



Full Length Article

The effects of Salicylic Acid (SA) and Sodium Nitroprusside (SNP) on physical and growth characteristics of *Pinus eldarica*

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ABSTRACT

The present study was conducted to study the effects of sodium nitroprusside (SNP) and salicylic acid (SA) on physiological and growth characteristics under salinity conditions on *Pinus eldarica*. For this aim, the factorial experiment was randomly designed in three replicates and different salinity treatments (0, 5, 10, 20 ds/m). Results showed the amount of proline significantly increased in dose 10 ds/m. Applying SA increased the amount of proline six times more than control in dose 20 ds/m. Root proline increased in dose 10 and 20 ds/m salinity and using SA in dose 20 caused an increase three times more than control. Increasing salinity suggested a significant augmentation of sugar in 10 and 20 ds/m. Increasing salinity was occurred by applying SA in dose 5 and 20 and using SNP in 10 ds/m increased sugar. There was not found any change in RWC, leaf potassium, root sodium, stem length, root length, wet and dry weight of stem and root, carotenoid and chlorophyll with increasing salinity. The results of this study can be used by managers in order to choose appropriate species for plantation and afforestation.

Key words: Salinity stress, *Pinus eldarica*, Salicylic acid, Sodium nitroprusside

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INTRODUCTION

Salinity stress is the certain factor that seriously constrains agricultural production in various regions especially in arid and semi-arid areas [10]. 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 [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*, whereas this value was a reduction of 82 percent for *S. sudanense* compared with control [13]. In addition, the chlorophyll has a reduction in salinity stress [4]. 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. Hence, decreasing growing features is related to a fall in photosynthesis rate. The study of the oxidative stress of corn showed that increasing salinity made a reduction of chlorophyll in leaves [8]. The leaf contents have a strong correlation with leaf water, 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 results in an adverse condition for plants [9]. The salinity effects on physiological and morphological characteristics of grape showed that increasing salinity significantly decrease water in leaves and chlorophyll index.

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 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 (Wellbum, 1983). Salicylic acid is one of the useful compounds for plants that play an important role in the plant resistance to environmental stresses such as salinity. This acid is 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].

Pinus eldarica is the coniferous species that is widely planted in various parts of Iran especially in arid and semiarid areas and also there is not comprehensive information about the different stresses on the species. The present study is aimed to evaluate the effect of SNP and SA on some physiological and growth characteristics of *Pinus eldarica* under salinity stress.

MATERIALS AND METHODS

The present study was carried out in 2011 in a greenhouse located in Yazd city. One year old seedlings of *Pinus eldarica* from nursery of Yazd city with same weather condition were selected. The seedlings were used in the treatments of 0, 5, 10 and 20 ds/m sodium chloride in the solution of a day. To produce salinity 5, 3.2 gr salt in one liter water were solved and also 8 and 16 gr salt in one liter water were solved for salinity 10 and 20, respectively. To make 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 [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.

$RWC (\%) = (\text{wet weight} - \text{dry weight}) / (\text{inflammation weight} - \text{dry weight}) * 100$

Sartorius 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 t-dant was used for comparing treatments with control. Excel software was used in order to draw graphs.

RESULTS

Increasing salinity made significant increment, six times more than control, of leaf proline in dose 20 ds/m. Using SA in dose 20 ds/m caused to increase proline rather than control three times. Root proline had a significant increase in 10 and 20 ds/m. With increasing salinity, the root sugar significantly enhanced in 10 and 20 ds/m and applying SA and SNP in dose 5 ds/m made a significant amplification of sugar in root. With increasing salinity, leaf sugar would increase in all doses. Increasing salinity was occurred by applying SA in dose 5 and 20 and using SNP in 10 ds/m increased sugar. There was not found any change in RWC, leaf potassium, root sodium, stem length, root length, wet and dry weight of stem and root, carotenoid and chlorophyll with increasing salinity, but there was found an increase of sodium in dose 10 ds/m. Applying SA and SNP without salinity treatment increased the leaf and root sugar. In addition, using SA without salinity treatment significantly augmented root proline (table 1, 2).

