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Effect of salicylic acid on photosynthetic pigments of *Myrtus communis* L. under salt stress condition

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Abstract

Salicylic acid is known as an important signaling molecule that regulates plant reactions to salt stresses. It plays a critical role in the regulation of physiological functions as non-enzymatic antioxidant. In this study we investigated the effects of different concentrations of salicylic acid (0, 50, 100 and 150 ppm) that were sprayed on one age seedlings of myrtle (*Myrtus communis* L.) with different levels of NaCl (0, 25, 50, and 100 mM) on activities of total chlorophylls, chlorophyll a, chlorophyll b and Carotenoid. Total chlorophylls showed the most significant decrease at 100 mM of salinity concentration and salicylic acid treatments was significant decrease at 100 and 150 ppm concentrations in all treatments. Chlorophyll a had the highest content in 25 Mm and was the least content at 100 Mm of salinity treatment. There was not a significant differences in chlorophyll b content between different levels of salinity applied. But showed significant decrease in 150 ppm salicylic acid concentration. Carotenoid content had a significant increase in 100 Mm of salinity but had not in salicylic acid treatment. Total chlorophylls and chlorophyll a had the highest decrease and significant interactions in 100 mM of salinity with 150 ppm salicylic acid concentration.

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Introduction

Soil salinity and alkalinity are main problems in almost all of irrigated areas and might be occur in agricultural fields and grasslands. In other words, all lands are capable to be salted. Soil salinity, one of the most serious problems attracts many scientists to overcome this obstruction by improving salt tolerant lines (Gholizadeh and Navabpour, 2012). Salinity stress reduces the plant growth and because of interruption in equivalence of essential elements absorption, water and oxidative tension, decrease the yield (Parida and Das, 2005; Mollassiotis *et al.*, 2006). The metabolic effects of salicylic acid and its compounds depend on the type of plant, the amount and manner of use of salicylic acid (Hayat and Ahmad, 2007). Salicylic acid has its effect on photosynthesis through its effect on stomatal factors, colorants and the structure of chloroplast and the enzymes involved in the photosynthesis process. The use of salicylic acid on canola leaves increased chlorophyll content (Ghai *et al.*, 2002). Wheat also increased salicylic acid at a concentration of 10^{-5} molar and increased the amount of photosynthetic pigments, but higher concentrations of salicylic acid reduced the amount of pigments (Hayat and Ahmad, 2007). In mustard plant, the concentration of 10 -5 molar salicylic acid also increased the chlorophyll content by 20%, but decreased the chlorophyll content from 10^{-3} molar to control (Fariduddin *et al.*, 2003). But in corn and soybean leaves treated with acetylsalicylic acid and gentamic acid there was no change in the amount of chlorophyll (Khan *et al.*, 2003) and in plants *Vinga mungo* and *barley*, salicylic acid treatment reduced the amount of chlorophyll and carotenoid In the leaves (Hayat and Ahmad, 2007). In a study on corn, chlorophyll a and chlorophyll b were also reduced by salinity, which increased with salicylic acid treatment (Khodary, 2004).

In a study on olive trees, chlorophyll content of leaf and carotenoid was also negatively correlated with salinity (Ben Ahmed *et al.*, 2010). The effects of salinity of 0, 125 and 250 mM NaCl on the plant indicated that chlorophyll content decreased with increasing salinity but this decrease was only significant in comparison with the control, but not significant between salinities. Regarding the amount of carotenoid, the decrease in salinity was observed, but this decrease was not significant even with the control (Dicori *et al.*, 2013). The study on wheat with salinity treatments of zero, four and eight dS / m, and salicylic acid at 1 mM and zero showed that with increasing salinity levels, the amount of chlorophyll b, a decreases and the application of SA alone Increases the amount of chlorophyll b, a. The application of salicylic acid and salinity together in all salinity levels increased the amount of chlorophyll b, a relative to sole salinity (Morad *et al.*, 2013).

Soil Salinity and alkalinity are main problems in almost of irrigated areas and might be occur in agricultural fields and grasslands. In other words, all lands are capable to be salted. So, it is vital and important to manipulate this kind of problems and new methods to utilize this type of soils and salted water resources that unfortunately this resources are increasing and become intensive (Pessarakli and Azabolecs, 1999).

