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Studying the salinity stress on physical and growth characteristics of *cupressus sempervirens*

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Key words: Salinity stress, *Cupressus sempervirens*, Salicylic acid, Sodium nitroproside.

Abstract

This study investigated the effects of some physiological and growth characteristics under salinity conditions. For this aim, the factorial experiment was randomly designed in three replicates and different salinity treatments (0, 5, 10, 20 ds/m) with and without Sodium nitroproside (SNP) and salicylic acid (SA). Results showed the amount of proline significantly increased in dose 20 ds/m. Applying SA increased the amount of proline three times more than control in dose 20 ds/m. Proline in roots increased in dose 20 ds/m salinity and using these two substances on proline content in roots showed applying SA didn't increase the amount of sugar in dose 20 ds/m. Sugar in leaves increased with increasing salinity dose 10 and 20 ds/m and applying SA in dose 10 and 20 ds/m and SNP in dose 5 ds/m increased the amount of sugar. Increasing salinity did not change root length and carotenoids, however; leaf Na increased in dose 20 ds/m with increasing salinity and decreased K in dose 10 and 20 ds/m. Increasing salinity did not change the amount of chlorophyll and it caused to decrease wet weight of roots and stems and also using SA decreased dry weight of stems.

Introduction

Salinity stress is the certain factor that seriously constrains agricultural production in various regions such as arid and semi-arid areas (Comba & Tomaro, 1998). Plants apply different ways in order to reduce salinity stresses. Increasing adaptive osmolyte in different organs of plants is one of the main 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 to drought and salinity stresses (De Lacerda *et al.*, 2005). Proline is the most popular solution that widely has various application associated with drought and salinity (Raskin, 1992).

The salinity study on physiological characteristics of *Sorghum bicolor* and *Sorghum sudanense* showed that there was an increase of sugar by 118 percent for former, although there was not found any increase for later. There was found an increase of proline by34 percent for *S. bicolor*, whereas this value was a decrease to 82 percent for *S.sudanense* compared to control (De Oliveira *et al.*, 2013). In addition the chlorophyll has a reduction in salinity stress (Amin, 2008). Reducing chlorophyll concentration is an important and efficient agent in photosynthesis capacity and also increasing saline level cause to accelerate the injuries of salinity stresses. Therefore, decreasing growing features is related to a fall in photosynthesis rate (Abdol-Baky, 2008). The study of the oxidative stress of corn showed that increasing salinity make a reduction of chlorophyll in leaves (Cengiz Kaya, 2013). The leaf contents have a strong correlation with water been in leaves, so that plants have an appropriate mechanism face to stresses such as salinity. For example in high salinity concentration the stoma are closed and this value can make drought stress and creates an adverse condition for plants (Colomm & vazzana, 2003). The salinity effects on physiological and morphological characteristics of grape showed that increasing salinity significantly decrease water in leaves and chlorophyll index (Karimi &Yusef-zadeh, 2013).

Sodium is a soluble ion in many desert soils that during long-term stress in plants the large amounts of this ion would accumulate in underground organs, especially roots and the little amount of it can transfer to aerial organs. 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 plant organs 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 adjust plant growth (Grattan & Grieve, 1994).The study carried out on pepper indicated that increasing salinity has an essential function to reduce and plant height and potassium (Wellbum, 1983). Salicylic acid is one of the useful compounds for plants that play an important role in the resistance of plants to environmental stresses such as salinity. This acid is classified among plant growth regulators. Therefore, ortho-hydroxy benzoic acid and salicylic acid are an endogenous growth regulator of plant that have a suitable role in physiological processes (Magdy, 2012).

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 (Delavari parizi, 2012). 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 (Nasibi *et al.*, 2011). SNP could improve the effects of salinity and increased chlorophyll in cotton (Lichtenthaler, 1987). This study is aimed to the effect of SNP and SA on some physiological and growth characteristics of *cupressus sempervirens* under salinity stress.

Material and methods

Materials

The study was carried out in 2011 in a greenhouse located in Yazd city. One year old seedlings of *cupressus simpervirens* from nursery of Yazd city with same weather condition were selected.

methods

The seedlings in the treatments of 0, 5, 10 and 20 dS/m sodium chloride in the solution of a day were used. For producing 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. For producing SNP, 150 mg SNP were solved in one liter water and also 160 mg SA first was solved in ethanol after that the solution by water up to one liter. Working on treatments lasted 2 months. Root and leaf proline based on bates methods (Bates *et al.*, 1973) and the amount of leaf and root sugar were measured based on Kochret, 1987. Sodium and potassium with phylum photometer and for RWC below equation was used.

