma, 1993).

The effects of increase in proline production on resistance to drought and salinity are still controversial, and beside the increases of proline synthesis, a proline catabolism reduction can be attributed to its accumulation at low water potential (Blum et al., 1996).

MATERIALS AND METHODS

The experiment was conducted in 1391 in the laboratory of Islamic Azad University of Ardabil branch. The used plants were obtained from the institute of modification and production of sugar beet seeds of Karaj. The used experiment was in a random factorial block design. The experiment was repeated three times. The first stress factor was in the five levels (1- Control, 2- 50 mM, 3- 75 mM, 4- 100 mM and 5- 150 mM) and the second genotypes factor included (1: Talaye 2: Okoti, 3: Tasiv). For sampling we used leaves, so that samples were wrapped in aluminum and immediately frozen by liquid nitrogen and after powdering it was transferred to a freezer of minus twenty degrees. Laboratory measurements were done as follows. Leaf proline was determined by modified method of Bates et al (1973).

The 0.5 g leaf samples after sampling was wrapped in aluminum paper and was put into liquid nitrogen and had been kept at the temperature of minus 80, and was grounded inside a ground pounder and was homogenized with ten ml solfosalisilik (3%) acid. Homogeneous solution at a temperature of four degrees Celsius, and 5000 rpm for 20 min by rpm Model 6900 Made in Japan was centrifuged, and then for KUBOTA purification, the Whitman filter paper number two was used.

About 2 ml of the supernatant 2 ml of Glacial acetic acid and 2 ml morafe nin reaction mixture at 100 ° C (water bath) for one hour. Hidrin was added and boiled then immediately placed on ice for 30 min and then 4 ml of toluene were added and vortexes for 20 seconds. During the mixing, the kromofer of toluene was separated and was absorbed at 520 nm was measured in spectrophotometer compared with control containing toluene. Proline density was determined using the standard curve.

To set the spectrophotometer in zero the control sample containing all ingredients except leaf samples were used and Utilizing the proline standards which were prepared using the same method, a standard curve was obtained based on the degree of dilution of the leaf and test weight mg wet weight of the sample proline was calculated. Statistical analysis was done using SPSS-16 and MSTAT-C software. Drawing graphs and statistical tables were performed by Excel and Word software.

RESULTS AND DISCUSSION

Analysis of variance of evaluated properties showed (Table 2) that there was no significant difference in levels of salinity. Despite being the fact that levels of salinity are meaningless the amount of proline when using 150 mM sodium chloride had the highest amount of proline. As shown in Table 3, sodium chloride increased proline about 8% in plants. Several adjustments to maintain torghsans in plants under salinity tension are involved. Praline is the most effective regulator of osmotic stress in plants under salinity (Chandler and Thorpe, 1987).

The results showed that the interaction between genotype and mutual effects of stress levels × genotype there are meaning full differences in the levels of 5% probability can be seen (Table 2). Also considering the examined proline, the Okoti genotype with the mean value 0.7367 gets the maximum amount, and is allocated to the Class A and tala genotype with the average number of genotypes 6793/0 was found with the lowest proline (Fig. 1). In general it can be concluded that the increase in production of proline as an osmotic regulatory mechanism would reduce plant growth. This implies that higher maintenance requirements for plant survival under salinity stress conditions are required

Table 2- Analysis of variance for different levels of salt stress on proline

S.O.V	df	proline
Salinity levels	4	0.009
Genotypes	2	0.016*
Genotype × salinity levels	8	0.012*
Error	30	0.006
CV%		10.45%

** Significantly at $p < 0.01$, respectively*

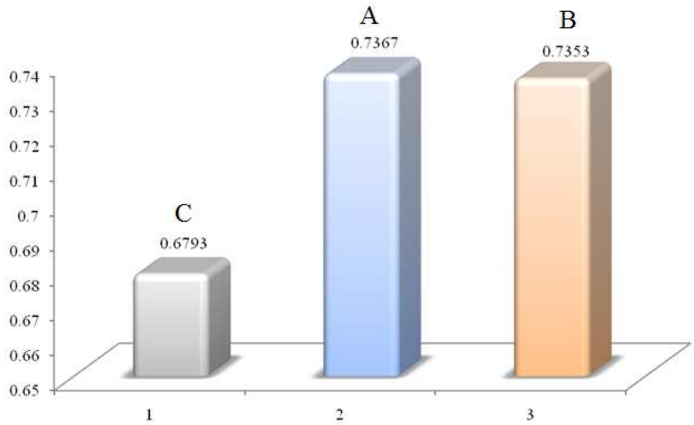

Figure 1 - Comparison mean valueof evaluated genotypes for proline
(1: Talaye 2: Okoti and 3: Tasiv)

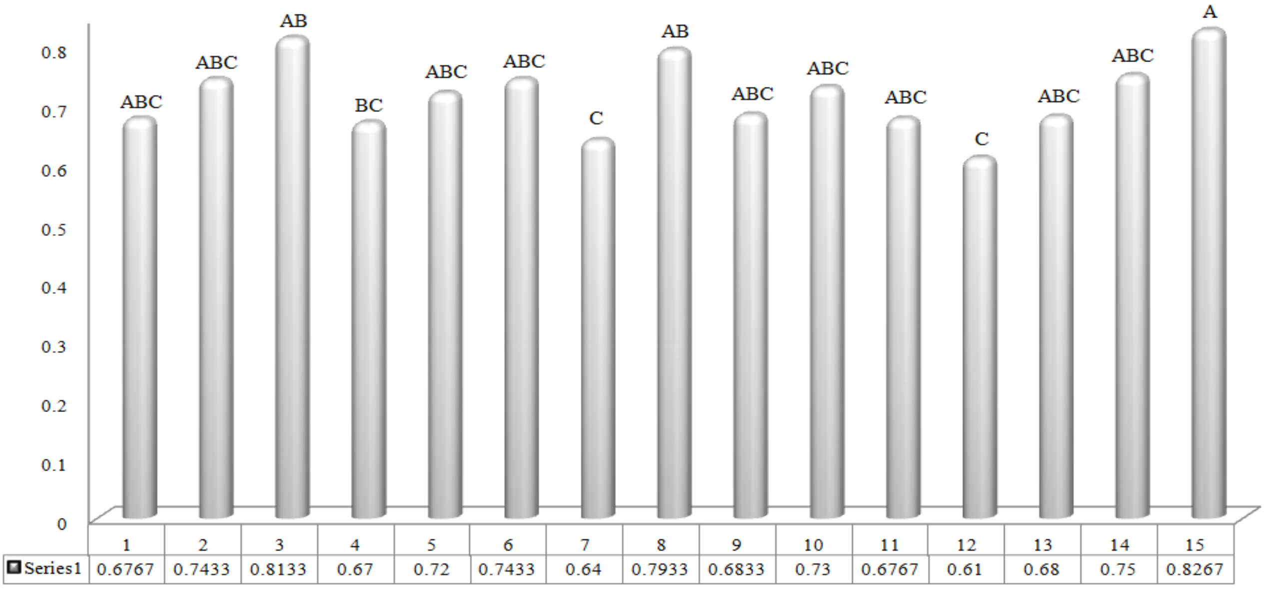

Figure 2 – Comparison of mean value mutual effects of salinity levels × evaluated genotypes for proline

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