Salinity stress reduce the plant growth and because of interruption in equivalence of essential elements absorption, water and oxidative stress, decrease the yield (Parida and Das, 2005; Mollassiotis *et al.*, 2006). Salinity has contribution on related enzymes in glycolysis stages, respiration enzymes and mitochondrial electron exchange chain; so, the total carbon metabolism influenced by salt stress (Parida and Das, 2005; Orcutt and Nilsen, 2000). The researches about impact of different stress on grassland and forest plants are less (Winicow, 2005). Less proficiency of products such as subordinate forestall products, may be due to abiotic stresses (Shanker and Venkteswarlu, 2011). One of the physiologic methods that in recent years had been used for diminishing the environmental stresses on different plants is application of stress extenuating materials (Yuan and Lin, 2008). One of these materials is salicylic acid that is an important messenger molecule and it helps plant to response to the environmental stress, and has a consequential role in adjustment of physiological activities in plant, such as a non-enzymatic antioxidant (Arfan *et al.*, 2007).

Myrtle (*Myrtus communis* L.) is an evergreen shrub that reaches a height of 3 meters. Myrtle is a compatible plant in sub-humid areas of provinces included: Lorestan, Fars, Kerman, Sistan and Isfahan (Azadbakht, 1999; Zargari, 1996). This plant due to the special conditions and development in the areas of warm and unsuitable habitat has vital role in retention and preservation of environmental elements in this region. This shrub prevents both soil erosion and washes ions and protects dams and also cause to infiltration water into the soil.

Myrtle has a considerable importance, given the role of in semi-arid ecosystems in terms of soil conservation and prevents soil erosion and also its importance to use in multipurpose culture. This genius is herbal and in cancer, infectious diseases, liver diseases and diseases of the respiratory system has many practical uses. This idea justified with tends to grow by: villagers, essence production centers and medicinal plants factories. Unfortunately, the distribution of this species in many areas, due to changes in habitat conditions did not have a pioneer progress.

Materials and methods

Plant materials and treatments

In this study, seeds were collected from maternal plants growing in Jiroft, Kerman province of Iran. Pots were in green house of agricultural research and natural resources center of Kerman. During the emergence of seedlings, the weak seedling were removed to have a suitable room in each pot. Pot experiment done by 12-centimeter vases that were filled by sand, peat and field soil (proportion was 1:1:1). At the age of one-year, salicylic acid was sprayed to pots at levels of: zero, 50, 100 and 150 ppm then salinity treatments at amounts of: zero, 25, 50 and 100 mM of sodium chloride were applied. The plants were irrigated by 3:1 Hoagland solution two times per week for two months and salicylic acid was sprayed on the plants two times per weeks. Sodium chloride was added to 3:1 Hoagland solution (Tattini *et al.*, 2006).

Chemical analyses

The amount of Glysin-betaein in dried leaves (third leaf from plant top) was measured (Gerive and Grattan, 1983) and at a wavelength of 365 nm was measured. Measurement of prolin was done via spectrophotometry at 25 ± 1 °C (Bates, 1973) and at a wavelength of 520 nm was measured. To measure damage to membrane, ion leakage was assessed (Campos *et al.*, 2003).

Chlorophy measurement was based on Arnon *et al.* (1994). First, a gram sample of leaves of control plants and tension in Chinese mortars were thoroughly washed with 5 ml of 80% acetone and after straightening the final volume of the extracted extract (Centrifuge 8000 round for 10 minutes) to 10 ml Increased. One milliliter of the dark extract was removed and 9 ml of acetone was added to make up to 10 ml. At first, the spectrophotometer was zero with 80% acetone, and then the extract absorption was extracted and read at 645 nm, 663 nm, 470 nm, 480 nm and 510 nm. Then, using the following relationships, chlorophyll a, chlorophyll b, total chlorophyll and carotenoid were calculated.

$[(12.7 \times A663) - (2.69 \times A645)] \times V / 1000 \times W =$ mg of chlorophyll a per gram of leaf

$= [(22.9 \times A645) - (4.69 \times A663)] \times V / 1000 \times W$ mg of chlorophyll b per gram of leaf

$[(20.2 \times A645) + (8.02 \times A663)] \times V / 1000 \times W =$ milligrams of total chlorophyll per gram of leaf

$[7.6 \times (A480) - 14.9 \times (A510)] \times V / 1000 \times W =$ mg carotenoid per gram fresh leaves

Where, A is the absorbance at the desired wavelength, V is the final volume of acetone 80% in milliliters, and W is the fresh leaf size in grams.

Statistical analysis

This study was done using a factorial experiment in a randomized complete block design with four replications. Factors were included: four levels of salinity (0, 25, 50 and 100 mM) and four levels of salicylic acid (0, 50, 100 and 150 ppm). Analysis of variance was done by SAS (ver. 9.1) and the comparison of means was carried out using Duncan's multiple range test.

