Comparing the effects of SNP and SA under salinity stress on proline, sugar, Na, K and chlorophyll of leaves of Pinus eldarica and Cupressus sempervirens in Iran

Zamani1, M., Hakimi2, M.H, Mosleh Arany3, A., Kiani2, B. and Rashtian2, A.
1- Forestry M.Sc. student, Faculty of Natural resources, Yazd University
2- Assistance. Prof. Faculty of Natural resources, Yazd University
3- Associate Prof. Faculty of Natural resources, Yazd University

ABSTRACT
The study aimed to evaluate the effects of salinity stress on proline, soluble sugar, the absorption of Na and K, chlorophyll of leaves of Pinus eldarica and Cupressus sempervirens. For this aim, the factorial experiment was randomly designed in three replicates and different salinity treatments (0, 5, 10, 20 ds/m) with Sodium nitroproside (SNP) and salicylic acid (SA). Results showed the amount of proline significantly increased in concentration of 20 ds/m in both species. Applying SA increased the amount of proline three times more than control in dose 20 ds/m. Increasing salinity resulted in an increase of leaf sugar and using SNP in 20 ds/m caused a reduction of soluble sugar. Subsequently, increase of salinity resulted in a decrease of Na and K of leaves. Moreover, applying SNP in 5 ds/m resulted in an augmentation in chlorophyll of both species.

Key words: Salinity stress, Cupressus sempervirens, Pinus eldarica, SA, SNP

INTRODUCTION
Various stresses particularly salinity stress is the main factor that seriously constrains agricultural production and yield in different regions especially in arid and semi-arid areas [10]. Plants use different ways in order to reduce salinity stresses. Increasing adaptive osmolyte in different organs of plants is one of the certain solution responses to salinity stress. The adaptive osmolyte, such as proline and glycine betaine amino acids as well as soluble sugars, control actions like regulating osmotic acts, protecting intercellular structure and reducing oxidative damages producing free radicals respond drought and salinity stresses [11]. Proline is the most popular solution that widely has various application associated with drought and salinity [27]. The salinity study on physiological characteristics of Sorghum bicolor and Sorghum sudanense indicated there was an increase of sugar up to 118 percent for Sorghum bicolor, although there was not found any increase for Sorghum sudanense. There was found an increase of proline up to 34 percent for S. bicolor. Whoever, this value acted a reduction of 82 percent for S.sudanense compared with control [13]. Moreover, salinity stress results in an reduction of chlorophyll [4]. Reducing chlorophyll concentration is an important and efficient agent in photosynthesis capacity and also increasing saline level causes accelerate the downsides of salinity stresses. Hence, reduction of growing features is associated to a fall in photosynthesis rate. The study of the oxidative stress of corn showed that increasing salinity causes a reduction of chlorophyll in leaves [8]. The leaf contents have a significant correlation with leaf water, so plants have an appropriate mechanism face stresses such as salinity. For example in high salinity concentration the stoma are closed and this value can make drought stress and results in an adverse condition for plants [9]. The salinity effects on physiological and morphological characteristics of grape showed that increasing salinity significantly decrease leaf water and chlorophyll index.

Sodium as a soluble ion is in a large number of desert soils that lots of this ion would accumulate in under ground organs especially roots and the little amount of it can transfer to aerial organs during long-term stress in plants. Potassium is another important ion in salinity stresses that has a fundamental role in
osmotic regulation and stomata opening and closing. The potassium concentration in organs of plant can be increased by salinity stresses. In the condition with a large number of ions in root area, potassium and sodium play a similar role in osmotic stress that adjusts plant growth [16]. A study carried out on pepper indicated that increasing salinity has an essential function to reduce and plant height and potassium. Salicylic acid is one of the useful compounds for plants that plays an important role in the plant resistance to environmental stresses such as salinity. It can be classified among plant growth regulators. Therefore, ortho-hydroxy benzoic acid and salicylic acid are endogenous growth regulators of plant that have a suitable role in physiological processes [25]. The effect of salicylic acid on resistance and oxidative role on green basil showed that the Na in treatment SA and salinity 100 and 200 mM would be decreased significantly that this value indicates adjusting salinity with salicylic acid [12]. Sodium nitroprusside(SNP) is used as a releasing compound of nitric oxide. Many studies have shown that this compound can protect plant under oxidative stresses and maintain chlorophyll [26] SNP could improve the effects of salinity and increased chlorophyll in cotton [24]. *Pinuseldarica* and *Cupressus sempervirens* are the coniferous species that are widely planted in various parts of Iran especially in arid and semiarid areas. On other hand, there is no comprehensive information about the different stresses on the spices. Therefore, the study aimed to evaluate the effects of SNP and SA under salinity stress on proline, sugar, sodium, potassium and chlorophyll of *Pinuseldarica* and *cupressus sempervirens*.