RWC (%) = (wet weight- dry weight) / (inflammation weight-dry weight)*100 .

Sartarius scale (BP211) was used for measuring wet and dry weight of root and stem. SPSS20 was applied for analyzing data. Duncan test was selected for mean comparison and also dant was used for comparing treatments with control. Excel software was used in order to draw graphs.

[1]*Cupressus sempervirens*

Results

With increasing salinity, leaf proline was significantly increased. Using SA in 20 ds/m caused to increase proline rather than control tree times. Root proline had a significant increase in 20 ds/m and using these two materials didn't have a significant effect on root proline. Applying SA in dose 20 made a significant increase of sugar in root. With increasing salinity, leaf sugar would increase. Increasing salinity in dose 10 and 20 were occurred and applying SA in dose 20 and 10 as well as SNP in 5ds/m caused to increase sugar. There was not found any change in root length and carotenoid as well as chlorophyll with increasing salinity, but there was found an increase in sodium and a reduction in potassium in dose 10 and 20 ds/m. Wet and dry weight of stem and root were decreased when salinity was increased. On other hand, using SA caused to make an increase of dry weight of stem. In addition, using two substances without salinity treatment increased sugar in leaves and roots (table 1, 2, 3).

Table 1. The effect of salinity on proline, soluble sugar, relative content and root/stem.

Treatment	Relative content (%)	Leaf sugar	Root sugar (mg/gfw)	Root proline (mg/gfw)	Leaf proline (mg/gfw)	Root/Stem (cm)
Control	70^{A}	15.55^{G}	35.37^{CDE}	13.09^{BC}	31.24^{EF}	1.5^{AB}
Salinity5	76.6^{BC}	24.73^{FG}	19.53^{F}	9.63^{C}	27.97^{EFG}	1.42^{AB}
Salinity5+SNP	98.24^{AB}	66.12^{B}	26.01^{EF}	8.59^{C}	22.86^{FG}	1.61^{AB}
Salinity5+SA	71.67^{C}	26.50^{EFG}	24.84^{ABC}	20.94^{BC}	7.50^{H}	1.09^{AB}
Salinity10	97.98^{AB}	37.72^{DEF}	37.19^{CDE}	8.96^{C}	30.48^{EF}	1.37^{AB}
Alinity10+SNP	83.58^{ABC}	29.59^{EFG}	35.71^{CDE}	9.50^{C}	35.38^{DE}	0.84^{AB}
Salinity +SA	94.86^{AB}	51.80^{BCD}	43.58^{ABCC}	7.86^{C}	19.91^{G}	0.86^{AB}
Salinity20	79.09^{ABC}	42.02^{CDE}	41.09^{BCD}	35.99^{A}	80.11^{B}	0.53^{B}
Salinity+SNP	90.46^{ABC}	57^{BC}	$27/99^{DEF}$	26.32^{AB}	45.58^{C}	0.56^{B}
Salinity+SA	91.56^{ABC}	82.02^{A}	55.82^{A}	21.80^{BC}	99.1^{A}	2.43^{A}
SNP	96.73^{AB}	33.31^{EF}	43.03^{ABC}	10.76^{C}	35.05^{DE}	1.56^{AB}
SA	97.01^{AB}	66.32^{B}	54.14^{AB}	11.73^{C}	41.64^{CD}	1.80^{AB}

Table 2. The effect of salinity on potassium, carotenoid, total chlorophyll, root and stem length and dry weight of root.