Results and discussion

Table of analysis of variance showed that Salicylic acid and Salinity treatments, as well as their interactions at 1% level on total chlorophyll and chlorophyll a were significant and only the interaction effect of treatments on chlorophyll b was significant. The effect of salinity was significant at 5% level on carotenoid, but the effect of salicylic acid treatments and the interaction of salinity and salicylic acid on carotenoid content was not significant (Table 1).

Table 1. Analysis of variance mean squares of myrtle traits.

(MS)				Df	Source
Carotenoid	Total chlorophyll	Chlorophyll b	Chlorophyll a		
0.0001ns	0.002 ns	0.0002 ns	0.002 ns	3	Replication
0.0003*	0.015**	0.0008 ns	0.04**	3	S
0.0001ns	0.025**	0.001 ns	0.04**	3	SA
0.00007ns	0.004**	0.002**	0.009**	9	S*SA
0.0001	0.001	0.0005	0.0008	3	Error
20.97%	17.28%	30.64%	17.71%	45	CV

ns=non-significant , P distinctly significant, S=Salinity, SA= Salicylic acid.

Salicylic acid and interactions were not significant in any of the treatments on carotenoid but the effect of salinity was significant at 5% level (Fig. 1). In the main effect of salinity on total chlorophyll at a concentration of 100 mM, salinity was significantly reduced compared with other treatments and there was no significant difference in total chlorophyll content among other salinity concentrations (Fig. 2). There was no significant difference in total chlorophyll content between Salicylic acid levels of 0 and 50 ppm. However, in 100 and 150 ppm treatments, the decreasing trend is significant in comparison with the control.

In chlorophyll a, the highest amount of chlorophyll was observed in salinity concentration of 25 mM and then showed a decrease in 0, 50 and 100 concentrations (Fig. 2). There was no significant difference between 0 and 50 mM treatments. However, in the salinity concentration of 100 mM, the lowest chlorophyll a was observed, which showed a significant difference with other concentrations (Fig. 3). Chlorophyll a had the highest concentration in 50 ppm Salicylic acid.

Then, in the concentration of 0, 100, and 150 ppm, the reduction trend was observed significantly Respectively (Fig. 3).

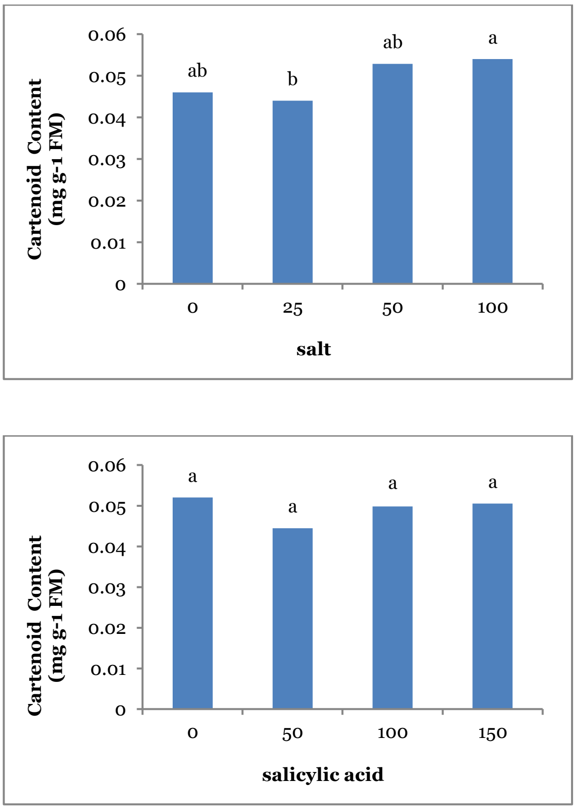
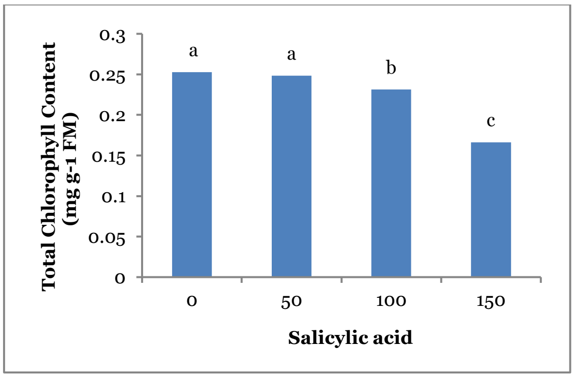


Fig. 1. Effect of salinity and salicylic acid treatments on carotenoid.



