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Evaluation of Interaction Effect of Drought Stress with Ascorbate and Salicylic Acid on Some of Physiological and Biochemical Parameters in Okra (*Hibiscus esculentus* L.)

[1]Baghizadeh Amin, [2]Ghorbanli Mahleghah, [1, 3]Haj Mohammad Rezaei Mahmood and [1]Mozafari Hossein
[1]International Center for Science, High Technology and Environmental Sciences, Kerman, Iran
[2]Department of Biology, Islamic Azad University, Gorgan Branch, Iran
[3]Department of Biology, Payam Noor University, Tehran Branch, Iran

Abstract: Drought stress causes oxidative damages in plant cells. Two of compounds which have antioxidative characteristic are Salicylic Acid (SA) and Ascorbic acid (As). These compounds can decrease drought effects in plants under stress. In this research, the drought stress was treated in 3 levels of drain (Control, 1/3 Field Capacity, 2/3 Field Capacity). Then, interaction effects of drought stress with SA and As on some of physiological and biochemical parameters of *hibiscus esculents* L plants were investigated. In recent research, we evaluated parameters such as; leaf area, Dry and Fresh Weight of leaf (DW and FW), lipid per oxidation amount, proline content, sugar content, ion leakage, protein content and photosynthetic pigments content. The results shown that leaf area, FW and DW parameters decreased compared to check under stress significantly. Lipid per oxidation, sugar and proline content and ion leakage increased in plants were under with drought stress. Reduction of protein content in this condition showed that drought stress effected protein biosynthesis and degradation. Moreover, significantly reduction of photosynthetic pigments content showed that stress effected photosynthetic process. Based on our results it seems that salicylic and ascorbic acid can considerably alleviated oxidative damage that occurred under drought stress condition. Therefore, we concluded that salicylic acid and ascorbic acid in the concentration 1 mM have in mitigation of stress caused by drought stress.

Key words: *Hibiscus esculentus* L, drought stress, salicylic acid, ascorbic acid

INTRODUCTION

When the plant tissues are subjected drought stress, some Physiological and biochemical changes occur. Some of these changes include: accumulating ABA, closing stoma and reducing leaf area (Premachandra *et al*., 1995). Drought also causes decreasing the speed of leaves growth and the walls of cells become sclerotic. These changes increase the plants resistance on drought stress. Drought stress causes decreasing biomass in plants. Increasing lipids per oxidation was regarded as an index of increasing oxidative stress. Also, because of drought stress, the concentration of proline increase very much (Larcher, 2001). Proline is as osmo regulation in abiosis Stress in plants (Bates *et al*., 1973). Protective mechanisms of proline still are not distinguished completely but his roles are more include: the mitigation of osmotic pressure Fixation of macromolecules and broom of oxygen types (Zhang *et al*., 2000). It is reported that drought stress causes many changes in the amount of plants carbohydrate and it become clear that with increasing drought stress on leaves, the amount of starch decrease One of the parameter, which is measured as an index of destruction membrane is ion leakage that the amount increases along stress (Caruso *et al*., 1999). When resistant plant after a period of anhydrous is in a good irrigation condition, show some signs of cell damage. One of these signs is ion leakage. Recent studies show that during cells dehydration, amphiphylic molecules enters membrane from cytoplasm humor that the existence of these molecules in membrane cause ion leakage and again in a good irrigation condition, these molecules come out of membrane and in this case the ion leakage stop (Black and Prithard, 2002). Under stress conditions, protein synthesis causes and degradation happen rapidly (Black and Prithard, 2002). The different types of oxygen which are produced during drought stress cause decreasing and chemical analyzing photosynthetic pigments. The chlorophyll pigment has a main role in photosynthetic (Sairam *et al*., 1998). Te Carotenoides are

Corresponding Author: Baghizadeh Amin, International Center for Science, High Technology and Environmental Sciences, Kerman, Iran

from co pigments of photosynthesis process which absorb wavelength that chlorophyll can't absorb and transfer it to central reaction in phenosystem II. Also, carotenoieds protect cells with absorbing radicals of oxygen and stopping these radicals (Develin and Withman, 2002). Moreover, carotenoieds by xanthophylls cycle with epoxidation and depoxidation reactions cause consuming oxygen and protecting from chlorophyll against phetoxidation (Sairam *et al*., 1998). Under drought stress the produced super oxide radicals cause lipids per oxidation (Sairam *et al*., 1998). One product of lipids per oxidation is malon D-alloide (Borsani *et al*., 2001; Sairam *et al*., 1998). Electrogenic pumps and ATP membrane azes have some problems because of lipids per oxidation so membrane's potential destroy (Sairam *et al*., 1998).

