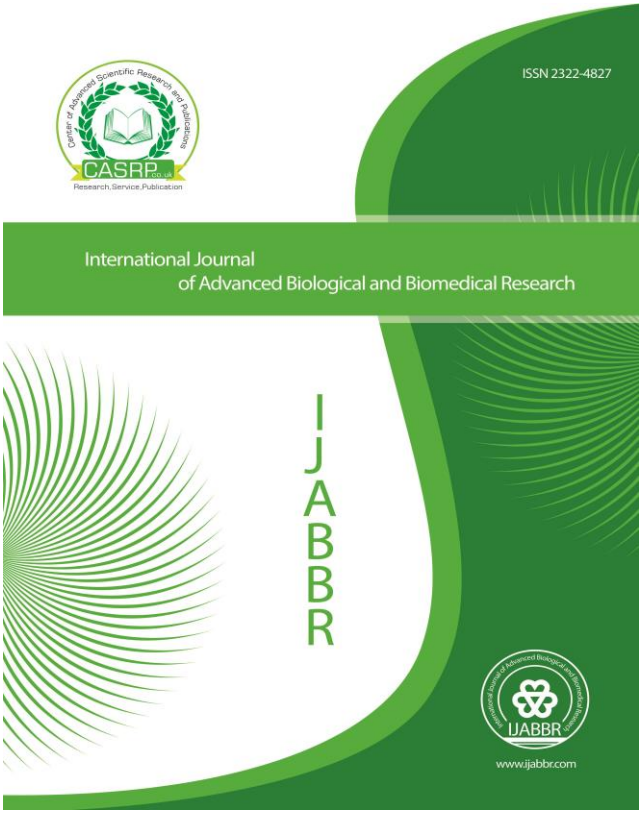

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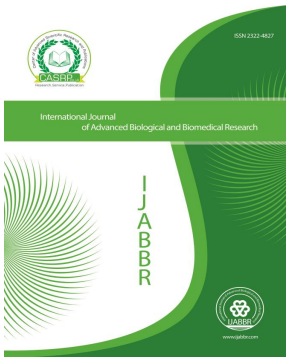

Original Article **Open Access**

Effect of salinity on some physiological and biochemical traits in grape (*Vitisvinifera*)

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Abstract

The present study was laid out in order toevaluate the effect of salinity on some biochemical characterize of grapevine c.vYaghouti. The experiment was done at Agricultural and Natural Resources Research Center, Ilam, Iran at 2014. The experiment was a completely block design (CRD) with four replications. Saline factor was NaCl salinity in seven levels (Zero, 50, 100, 150, 200 and 250 mM). Grape scions were planted in beds containing equal proportions of sand, perlite and vermiculite in 20-L vases. The vases were nourished by half Hoagland nutrient solution for first three weeks after plantation. In order to create the desired salinity levels, another half of the Hoagland nutrient solution was salinized with 0, 50, 100, 150, 200 and 250 mMNaCl. During growth stages, measurement was done on physiological and biochemical parameters such as soluble sugar, proline, chlorophyll, catalase, peroxidase and ascorbat peroxidase enzymes. The results showed that maximum chlorophyll a and b were obtained for control treatment and decreased gradually with increasing of saline level. The proline and soluble content as osmolits increased gradually by increasing of saline levels. However, CAT, APX and POX activity increased by salinity levels until 150mM, 150mM and 250mM saline treatment respectively. The results of the present study reviled that salt stress decreased some biochemical traits and decreased some damages by increasing of osmolits content and antioxidant enzymes activity.

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1. Introduction

Grapevines (*Vitisvinifera* L.) are one of the oldest and most important perennial crops in the world. An estimated 5000 to 6000 varieties of the *Vitisvinifera* species exist around the world, although only a relatively small number of these are used commercially (Dettweiler and Eibach, 2003). So far identify 30555 grapevine cultivars and many species on the world then naming and evaluated. Iran is one of the creation and distributioncenters of grapes in the world that had high genetic diversity (Dettweiler and Eibach, 2003). Among the Iranian grapevine cultivars, only good quality cultivars for fresh grapes, raisins and industrial processing seem to have attracted farmers and others less attention.