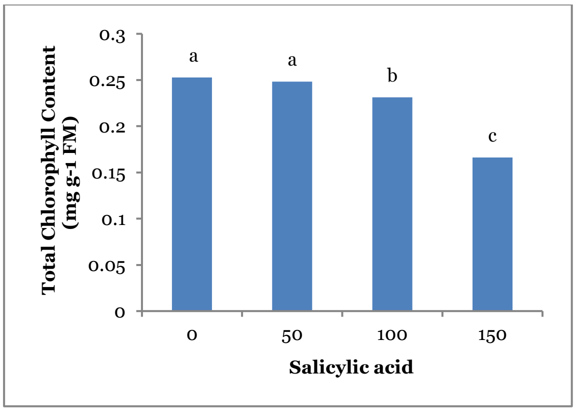
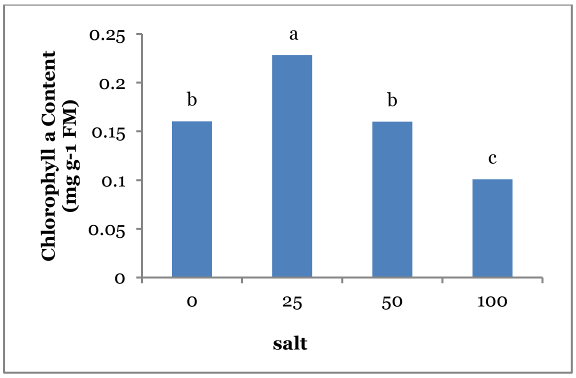


Fig. 2. Effect of salinity and salicylic acid treatments on total chlorophyll.



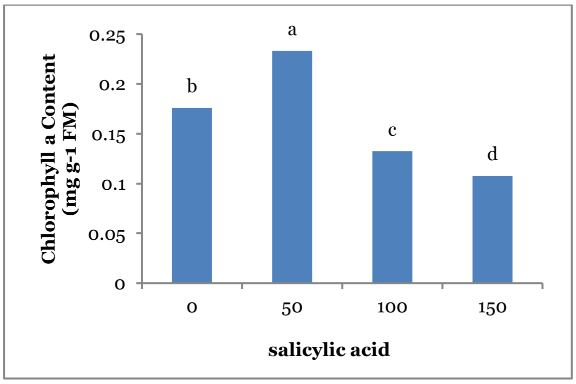
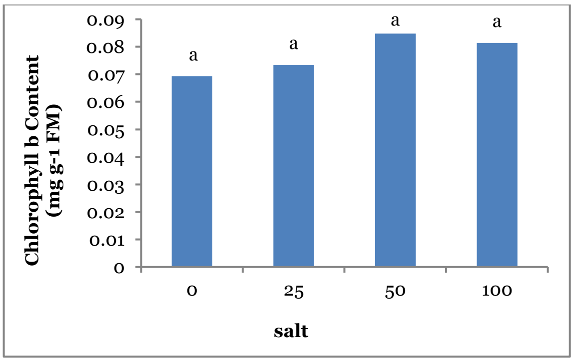


Fig. 3. Effect of salinity and salicylic acid treatments on chlorophyll a.



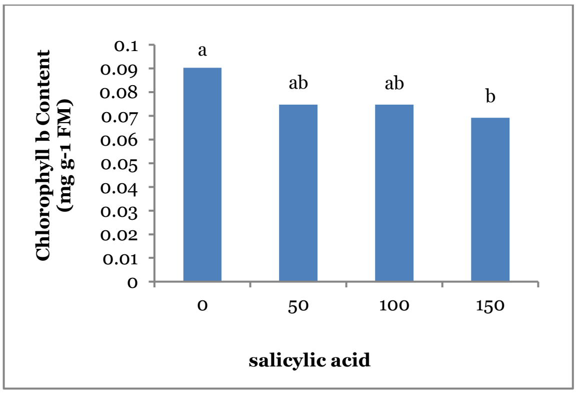


Fig. 4. Effect of salinity and salicylic acid treatments on chlorophyll b.

Salinity did not show any significant differences in chlorophyll b content at any of the levels (Fig. 4). And the effect of salicylic acid was only significant at 150 ppm as compared to control (Fig. 4). In the study of interaction effect on total chlorophyll content, salicylic acid treatment of 150 ppm and salinity of 100 mM had the most effect on the content of total chlorophyll and chlorophyll a, in fact, salicylic acid at this concentration had a more negative effect on salinity.

Makes However, no significant reduction was observed for carotenoid in the interaction effect, which indicates the auxiliary of this pigment. The interaction between salinity and salicylic acid in chlorophyll b was also significant. In the study of interaction effect on total chlorophyll content, salicylic acid treatment of 150 ppm and salinity of 100 mM had the most dropping effect on the content of total chlorophyll and chlorophyll a, in fact, salicylic acid at this concentration had a more negative effect on salinity. However, no significant reduction was observed for carotenoid in the interaction treatments, which indicates the auxiliary of this pigment.