MATERIALS AND METHODS

In this research, *Hibiscus esculentus*. L plants were sown in plastic pots containing sand, clay and peat (2:1:1). After one month, when four fully expanded leaves were appeared, salicylic acid and ascorbic acid were sprayed to the leaves at tow levels of concentration (0 mM: control and 1 mM) for 10 days. Three levels of drought stress (control, 1/3 filed capacity and 2/3 field capacity) were applied. Then the interaction effects of drought stress with salicylic acid and ascorbic acid investigated on some of physiological and biochemical parameters. For dry and fresh weight of third leaf, after separating leaves, the weight of each one was measured with 0.001 preciseness in gram with sartorius scale, BPSIID. Then samples twist in aluminium fuyl and was dried in avan in 70°C for 72 h. Then dry weight of types was measured with 0.001 preciseness in gram. For measuring leaf area, we provide papers copy from leaves. Then we calculate the weight of 1×1 cm square paper and specify leaf area according to its weight, with ratio relation and were reported with square centimeter. For evaluating proline content in leaf tissue, we use method of Bates *et al*. (1973) and sugar content of leaf tissue and root was calculated with method of Somogy (1952). The results of measuring proline content was calculated and presented with gram of fresh weight. The result of measuring sugar content was calculated and presented with milligram on gram of fresh weight. Ion leakage content for measuring leakage of cell membrane was evaluated by method of Marty's *et al*. (2005). For evaluating chlorophyll and Carotenoides content we used method of Lichtenther (1987). About 0.2 g of fresh tissue of plant leaf was abraded in china mortar with 15 mL acetone 80% and after filtering, the absorption of it was read by spectrophotometer process from Germany, Cary 50 in 470, 663, 646 nm. For regulating device, we used acetone 80%. The concentration of pigments was calculated with these relations

$$\text{Chla} = (12.25\text{A}\,663.2 - 2.79\text{A}646.8)$$
$$\text{Chlb} = (21.21\text{A}646.8 - 5.1\text{A}663.2)$$
$$\text{ChlT} = \text{chla} + \text{chlb}$$
$$\text{Car} = \frac{1000\text{A}470 - 1.8\text{chla} - 85.02\text{chlb}}{198}$$

in this formula chla, chlb, chlT and car show the concentration of chlorophyll a, chlorophyll b, the total chlorophyll and Carotenoides (include carotene and xanthophylls), respectively. The results of measuring photosynthetic pigments content was calculated and presented in fresh weight in gram. Then for calculating the content of per oxidation in membrane fats, the concentration of Malon-Dialdehyde (MDA) from this reaction was measured. The measurement of MDA concentration was done in Heat and Pacher (1969) method. According to this method, 0.2 g of leaf fresh tissue was weighted and was abraded in china mortar of 5 mL Trichloroacetic Acid (TCA) 0.1%. The achieved juice was centrifuged by centrifuge device in 10000 G for 5 min. About 5.4 mL TCA solution which is 20% and contains 0.5% Thiobarbituric Acid (TBA) was added to 1 mL of the above solution from centrifuge. The achieved compound was heated in hot bath in 95°C for 30 min. Then become cool in ice immediately and again the compound was centrifuged in 10000 G for 10 min. The intensity absorption of this solution was read by spectrophotometer in wavelength of 532 nm. The specified compound for absorption this wavelength is red complex (MDA-TBA). The absorption of other nonspecific pigments was Measured in 600 nm and reduced from this content for calculating MDA concentration from silence coefficient was used and the results from measuring was calculated and presented according to microgram on fresh weight gram. Then for calculating protein concentration (Bradford, 1976), proteins were extracted from aerial organ (leaves) (3 repetitions) in 0-4 centigrade separately. For this purpose, 1 g fresh tissue was abraded in a china mortar which contain 5 mL Tris-Hcl Buffer 0.05 M in PH 7.5 for 30 min. The homogeneous solution was transferred to centrifuge tube and the samples were done with glacier centrifuge device after 10 min silence for 20 min in 13000 G and in 4°C (Lieberman and Wang, 1982). At the end stage of centrifuge, the tubes come out from device slowly and the above solution was filtered from some cloth layer and was distributed in some small vial.