Table 1: The salinity effect on proline, sugar, relative content, stem length, sodium and potassium absorption of leaf and root

Treatment	Root sodium)	Leaf sodium	Leaf potassium	Relative content	Leaf sugar	Root sugar	Root proline	Leaf proline	
Control	0.014 ^A	0.0104 ^{CD}	0.0596 ^A	69.96 ^A	15.28 ^E	23.91 ^H	11.49 ^C	14.3 ^{DE}	19 ^A
Salinity5	0.017 ^A	0.0069 ^D	0.015 ^A	104.5 ^A	26.11 ^{CD}	24.66 ^H	11.83 ^C	14.21 ^{DE}	23 ^A
Salinity5+SNP	0.016 ^A	0.0116 ^{BCD}	0.055 ^A	103.8 ^A	30.95 ^{CD}	39 ^G	17.42 ^B	14.95 ^{DE}	19 ^A
Salinity5+SA	0.014 ^A	0.0129 ^{ABCD}	0.05 ^A	108 ^A	23.99 ^{DE}	42.48 ^{FG}	11.48 ^C	18.94 ^{DE}	21.3 ^A
Salinity10	0.014 ^A	0.0172 ^{AB}	0.289 ^A	94.8 ^A	34.05 ^{CD}	55.82 ^D	22.45 ^B	50.32 ^B	23 ^A
Alinity10+SNP	0.023 ^A	0.0138 ^{ABCD}	0.164 ^A	90 ^A	56.47 ^B	45.67 ^{FG}	21.82 ^B	41.77 ^{BC}	23 ^A
Salinity +SA	0.023 ^A	0.0179 ^{AB}	0.0435 ^A	85.4 ^A	23.05 ^{CD}	50.14 ^E	7.16 ^C	14.62 ^{DE}	20 ^A
Salinity20	0.023 ^A	0.0161 ^{ABC}	0.0235	94	55.75	97.04	22.48	31.54	20 ^A
Salinity+SNP	0.019 ^A	0.0192 ^A	0.057 ^A	97.3 ^A	37.1 ^C	50.16 ^E	20.76 ^B	28.89 ^{CD}	20.3 ^A
Salinity+SA	0.022 ^A	0.0158 ^{ABC}	0.05 ^A	95.1 ^A	76.93 ^A	88.99 ^B	38.29 ^A	80.83 ^A	23 ^A
SNP	0.137 ^A	0.0115 ^{BCD}	0.0535 ^A	105 ^A	26.63 ^{CD}	97.31 ^A	7.08 ^C	10.18 ^E	21.3 ^A
SA	0.021 ^A	0.0098 ^{CD}	0.073 ^A	93.6 ^A	35.62 ^C	77.94 ^C	18.56 ^B	20.44 ^{DE}	18.6 ^A

Table 2: The effects of salinity on root potassium, chlorophyll, carotenoid, dry and wet weight of stem and root

Treatment	Root length	Root/Stem	Wet weight of Stem	Wet weight of Root	Dry weight of Stem	Dry weight of Root	Carotenoid	Chlorophyll	Root potassium	
Control	27.33 ^{AB}	1.23 ^B	3.13 ^A	4.79 ^A	0.673 ^A	1.42 ^A	1.79 ^A	13.44 ^A	0.227 ^{AB}	
Salinity5	34 ^{AB}	1.45 ^B	2.79 ^A	5.89 ^A	1.09 ^A	1.92 ^A	1.46 ^A	8.3 ^A	0.226 ^{AB}	
Salinity5+SNP	31.33 ^{AB}	1.02 ^B	1.62 ^A	3 ^A	0.673 ^A	0.673 ^A	1.19 ^A	18.91 ^A	0.227 ^{AB}	
Salinity5+SA	26.33 ^{AB}	1.8 ^B	2.38 ^A	5.26 ^A	0.853 ^A	1.266 ^A	2.05 ^A	9.97 ^A	0.215 ^{ABC}	
Salinity10	38 ^{AB}	1.25 ^B	3.34 ^A	6.46 ^A	1.265 ^A	1.57 ^A	2.01 ^A	13.87 ^A	0.155 ^{BCDE}	
Alinity10+SNP	16.5 ^B	1.36 ^B	2.92 ^A	3.48 ^A	1.13 ^A	1.5 ^A	1.18 ^A	10.66 ^A	0.143 ^{CDE}	
Salinity +SA	29.33 ^{AB}	1.62 ^B	2.63 ^A	5.52 ^A	0.946 ^A	1.49 ^A	1.26 ^A	15.4 ^A	0.119 ^{DE}	
Salinity20	41.66 ^A	2.2 ^B	2.18 ^A	6.89 ^A	0.906 ^A	2.16 ^A	0.97 ^A	15.87 ^A	0.179 ^{ABCD}	
Salinity+SNP	0.019 ^A	0.019 ^A	0.0192 ^A	0.057 ^A	97.3 ^A	37.1 ^C	50.16 ^E	20.76 ^B	28.89 ^{CD}	20.3 ^A
Salinity+SA	0.022 ^A	0.022 ^A	0.0158 ^{ABC}	0.05 ^A	95.1 ^A	76.93 ^A	88.99 ^B	38.29 ^A	80.83 ^A	23 ^A
SNP	0.137 ^A	0.137 ^A	0.0115 ^{BCD}	0.0535 ^A	105 ^A	26.63 ^{CD}	97.31 ^A	7.08 ^C	10.18 ^E	21.3 ^A
SA	0.021 ^A	0.021 ^A	0.0098 ^{CD}	0.073 ^A	93.6 ^A	35.62 ^C	77.94 ^C	18.56 ^B	20.44 ^{DE}	18.6 ^A