Salinity has considerable adverse impacts on productivity of agricultural plants. Soil salinity, resulting from natural processes or from crop irrigation with saline water, occurs in many arid and semi-arid regions of the world (Koca et al., 2007). Soil salinity affects growth and yield in grapevine by osmotic and specific ion toxicities (Shani and Al, 2005). One of the most common stress responses in high plants is over production of some various compatible organic solutes (Serraj and Sinclar, 2002). These solutes are most commonly carbohydrates, like sugars, amino acids and proteins that acts as osmolytes. Proline and soluble sugar are two of them that known to occur widely in higher plants and normally accumulates in large quantities in response to environmental stresses (Kishor et al., 2005). It has also been reported that not only species but also varieties of a species show differences in salt tolerance (Schwarz, 1995). In plant cells, antioxidant enzymes, such as superoxide dismutase (SOD), peroxidase (POX), and catalase (CAT), are considered to form a defensive team, whose combined purpose is to protect cells from oxidative damage (Blokhina et al ., 2003). Moreover, Goel and Sheoran (2003) told that malondialdehyde (MDA) is considered sensitive marker commonly used for assessing membrane lipid peroxidation. CAT reduces H2O2 to water and dioxygen, and APX reduces H2O2 water and generates MDA (Noctor and Foyer, 1998). In a study, three European vinifera cultivars and four Argentinean cultivars were analyzed for their salt tolerance by in vitro culture and it was found that Argentinean cultivars showed better performance in growing under salinity, especially in the highest salt concentration (Cavagnaro et al., 2006). However, this study was conducted to study on some biochemical characterize ofgrapevine under salt stress.

2. Materials and methods

This experiment was laid out in order to evaluate the effect of salinity on some physiological and biochemical of grape (*Vitisvinifera*) c.vYaghoutiin Agricultural and Natural Resources Research Center, Ilam, Iran at 2014. The experiment was a completely block design (CRD) with four replications at greenhouse. Saline factor was NaCl salinity in seven levels (Zero, 50, 100, 150, 200 and 250 mM). Grape scions were planted in beds containing equal proportions of sand, perlite and vermiculite in 20-L vases. Tthe vases were nourished by half Hoagland nutrient solution for first three weeks after plantation. In order to create the desired salinity levels, another half of the Hoagland nutrient solution was salinized with 0, 50, 100, 150, 200 and 250 mMNaCl.

The vases were irrigated by the prepared solutions after the establishment of scions. During growth stages, measurement was done on physiological and biochemical parameters such as soluble sugar, proline, chlorophyll, catalase, peroxidase and ascorbat peroxidase enzymes. Soluble protein was estimated using the bovine serum albumin as standard. Extraction of protein from the sample was carried out using tris HCl buffer (pH 7.8) and the supernatant was used for the estimation and the absorbance was measured at 660 nm (Sadasavim et al., 1966).

To measure proline content, cotyledon (500 mg) were ground in mortal and pistil, homogenized in 10 ml 3% sulfosalicylic acid, and then centrifuged at 5 000 rpm for 5 min. The supernatant (200 µl) was mixed with 200 µl glacial acetic acid and 200 µl acidic ninhydrin, and then mixed well. The mixture was incubated at 100◦C for 60 min. The reaction was terminated by placing the mixture on ice, and then extracting the sample with 1.0 ml toluene. The absorbance of the mixture was measured at 520 nm. To prepare the standard curve, an L-proline stock solution (100 µg/ml) was diluted to 2, 4, 6, 8, 10, 12 µg/ml (Bates, 1973).