The protein juice was used for evaluating the concentration of protein solution. For providing biuret agent, 0.1 g coumacyberylliantblue (G 250) was solved in 50 mL ethanol 95% for 1 h. Then 100 mL phosphoric acid 85% was added drop by drop. At the end, the total volume of solution was transferred to 1 L by distilled water. The produced solution was filtered by filter paper vatman number one for drawing standard chart, 1.4 g standard cattle albumen was solved in one liter distilled water and from this solution 25, 50, 200, 400, 600, mg concentration in liter was made. All of the research stages were repeated by agent as previously was explained in unknown samples. Then absorption of every sample in wavelength 505 nm was read by spectrophotometer device. For specifying protein concentration of unknown samples, we use standard chart. $Y = 0.0008X + 0.01$ in this relation Y equals read absorption and X equals protein concentration according to milligram on liter.

Statistic operation: In this research, the total of experiments were done in different stages in Completely Randomized Design with 3 replications and test considers the reciprocal effect of salicylic acid, ascorbic acid and drought on different parameters as factorial. The levels of 0 (control), 1/3 FC and 2/3 FC of water stress were used and the levels of salicylic acid and ascorbic acid factors were 0 mM (control) and 1 mM. The comparison of mean was done with LSD test to spss 14.0 software in probability level of 1%. For drawing graph, we use Excel 2003 software.

RESULTS AND DISCUSSION

In this study, drought stress cause reduction of dry and fresh weight of leaf and the leaf area of okra. Ascorbic acid causes increasing dry and fresh weight of leaf compared to check type. Also, treatment Salicylic with ascorbat alleviated drought stress damages on dry and fresh weight of leaf and leaf area (Fig. 1a-c). Moreover, drought stress increase sugar content in root and decrease in leaf. Salicylic acid, ascorbic acid and the compounds of these 2 treatments cause mitigation and improving damage from drought stress on sugar concentration in root and leaf of okra. Drought stress also causes increasing of proline content and ion leakage compared to check plants. Use of salicylic acid, ascorbic acid and the compound of these 2 substances on the plant, which are in drought stress conditions cause increasing of proline content of plant leaves (Fig. 1 d-g). These results have significant in level of 1% statistically. Moreover, drought stress alone causes decreasing chlorophyll a, b, total and Carotenoides in compare with check plants. the decrease of these pigments content on plant that are drought stress conditions are improved more with treatment salicylic acid, ascorbic acid and the compound of these 2 substances (Fig. 2 a-d). The MDA has significant increasing under drought stress. The Treatment salicylic acid, ascorbic acid and drought stress cause decreasing MDA content (Fig. 2 e). The results become significant in 5% level statistically. Drought stress decrease protein content considerably (Fig. 2 f). But salicylic acid, ascorbic acid and the compounds of salicylic acid with ascorbic acid treatments on okra leaves cause alleviating the effects of drought stress on protein content of leaf. The results become significant in 1% level statistically.

In this study, the leaf area, dry and fresh weight of leaf was decreased significant under drought stress. These results correspond with finding of some researchers. For example, Liberman and Wang (1982) reported that the decreasing of leaf area in drought stress conditions is the result of decreasing division and cell expansion. Also, Herralde *et al.* (1998) believed that drought causes decreasing biomass in argyranthemum plant. The plant with decreasing leaf area alleviates water mortality and causes increasing resistance strength on drought.