DISCUSSION

Transforming materials due to reduced water availability leads to change in the concentrations of some metabolites during salinity and drought stresses. 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]. Various studies on the role of these materials under different stress conditions have been conducted that all of them imply on the role of cited compounds in osmoregulation [11]. The results of this study on *Pinus eldarica* showed that with increasing salinity, proline and soluble sugars had increased significantly. Kamalnejhad et al [22] 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 [19] on five rape seed cultivars reached the conclusion that with increasing salinity, proline contents in both stems and roots increased. 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 corresponded 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 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. Applying SNP and SA without salinity treatment increased sugar in leaves and roots. The application of salicylic acid without salinity treatment significantly increased root proline. This process reflects the positive role of salicylic acid on *Pinus eldarica*. SA made an increase of dose 20ds/min leaf and root proline. However, NSP didn't play any effective role on this issue. In addition, SA in dose 20 and 5 ds /m and SNP in dose 10 ds /m increased the sugar content. The effect of salicylic acid on growth and some morphological characteristics of *Gomphrena* showed SA improved 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 afzal 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. As a conclusion, the results of this research showed that *Pinus eldarica* with increasing proline and soluble sugars can tolerate salt solution and also applying the SNP and SA enhance plant tolerance to salinity.

REFERENCES

1. Abbaspour, H. H. Afshari & M.H. Abdel-Wahhab, (2012). Influence of salt stress on growth, pigment, soluble sugars and ion accumulation in three pistachio cultivars. *Journal of medicinal plants Research*, (12): 2468-2473.
2. Abdel-Baky, H.M., M. Hussein & G. El-Baroty, (2009). Algal extracts improve antioxidant defense abilities and salt tolerance of wheat plant irrigated with sea water. *Afr. j. Biochem* 2. 151-164.
3. Agarwal, S., R.K. Sairam, G.C. Srivastava & R.C. Meena, (2005). Changes in antioxidant enzymes activity and oxidative stress by abscisic acid and salicylic acid in wheat genotypes. *Biologia Plant* 49: 541-550.
4. Amin, A.L., (2008). Cadmium induced change in pigment content ion uptake, proline content and phosphoenolpyruvate carboxylase activity in *triticum aestivum* seedling. *Aust. j. Basic Appl. sci* 2:57-62.
5. Bates, L.S., R.P. Waldren & I.D. Tear, (1973). Studies plant and soil. Rapid determination of free proline for water stress, 39:205-20
6. Bohnert, H.J., D.E. Nelson & R.G. Jensen, (1992). Adaptation to environmental stresses. *The Plant Cell*, 7:1099-1111
7. Boyarshinov A. V. & E. V. Asafova, (2011). Stress Responses of Wheat Leaves to Dehydration: Participation of Endogenous NO and Effect of Sodium Nitroprusside. *Russian Journal of Plant Physiology*, 2011, Vol. 58, No 6, pp. 1034-1039.
8. Cengiz, K., O. Sonmez, S. Aydemir & M. Dikilitas, (2013). Mitigation effect of glycinebetaine on oxidative stress and some key growth parameters of maize exposed to salt stress. *Turkish Journal of Agriculture and forestry* 37:188-194.
9. Colom, R. & C. Vazzana, (2003). Photosynthesis and psii functionality of drought resistance and drought sensitive weeping lovegrass plants. *Environmental and Experimental Botany*, 49:135-144.
10. Comba, M.E., M.P. Benavides & M.L. Tomaro, (1998). Effect of salt stress on antioxidant defence system in soybean root nodules. *Aust. j. plant physiol.* 25:605-671