The interaction between salinity and salicylic acid in chlorophyll b was also significant. Chlorophyll a in salinity of 25 mM and salicylic acid of 50 ppm had the highest activity level. In the combination of 100 mM salinity with salicylic acid 150 ppm, almost chlorophyll a showed a decreasing trend indicating damage to the photosynthetic system. The lack of interaction effects on carotenoid is probably due to the fact that the pigment is helpful in photosynthesis. Particularly in the main effects of salinity and salicylic acid at the highest levels, it is not significant, which probably indicates that the destructive effects of these treatments are still not so much that the plant wants to use auxiliary pigment to compensate for this damage.

Carotenoid are known to support photosynthetic pigments and photosynthesis, which can convert excess energy of short wavelengths into one-to-one oxygen to three-fold oxygen and, by producing oxygen radicals, the role Anti-oxidants (Inze and Montagu., 1995). Environmental stresses reduce the photosynthesis pigmentation, which depends on the genotype of the plant (Juan *et al.*, 2000 (Romero et. Leaf chlorophyll and carotenoid usually decrease in salinity stress, but if the salinity period is prolonged, the old leaves are chlorinated and Fall down (Parida *et al.*, 2004). Another report also found that the amount of chlorophyll and carotenoid in carrots was reduced by salinity (Eraslan *et al.*, 2007). This reduction of chlorophyll is probably due to the activation of the chlorophyll catabolic pathway. Changes in chlorophyll a, and b depend on plant salinity during salt stress, salinity level, salt type and age of the plant. In stresses, the lowest chlorophyll content is related to salinity stress and chlorophyll content depends on the resistance of the plant to salinity (Sairam and Srivastava, 2002).

In this study, the lowest level of carotenoid was observed in 25 mM salinity, the carotenoid did not follow a specific pattern in response to salinity, which is probably due to the auxiliary nature of this pigment. Karimi *et al.* in Kochia plant Reported that the maximum levels of chlorophyll a and b were observed in control plants and the lowest chlorophyll content was observed in salinity at a concentration of 200 mM of sodium chloride (Karimi *et al.*, 2005).

In the present study, chlorophyll a and total chlorophyll content were observed at a stress level of 100 mM. These results indicate that increased stress has a greater effect on photosynthetic pigments. The effect of salinity has been demonstrated on the absorption of some ions, such as Fe and Mg, that have been used in the chloroplasts building, so, after lowering these ions, the chlorophyll content of the leaves decreases and as a result the photosynthesis decreases (Hanafy *et al.*, 2002). Reducing the amount of chlorophyll can be due to the fact that the plant begins to decompose chlorophyll. In particular, the closure of the stomata and the reduction of stomatal conduction lead to the release of radicals. On the other hand, the reduction of chlorophyll and the efficiency of the photocyte II lead to the decomposition of chlorophyll, and if continued, will have a serious effect on plant growth. In some salinity experiments, chlorophyll a and b decreased, pure photosynthesis and stomatal conductance and transpiration were found to be between 75% and 94% (Netondo *et al.*, 2004). In more severe stresses, a factor other than the chlorophyll degradation enzyme, such as the effects of osmotic stress on thylakoid chloroplasts, can affect chlorophyll decomposition (Santos, 2004).

Carotenoid can also directly oxidize themselves by these oxygenates. Therefore, they indirectly reduce the production of free radicals. Carotenoid, through the xanthophyll cycle, also causes oxygen consumption and chlorophyll protection against photo-oxidation. Therefore, the protective role of carotenoid in salt stress and salicylic acid consumption is significant (Khadri *et al.*, 2006). Such results are confirmed by El-Tayeb (2005) and Yildirim and Guvenc (2008). They stated that salicylic acid treatment increased the amount of chlorophyll and carotenoid in plants and increased the rate of photosynthesis under salinity stress conditions.

In this study, was the highest chlorophyll in salinity 25 mM and salicylic acid 50 ppm. Reduction of carotenoid in salt stress due to the destruction of beta-carotene and the formation of zeaxanthin (Sultana *et al.*, 1999) (Sharma and Hall., 1991). In Kochia, the maximum carotenoid was observed in the control plant and at least in the salinity treated plant with a concentration of 200 mM sodium chloride (Karimi *et al.*, 2005). On the other hand, salicylic acid activates the synthesis of carotenoid (Hayat and Ahmad, 2007).

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