In the case of salicylic acid role on growth parameters, it was reported that salicylic acid set the expansion, division and cell death (Zhang *et al.*, 2002). Furthermore, salicylic acid with effect on abcisic acid (Senaranta *et al.*, 2002), Jiberlin (Traw and Bergelson, 2003), methyl jasmonat (Traw and Bergelson, 2003) and ethylene (Zhang *et al.*, 2003) hormones, regulate many physiological process and plant growing. Also, it is reported that salicylic acid with effect on abcisic acid and accumulating of this hormone on plant causes plants adaptation upon environmental stress (Shah *et al.*, 2002). It is reported that ascorbic acid increase the cell division and causes increasing leaf area and dry and fresh weight of leaf on plants and also with antioxidant property decreases the damage from oxygen radicals, which are product by drought stress (Miguel *et al.*, 2006). It is reported that drought stress causes decreasing of sugar content. The alleviation of sugar can be as a result of photosynthesis reduction, because the decreasing of water cause alleviating of torger and losing torger pressure causes closing stoma and finally decreasing photosynthesis. Okra plant with increasing sugar content in root help decreasing osmotic potential on root

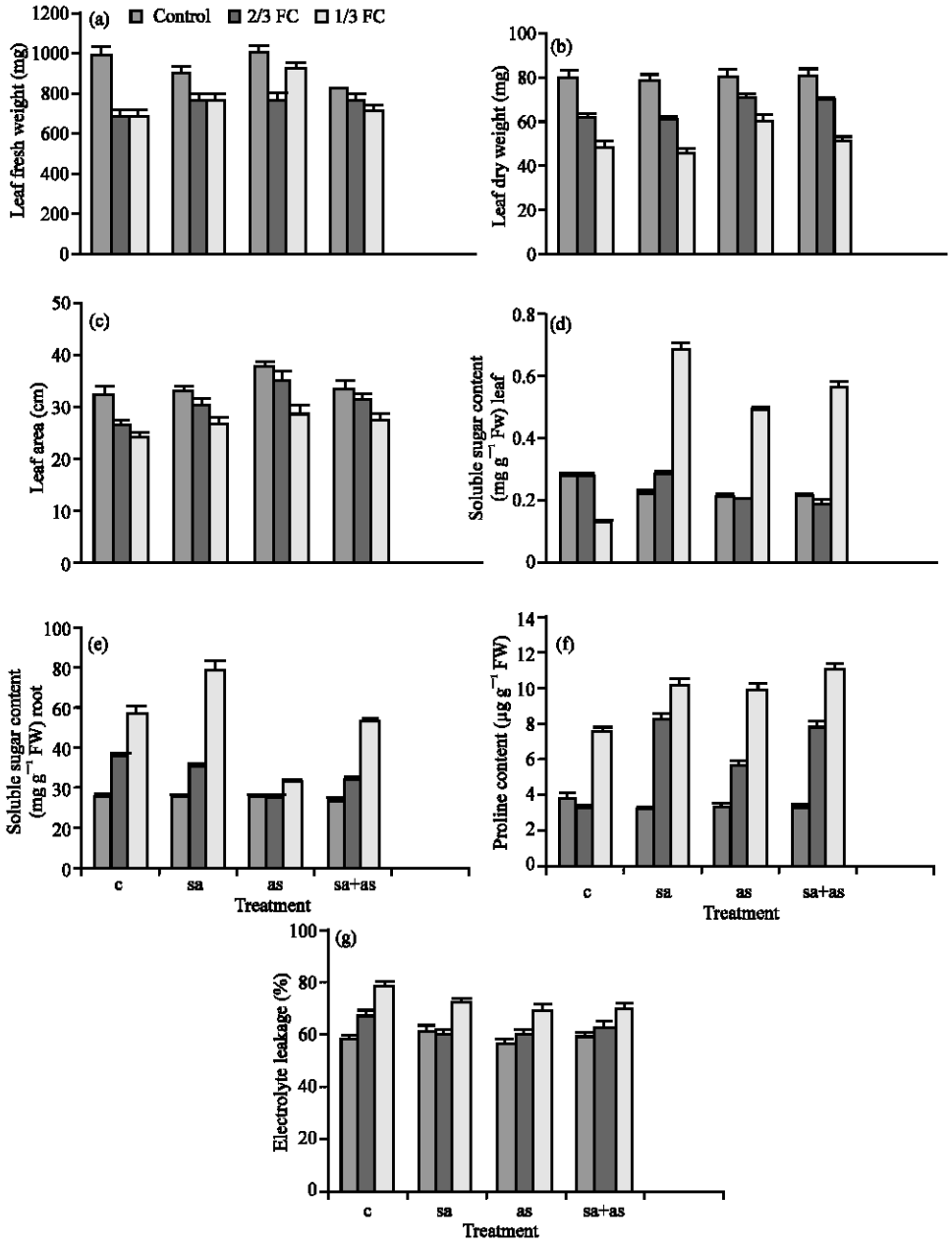

Fig. 1: a-g) The reciprocal effect of drought stress, salicylic acid and ascorbic acid on dry and fresh weight of third leaf, third leaf Area, the sugar content of leaf and root, proline content and ion leakage on leaf, respectively. (The mean comparison was done with LSD test)($p<0.01$), C: (Check), SA: (Salicylic Acid 1 mM), As: (Ascorbic Acid 1 mM), FC: (drought about 1/3 and 2/3 Field Capacity)

so absorb more water. Treatment with ascorbat and salicylic causes improving resistance of plant on stress and as a result sugar approach to its normal (Miguel *et al*., 2006) condition. One of the reason of decreasing carbohydrate in plant leaves which are under drought stress in this research is the result of the effects of these stress on Tilacoide membrane, photosynthesis pigments content and the amount of photosynthesis. In recent research, proline accumulation was observed because of drought on leaf. Redy *et al.* (2003) reported that proline content increase in drought stress time. Sairam *et al.* (1998) reported that in increasing proline causes increasing resistance on drought and salty. Plants have a series of antioxidant enzyme and no enzyme systems for