11. De Lacerda, C.F., J. Cambraia, M.A. Oliva & H.A. Ruiz, (2005). Changes in growth and in solute concentrations in Sorghum leaves and roots during salt stress recovery. *Environment and Experimental Botany*, 54:69-76.
12. Delavari, P., M.A. Baghizadeh, Sh. Enteshari & K. Manouchehrizadeh, (2012). The study of the interactive effect of salicylic acid and salinity stress on induction of oxidative stress and mechanisms of tolerance in *ocimum basilicum*. *Iranian Journal of plant Biology* 4(12):25-36.
13. De Oliveira, V., E. Camelo Marques, F. De Lacerda, J. Tarquinio Prisco & E. Gomes-Filho. (2013). Physiological and biochemical characteristics of *Sorghum bicolor* and *Sorghum sudanense* subjected to salt stress in two stages of development. *African Journal of Agricultural Research*, 8: 660-670.
14. Doring, H. (1992). Evidence for osmotic adjustment to drought in grapevines (*Vitis vinifera*). *Vitis*, 23:1-10
15. El-Tayeb, M.A. (2005). Response of barley grains to the interactive effect of salinity and salicylic acid. *plant Growth Regulation* 45:215-225.
16. Grattan, S.R. & C.M. Grieve, (1994). Mineral nutrient acquisition and response by plants grown in saline environments. In *Handbook of plant and crop stress* (ed) M. Pessarakli, Marcel Dekker, New York.
17. Hanan, E.D. (2007). Influence of salicylic acid on stress tolerance during seed germination of *Triticum aestivum* and *Hordeum vulgare*. *Biological Research*. 1:40-48
18. Jaziree, M.H. (2002). *Afforestation in arid areas*. Tehran university press. Second edition. 560p.
19. Heydari, M., Mesri, F. and Kaikha, Z. (2010). The salinity effects on metabolism of nucleotide acids, the activity of anti-oxidant enzymes in five varieties of rape. *Iranian Journal of Field Crop Science*. 41(3): 491-502.
20. Karimi, H. & A. Yusef-zadeh, (2013). The effect of salinity level on the morphological and physiological traits of Two Grape (*Vitis vinifera*) cultivars. *International Journal of Agronomy and plant production*. vol.4(5), 1108-1117.
21. Kafi, M., Damghani, M., Basra, A. and Basra, R. (2002). Resistance mechanisms of plants to environmental stresses. Second Edition, Ferdowsi University of Mashhad.
22. Kamalnehzad, J., Farahi-ashtiani, S. and Ghanati, F. (2006). Effects of salinity and potassium on the growth and proline accumulation in two barley cultivars. *Journal of Agricultural Sciences and Natural Resources*, Volume 13, Number 19.
23. Kochert, G., (1978). Carbohydrate determination by the phenol sulfuric acid method. In Helebut, J.A. Craig, J.S. (ed): *Handbook of physiological method*. Cambridge University Press Cambridge. 56-97.
24. Lichtenthaler, H.K., (1978). Chlorophyll and carotenoids: pigments of photosynthetic biomembranes. *Method Enzym.* 148: 350-382. Magdy, A., M.M. Shallan, H. Hazem., M. Alia., A. Namich & A. I. Alshaimaa., (2012). Effect of Sodium Nitroprusside, Putrescine and Glycine Betaine on Alleviation of Drought Stress in Cotton Plant. *American-Eurasian. J. Agric. & Environ. Sci.*, 12 (9): 1252-1265.
25. Nasibi, F., Manochehri, K. and Yaghobi, M.M. (2011). Comparing the effects of SNP and arginine pretreatment on some physiological responses of tomato plants under water stress. *Iranian Journal of Biology*. Volume 24, Number 6.
26. Raskin, I., (1992). Role of salicylic acid in plants. In *Annual Review of plant physiology and plant molecular Biology*. ISSN 1818-6769
27. Sudhakar, C., P.S. Reddy & K. Veeranjanyulu, 1993. Effect of salt stress on enzymes of proline synthesis and oxidation in green gram (*Phaseolus aureus*) seedling. *Journal of Plant Physiology*, 141:621-623.
28. Wellbum A. R., 1983. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *J. Biochem Soc Trans*, 11: 591 - 592.

29. Zhanikauther , N., H. A. Kezwan& H. Cherif, 2013. Evaluation of salt Tolerance (Nacl) in Tunisian chili pepper (capsicum frutescers) on growth, Mineral Analysis and solutes synthesis. Journal of stress physiology& Biochemistry,Vol.9 No.pp.209-228.