Chlorophyll a and b was measured using Arnonmethod. In this method, as little as a 0.5 gram of wet vegetative matter was mashed in liquid nitrogen, in a porcelain mortar. As much as 20mL of 80% aceton was added to the sample, and then the mixture was put into centrifuge device with 6000 rpm speed for 10 minutes. Supernatant was transferred into a glass balloon. Some of the samples in the balloon were read in

spectrophotometer for chlorophyll a at 663nm; for chlorophyll b at 645nm; and for Carotenoids at 470nm. Finally, the following formulas were used to calculate chlorophyll a and b and carotenoids contents in mg/g of fresh weight of the sample:

$$\text{Chlorophyll a} = (19.3 * A663 - 0.86 * A645)\ V/100W$$
$$\text{Chlorophyll b} = (19.3 * A645 - 3.6 * A663)\ V/100W$$

For the catalase (CAT) assay, soluble proteins were extracted by homogenizing 1 g (fresh weight) powdered sample in 3 ml of 100 mM mono sodium phosphate buffer (pH 7.5) containing 0.1% Triton X-100. Other materials for determined of CAT activity was 54 mM mono sodium phosphate buffer (pH 6.8) and 450 mM H_2O_2. CAT activity was measured following the change in the absorbance of the reaction mixture at 240 nm (Philippe et al., 2007). The assay was detected in a 3 ml reaction mixture containing 2.85 mL 54 mM mono sodium phosphate buffer (pH 6.8), 100 µL H_2O_2 (450 mM) and 50 µL of crude extract.

POX activity was measured by Sakharov and Aridilla (1999) method with slight modifications. A 3 mL mixture consisted of 100 µL of based solution guaiacol, 100 µL H_2O_2 (1.2 M) and 200 µL enzyme extract and the changes of absorbance at 470nm were measured using a UV/vis spectrophotometer. One unit of POX activity was expressed as 1.0 change in absorbance per minute. Total APX as Nakano and Asada (1981) method with slight modifications in a 1-ml total volume. The reaction mixtures included 25 µL ascorbic acid (0.03%), 25 µL EDTA (0.1%), 50 µL H_2O_2 (0.015%), 800 µL 312.5 mM sodium phosphate buffer (pH 7.0) and 100 µL protein extract. The changes of absorbance were read at 290 nm using a UV/vis spectrophotometer for 3 minutes.

The statistical analysis was conducted using JMP 5.0.1.2 (Statistical analyses system Institute incorporated, 2002). Statistical significance was declared at $P \leq 0.05$ and $P \leq 0.01$. Treatment effects from the two runs of experiments followed a similar trend, and thus the data from the two independent runs were combined in the analysis.

3. Results and discussion

The results showed that some physiological and biochemical traits of grape Yaghuti cultivar was changed by increasing of saline treatment. The results showed that effect of saline treatment on all traits was significant. However, mean comparison results showed that maximum chlorophyll-a was recorded for control treatment (8.1 mg/ml). Minimum chlorophyll-b was recorded for 250 mM saline treatment (2.72 mg/ml) (fig1).

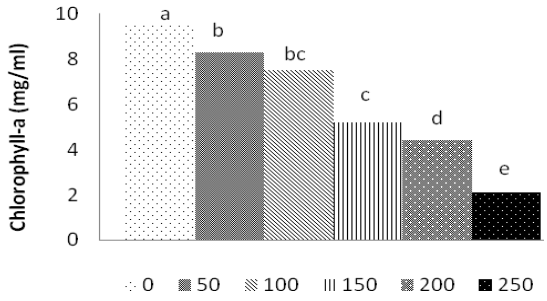

Fig. 1. Effect of salinity on chlorophyll-a in *vitisvinifera* leaves (Columns with same letters have not significant differences).

The results showed that maximum chlorophyll-b was recorded for control treatment (0.84 mg/ml). Minimum chlorophyll-b was recorded for 250 mM saline treatment (0.13 mg/ml) (fig2).