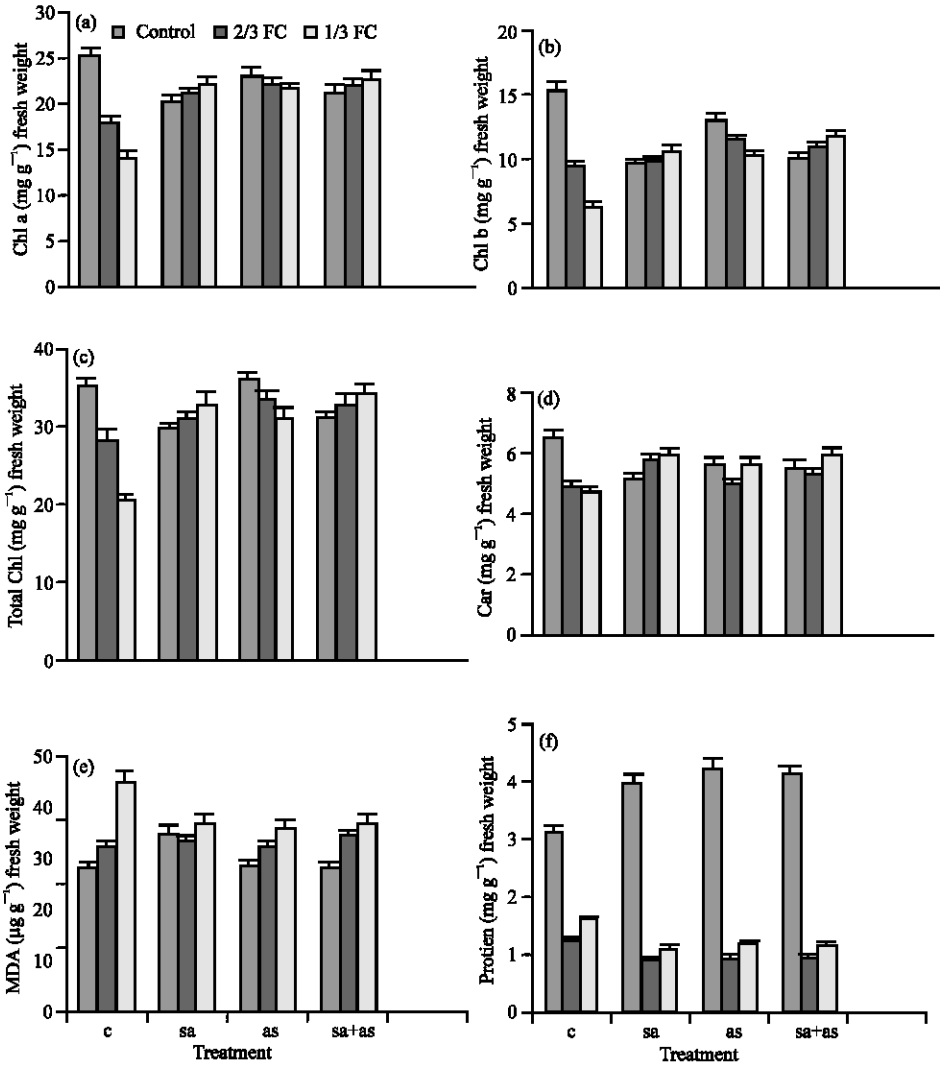

Fig. 2: a-f) The reciprocal effect of drought stress salicylic acid and ascorbic acid on chlorophyll a and chlorophyll b content, total chlorophyll, Carotenoides, MDA content and protein, respectively (The mean comparison was done with LSD test) ($p<0.05$), C: (Check), SA: (Salicylic Acid 1 mM), As: (Ascorbic Acid 1 mM), FC: (drought about 1/3 and 2/3 Field Capacity)

adapting drought stress and preventing from oxidation damage. The no enzyme systems include: ascorbat, glutathione, tocopherol, zazantin and anthrazantin (Wise and Naylor, 1987). It is reported that increasing proline and sugar cause protecting torger and the reduction of membrane damage on plants. So, osmo regulation is an adaptation that increase the tolerance toward drought stress (Inze and Montago, 2000) In barley, wheat, bean and tomato during oxidative stress the accumulation of proline content with treatment salicylic acid hormone and ascorbic acid was increased and increasing proline content and sugar and producing osmo dip in plants cause resistance against losing leaf water and increasing the speed of plant growing in stress conditions (Tasgin *et al*., 2006).

It is said that the accumulation of smolets (proline, sugar) has a direct and positive relation with highly resistance in plants which are in no environmental stress (Ramanjulum *et al*., 1998). One of the parameters that is measured as an index of damaging membrane is the leakage of electrolyte that its amount increase in stress time (Caruso *et al*., 1999) and in this case, it is said that proxidation of desaturation fat acids in membrane phospholipids causes increasing electrolyte leakage. So, leakage can be calculated as a norm for estimating the damage of bio-membrane (Caruso *et al*., 1999). The stability index of membrane that evaluated by ion secretion is a norm from damage to membrane because of fat proxidation by active types of oxygen (Jiang and Nhunag, 2001). Other researchers also reported