**MATERIALS AND METHODS**

The study was conducted in 2011 in a greenhouse of Yazd city, center of Iran. One yearold seedlings of *Pinuseldarica* and *cupressus sempervirens* were selected from nursery of Yazd city with same weather condition. The seedlings were used in the treatments of 0, 5, 10and20dS/m sodium chloride in the solution of a day. To produce salinity 5, 3.2 gr salt in one litter water were solved and also 8 and 16 gr salt in one litter water were solved for salinity 10 and 20, respectively. To produce SNP, 150 mg SNP were solved in one liter water and also 160 mg SA first was solved in ethanol after that the solution of water up to one liter. Working on treatments lasted 2 months. Root and leaf proline based on bates methods [5] and the amount of leaf and root sugar were measured based on Kochret, 1987. Sodium and potassium with phylum photometer and below equation was used for RWC.

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\text{RWC} \% = \frac{\text{wet weight} - \text{dry weight}}{\text{inflammation weight} - \text{dry weight}} \times 100
\]

To measure wet and dry weight of root and stem, Sartarius scale (BP211) was selected. SPSS20, Duncan, dant and Excel were used to analyze data, mean comparison, compare treatments with control and draw graphs.

**RESULTS**

Increasing salinity resulted in a remarkable increase of proline in both *Pinuseldarica* and *Cupressus sempervirens* in concentration of 20 ds/m. Using SA caused to increase proline rather than control several times. With increasing salinity, leaf sugar would increase in all concentrations. Increasing salinity was occurred by applying SA in concentration of 20 and using SNP in 10 ds/m increased sugar. In addition, using SA and SNP resulted in a decrease of sodium and potassium in concentration of 20 and 10 ds/m, respectively, in both *Pinuseldarica* and *Cupressus sempervirens*. Increasing salinity resulted in a reduction of chlorophyll, while SNP in concentration of 5 ds/m caused to increase chlorophyll in both species.

![Figure 1: Leaf proline of *Pinuseldarica* and *Cupressus sempervirens*](image)
DISCUSSION
During salinity and drought stresses transforming materials due to reduced water availability leads to change in the concentrations of some metabolites. On the other hand, the rate of adaptive solutions such as soluble sugars, in particular amino acids such as proline, glycine and betaine have been increased [14] and can increase the absorption of certain minerals[6]. Many studies on the role of the materials under
various stress conditions have been done that all of them imply on the role of cited compounds in osmoregulation. The results of the present study on *Pinuseldarica* and *Cupressus sempervirens* revealed that there was a significant increase of proline and soluble sugars by increasing salinity. Kamalnezhad et al[22] concluded, a study on the effect of salinity and potassium on the growth and proline accumulation in two barley cultivars, that with increasing salinity level, proline was significantly increased in both cultivars. Heydari et al[19] reached the conclusion that with increasing salinity, proline contents in both stems and roots increased in five rape seed cultivars. Abbaspour et al[1] studied the effect of salinity on growth of soluble sugar pigments and ion accumulation in three pistachio cultivars that it showed increasing salinity levels from 0 to 300 mM soluble sugars in variety AK of pistachio increased. The findings of this study correspond with the results. Increase insoluble sugars may be due to two reasons: it increases the photosynthesis of salt and thus accumulate sugar in the tissues; another reason is because of breaking larger sugars (starch) to smaller sugars (glucose). With increasing salt concentration, total chlorophyll decreased, resulting in decreased photosynthesis during that time. As a result of the increase insoluble sugars is due to breaking down large carbohydrates to small sugars. Using SNP and SA without salinity resulted in an increase of sugar in leaves and roots. The application of salicylic acid without salinity treatment significantly increased leaf sugar. This process reflects the positive role of salicylic acid on *Pinuseldarica* and *Cupressus sempervirens*. SA and SNP results in an increase in concentration of 20ds/m leaf and root proline. In addition, SA in concentration of 20 and 10ds/m and SNP in concentration of 5ds/m made increase the sugar content. The effect of salicylic acid on growth and some morphological characteristics of Gomphrena showed SA improves physiological traits and photosynthetic its resistance to harsh conditions due to increased salinity[27]. Results of SNP indicated making treatments with SNP increased phenol but it can’t affect proline and amino acids so that the results of this research are similar to those obtain by Nasibi et al[26] The results obtained by different researchers associate with the effects of SA on increasing root length [17], relative moisture [3], and dry weight of root [15] are not similar to the findings of this study, because the period of experiment and the growth of plant in this study was small. Application of SA reduced potassium uptake in roots. Similar results were obtained in a study of the effects of salinity in two barley cultivars showed that increasing salinity levels in the root decreased dry weight of roots, stomata conductance, transpiration rate and K Acclimatizing of basil and alfzal cultivars. Nasibi et al[26] The weight loss may be due to the negative effects of severe osmotic potential of soil solution, which decreases the absorption of water and nutrients and ultimately reduces the weight of the root and stem. Finally, the results showed that *Pinuseldarica* and *Cupressus* with increasing proline and soluble sugars can tolerate salt solution and also using the SNP and SA will increase plant tolerance to salinity.

REFERENCES


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