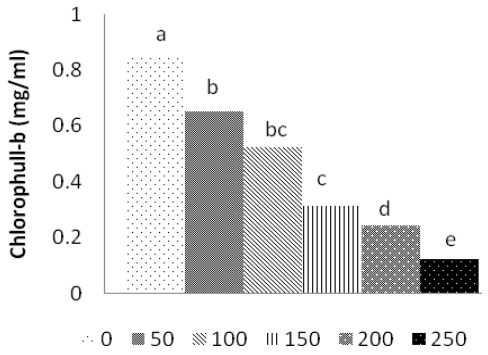

Fig. 2. Effect of salinity on chlorophyll –b in *vitisvinifera* leaves (Columns with same letters have not significant differences).

The results of the present study showed that maximum soluble sugar content was recorded for 250 mM saline treatment (366 mg/g). Minimum soluble sugar content was recorded for control treatment (146 mg/g) (fig3).

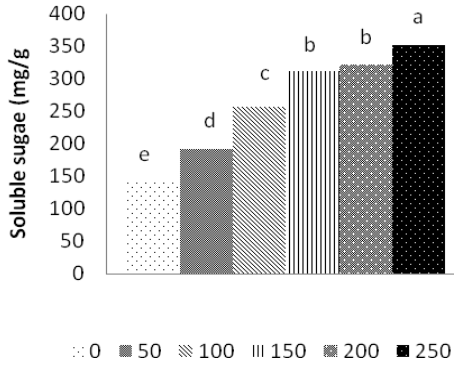

Fig. 3. Effect of salinity on soluble sugar content in *vitisvinifera* leaves (Columns with same letters have not significant differences).

Proline content as an osmolit increased gradually by increasing of saline levels until 250mM. However, maximum proline content recorded for 250 mM saline treatment (270 mg/g). Minimum proline content was recorded for control treatment (86 mg/g) (fig4).

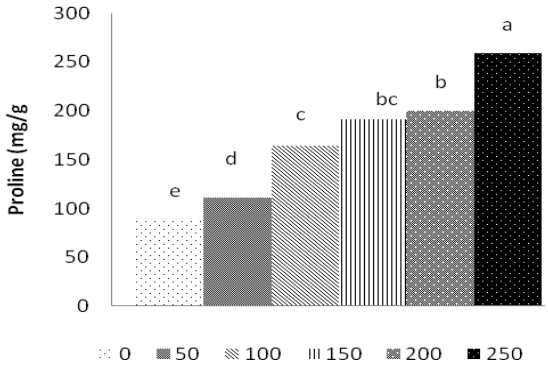

Fig. 4. Effect of salinity on proline content in *vitisvinifera* leaves (Columns with same letters have not significant differences).

Activity of CAT enzyme decreased by salinity levels until 150mM saline treatment then decreased. However, maximum CAT recorded for 150 mM saline treatment (2630 nMol.mn^{-1}.g^{-1} FW). Minimum CAT activity was recorded for control treatment (1860 nMol.mn^{-1}.g^{-1} FW) (fig5).

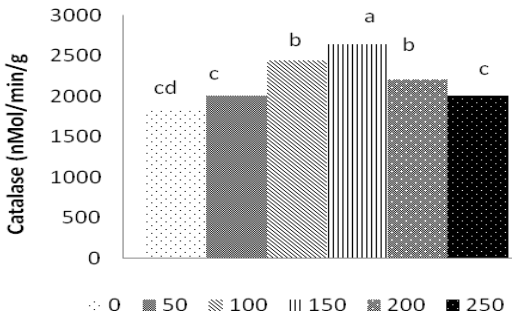

Fig. 5. Effect of salinity on CAT activity in *vitisvinifera* leaves (Columns with same letters have not significant differences).

POX activity increased by salinity levels until 250 mM saline treatment. However, maximum POX recorded for 250mM saline treatment (121 nMol.mn^{-1}.g^{-1} FW). Minimum POX activity was recorded for control treatment (61 nMol.mn^{-1}.g^{-1} FW) (fig6).