damage to cell membrane because of drought stress (Sairam *et al*., 1997; Sing *et al*., 1992). The compounds with antioxidant property like ascorbic acid (Miguel *et al*., 2006) and salicylic acid (Avancini *et al*., 2003) with increasing ability of antioxidant plant are able to mitigate during drought stress, the amount of damage from drought stress on cell membrane with booming the produced oxygen radicals. According to the observed negative correlation between the amount of damage to cell membrane, with yield in stress condition suggested that the more conserve of cell membrane in drought uniformity Condition, the more the yield. It is reported that in maize doesn't have a great change than a check sample lonely in ion leakage, but when the plant placed under drought stress, salicylic causes enough changes in ion leakage (Nemeth *et al*., 2002). Drought stress causes damage in enzyme systems of active oxygen types inhibitors which cause increasing fat proxidation and finally damage to cell membrane and pigments (Bowler *et al*., 1994). One of the important factors in preserving photosynthetic capacity is the chlorophyll content in alive plants (Jiang and Nhunag, 2001). It is supposed that the decreasing of chlorophyll content of drought stress is because of increasing the production of oxygen radicals that cause proxidation of these pigments and finally chemical analyzing them (Wise and Naylor, 1987). Similar, reports exist for decreasing chlorophyll in wheat, cicer and white mulberry in drought condition (Hyodo and Yang, 1971). The accumulation of active oxygen types that produce during drought stress, damage to many cell compounds like fat, protein and photosynthetic pigments (Sairam *et al*., 1998; Jiang and Nhunag, 2001). It is reported that in wheat, cause decreasing damage effects of drought strss on photosynthetic pigments under stress condition with ascorbic acid (Hamad and Hamda, 2001). There are contradictory reports about the role of salicylic acid on photosynthetic pigments. Lusia *et al*. (2005), reported that methyl salicylic don't have any effect on photosynthetic pigments but photosynthesis decrease under treatment salicylic acid. It is reported that salicylic acid causes increasing photosynthetic pigments in plant, under salty stress (El-Tayeb, 2005). Contradictory reports are from equivocally effects of salicylic acid which act as a phenol compound and probably with activation of O_3 cause damage of chloroplast proteins and proxidation of membrane lipids of tilacoide and finally decrease photosynthetic pigments under normal condition. But in the time of stress, Salicylic acid protect from photosynthetic device through increasing the ability of cell antioxidation and new proteins synthesis (Avancini *et al*., 2003). Increasing lipids proxidation was regarded as an index of increasing oxidative stress (Meirs *et al*., 1992). In recent research, drought stress increase MDA. Sairam *et al*. (1998) reported that MDA increased in 3 genotype of wheat under drought effect. It is proved that produced MDA content is different between cultivars of maize, banana and rice during drought stress; so that resistance spices on drought can broom h_2O_2 with increasing ability of antioxidation and by decreasing H_2O_2 produce less MDA. But the amount of produced MDA of sensitive cultivars are more (Sharam and Shanker, 2005). The observed increase of MDA in this research is in drought stress condition, which results from producing active oxygen types like super oxide radical, hydrogen peroxide and hydroxide radical. Ascorbic acid can refuse from lipids proxidation by omitting active oxygen types and decrease MDA (Miguel *et al*., 2006). In the case of salicylic acid role for solving oxidative stress, there are many reports. Salicylic acid protects Arabidopsis against drought stress by effect on antioxidation enzyme and preoxidation of lipids (Larkindale and Knight, 2002). The studies show that salicylic acid causes preventing from damage to desaturation fat acid, the reduction of membrane leakage and preventing from tilacoide membrane in the time of salty stress in Arabidopsis (Borsanio *et al*., 2001). Besides, salicylic acid causes the produced MDA was reduced during salty stress in leaves and roots of barley (Elstner *et al*., 1976). Against other reports there is a reduction antioxidation ability and as a result increasing free radicals in treatment salicylic acid (Lisia *et al*., 2005). In this research, the protein content of plant leaf was reduced significantly under drought stress than the check.

The active types of oxygen produced and accumulated under the contrary environmental conditions like drought stress. Along with it the increasing H_2O_2 causes increasing protein oxidation in some plant species. Drought is the case of reducing the activity of rubisco and also the content of on plant (Inze and Montagu, 2000). Drought stress causes decreasing the production of protein in some species of plants by decreasing cell polyzoms. Active radicals of oxygen with differing conditions of amino acid in proteins cause easing effect of analyzing proteins enzymes so one of the reason of decreasing protein content in plants which are in drought stress is producing free radicals of oxygen (Somogy, 1952). It is reported that salicylic acid effects on producing defensive proteins and different kinds of kinas and rubisco.

Also, it is proved that salicylic acid inculcates the synthesis of suppressive proteins of plants proteases (Popova *et al*., 1997).

El-Teyeb (2005) reported that the solution protein content and free amino acid in aerial organs and roots reduced in stress conditions. So, reducing protein content

or increasing analyzes or both of them can be related to increasing activity of antioxidant enzymes. In the manner that salicylic acid and ascorbic acid protect from oxidation of plant proteins by increasing the ability of antioxidant (Sairam *et al*., 1998; Miguel *et al*., 2006).

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