Treatment	Stem length (cm)	Root length (cm)	total chlorophyll	carotenoid (mg chl/ b)	Root potassium (meq/l)	dry weight of Root (gr)
Control	18.66^{AB}	40^{A}	8.29^{BC}	1.23^{A}	0.227	0.726^{ABC}
Salinity5	21.33^{A}	51.33^{A}	11.18^{AB}	1.69^{A}	0.226^{AB}	1.25^{A}
Salinity5+SNP	21^{A}	37.33^{A}	17.75^{A}	2.23^{A}	0.227^{AB}	0.926^{ABC}
Salinity5+SA	14.33^{B}	43^{A}	11.58^{AB}	2.48^{A}	0.215^{ABC}	0.406^{BC}
Salinity10	20.33^{AB}	38.66^{A}	8.34^{BC}	1.21^{A}	0.155^{BCDE}	0.683^{ABC}
Alinity10+SNP	15.66^{AB}	34^{A}	10.53^{BC}	1.9^{A}	0.143^{CDE}	0.273^{C}
Salinity +SA	16.66^{AB}	35^{A}	8.12^{BC}	1.29^{A}	0.119^{DE}	0.56^{ABC}
Salinity20	18^{AB}	43.66^{A}	7.79^{BC}	1.55^{A}	0.179^{ABCD}	0.313^{C}
Salinity+SNP	17.33^{AB}	30^{A}	3.31^{C}	1.63^{A}	0.148^{CDE}	0.213^{C}
Salinity+SA	20.33^{AB}	36^{A}	6.38^{BC}	1.32^{A}	0.105^{E}	0.906^{ABC}
SNP	21.33^{A}	52.33^{A}	8.28^{BC}	1.57^{A}	0.23^{A}	1.143^{AB}
SA	16.66^{AB}	47^{A}	7.30^{BC}	2.34^{A}	0.223^{AB}	0.525^{ABC}

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 (During, 1992) and can increase the absorption of certain minerals (Bohnertet *et al.*, 1992). Many studies on the role of these materials under various stress conditions have been done that all of them imply on the role of cited compounds in osmoregulation. The results of this study on *Cupressus sempervirens* showed that with increasing salinity significantly proline and soluble sugars had increased. Kamalnezhad *et al.*, (2006) conducted a study about the effect of salinity and potassium on the growth and proline accumulation in two barley cultivars that the results showed with increasing salinity level proline in both cultivars significantly had increased. In another study that was performed by Heydari *et al.* (2010) on five rape seed cultivars reached the conclusion that with increasing salinity, proline contents in both stems and roots increased. Abbaspour *et al.* (2012) 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 in soluble 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 in soluble sugars is due to breaking down large carbohydrates to small sugars. Applying SNP and SA without salinity increased 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 *Cupressus sempervirens*. SA and SNP causes an increase in dose 20 ds/m leaf and root proline. In addition, SA in dose 20 and 10ds /m and SNP in dose 5 ds /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 (Raskin, 1992). 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.* (2011). The results obtained by different researchers associate with the effects of SA on increasing root length (Hanan, 2007), relative moisture (Agarwal *et al.*,2005) and dry weight of root (EI-Tayeb, 2005) are not similar to the findings of this study, because the period of experiment and the growth of plant in this study was small.

Table 3. The effect of salinity on sodium, potassium of leaves and roots, dry and wet weight of roots and stems.

wet weight of Stem (gr)	dry weight of Root (gr)	Leaf potassium (meq/l)	Root sodium (meq/l)	Leaf sodium (meq/l)	dry weight of Stem (gr)
1.36^{ABC}	2.61^{AB}	0.23^{A}	0.125^{BC}	0.0128^{FG}	0.45^{B}
2.63.	1.27^{A}	0.229^{A}	0.211^{AB}	0.0188^{CDE}	1.83^{A}
2.74^{A}	3.64^{A}	0.237^{A}	0.173^{ABC}	0.0117^{DEF}	0.58^{B}
1.29^{ABC}	2.23^{AB}	0.186^{B}	0.191^{AB}	0.0176^{DEF}	0.39^{B}
1.66^{ABC}	2.53^{AB}	0.143^{CD}	0.163^{ABC}	0.0213^{BCD}	0.51^{B}
0.806^{C}	1.2^{B}	0.135^{D}	0.151^{ABC}	0.0177^{DEF}	0.30^{B}
1.380^{ABC}	1.38^{B}	0.191^{B}	0.213^{AB}	0.0234^{ABC}	0.58^{B}
1.27^{ABC}	1.89^{B}	0.164^{BC}	0.241^{A}	0.0254^{AB}	0.49^{C}
1.29^{ABC}	1.85^{B}	0.122^{DE}	0.185^{AB}	0.028^{A}	0.44^{B}
1.71^{ABC}	3.42^{AB}	0.101^{E}	0.155^{ABC}	0.0243^{AB}	0.59^{B}
2.4^{AB}	3.63^{AB}	0.233^{A}	0.13^{BC}	0.015 ^{EFG}	0.7^{B}
1.11^{BC}	2.88^{AB}	0.224^{A}	0.905^{C}	0.0104^{G}	0.35^{B}

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 afzal cultivars. Nasibi *et al.*, (2011). 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 *Cupressus sempervirens* with increasing proline and soluble sugars can tolerate salt solution and also applying the SNP and SA amplifies plant tolerance to salinity.

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