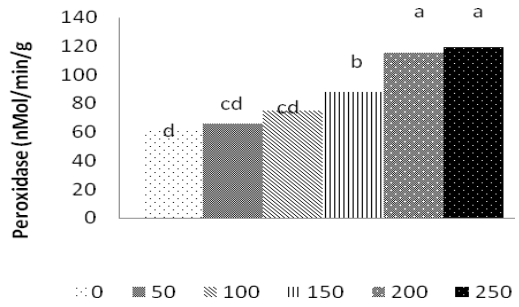

Fig. 6. Effect of salinity on POX activity in *vitisvinifera* leaves (Columns with same letters have not significant differences).

APX activity increased by salinity levels until 150mM saline treatment then decreased. Maximum APX recorded for 150 mM saline treatment (69 nMol.mn^{-1}.g^{-1} FW). Minimum APX activity was recorded for control treatment (31 nMol.mn^{-1}.g^{-1} FW) (fig7).

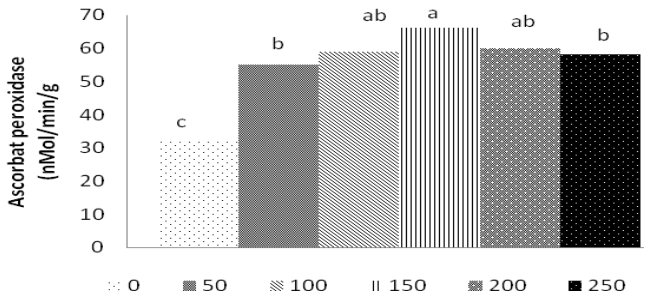

Fig. 7. Effect of salinity on APX activity in *vitisvinifera* leaves (Columns with same letters have not significant differences).

The results of the present study showed that saline treatment had the significant effect on physiological and biochemical parameters such as soluble sugar, proline, chlorophyll, catalase, peroxidase and ascorbat peroxidase enzymes. Chlorophyll a and b were decreased by increasing of saline treatment (fig 1 and 2). One of the remarkable effects of saline treatment is decline of chlorophyll a and b. Increasing salinity level leads to decreased chlorophyll biosynthesis through increased salt.Increasing of saline treatment, lead to increasing of some osmolites such as proline and soluble sugar content (fig 3 and 4). Under high salt stress plant cells decrease their osmotic potential by accumulation of some solutes such as proline and soluble sugars (Youssef and Al-Fredan, 2008). Proline plays an adaptive role in mediating osmotic adjustment and protecting the sub-cellular structures in stressed plants (Voetberg and Sharp, 1991). Proline is more efficiency to protection against stress comparison with other conventional osmolit compatible such as sugars and sugar alcohols (Verma, 2003). Some enzymes such as CAT, POX and APX had the anti-oxidative roles in front of oxidative stresses. In the present study, results showed that with increasing of saline treatment until 150 mMCAT and APX activities increased than decreased. However POX activity was increased by increasing of saline treatment significantly. Salt stresses could cause oxidative damage. Therefore, plant cells need different mechanisms, which enable the detoxification of excess reactive oxygen species (ROS) and keep the balance of the formation and removal ROS. The antioxidant enzymes remove some dangerous reactive oxygen species produced by oxidative stresses. The imbalance between the production of reactive species and antioxidants is called oxidative stress; this condition is known as a cause of damage in biomolecules (DNA, lipids and proteins) and is involved in the generation or aggravation of more than a hundred pathological conditions (Berger, 2005). In this study, the changing of CAT activity was related with the level of salinity. There were significant changes in CAT, POX and APX activities with increasing of salinity levels as oxidative stress. In final the results of the present study reviled that salt stress decreased some biochemical traits and decreased some damages by increasing of proline and sugar content and antioxidant enzymes such as CAT, POX and APX.

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