Assessment of salicylic acid impacts on growth rate and some physiological parameters of lettuce plants under drought stress conditions

Mohammad Sayyari¹, Mojtaba Ghavami², Fardin Ghanbari² and Sajad Kordi³

ABSTRACT: Drought stress is a major constraint for crop production in arid and semiarid regions, such as Iran. In this study, the effect of salicylic acid (SA) on lettuce (Lactuca sativa L.) plants under drought stress was studied. The layout was factorial experiment in randomized complete block design (RCBD) with 3 levels of drought stress including stress-free conditions, mild and severe stress and 3 concentrations of SA (0, 0.75 and 1.5 mM) as main factors and 3 replication. The experiment was conducted in the greenhouse of agricultural faculty of Ilam University. Statistical analysis showed significant effects of the drought and SA on plant growth parameters, photosynthetic pigments, leaf relative water content (RWC), electrolyte leakage (EL), proline and malondialdehyde (MDA) content. Results showed that drought stress imposed negative effects on plant growth and productivity. In drought conditions, fresh and dry weight, leaf area, photosynthetic pigments and RWC reduced but EL, proline and MDA increased. SA application significantly increased plant fresh and dry weight, leaf area, Carotenoids and proline and decreased MDA accumulation and EL. In this experiment, SA treatment, with enhancing growth Rate and changing physiological parameters decreased adverse effects of drought stress on lettuce plants.

Keyword: Electrolyte Leakage, Malondialdehyde, Photosynthetic Pigments, Proline Content

INTRODUCTION

Environmental stresses, such as drought, salinity, high and low temperatures, cause adverse effects on plants growth and productivity of crops. Abiotic stress is the primary cause of crop loss worldwide, reducing average yield for most major crop plants by more than 50%. Among abiotic factors that have shaped and continued shaping plant evolution, water availability is most important (Rodrigue et al., 2005). Drought stress causes an increase of solute concentration in environment, leading to an osmotic flow of water out of plant cells. This in turn causes high solute concentration inside plant cells, then low water potential and membranes disruption along with essential processes like photosynthesis. These drought-stressed plants consequently exhibit poor growth and crop yield (Taheri Asghari et al., 2009).

Breeding, genetic engineering and use of plant growth regulators (PGRs) are some approaches to increase plants tolerance against stresses. In recent studies number of plant growth regulators have been under trial to alleviate drought stress injuries in plants, such as Mepiquat Chloride (Ahmed et al., 2009), Brassinosteroids (Bajguz and Hayat, 2009), Caronatine (Ai et al., 2008), Jasmonate (Wang., 1999), 5-aminolevalenic acid (Liu et al., 2011) and Salicylic acid (Senaratna et al., 2000).

Salicylic acid (SA) belongs to phenolic compound and is an endogenous growth regulator which participates in regulation of physiological processes in plants such as seed germination, fruit yield, glycolysis, flowering and heat production in thermogenic plants (Klessing and Malamy, 1994). Ion uptake and transport (Harper and Balke, 1981), photosynthesis rate, stomatal conductivity and transpiration (Khan et al, 2003) could also be affected by SA application.

Several methods of SA application (seeds soaking prior to sowing, adding to the hydroponic solution, irrigating or spraying with SA solution) have been shown to protect various plant species against abiotic stress by inducing a wide range of processes involved in stress tolerance mechanisms (Horvath et al., 2007). Agarwal...
et al. (2005) demonstrated the enhanced chlorophyll levels and relative water content (RWC) as well as the lessened hydrogen peroxide (H₂O₂) and lipid peroxidation when the wheat leaves were treated with SA under water stress conditions. Application of SA significantly increased growth parameters, photosynthetic pigments and proline content and decreased lipid peroxidation in sweet basil under salinity stress condition (Delavari et al., 2010). In addition, Yazdanpanah et al. (2011) reported that SA application declined adverse effect of drought in savory by increasing sugar, protein and proline accumulation and decreasing Malondialdehyde (MDA) and other aldehydes. Several studies also supported a major role of SA in modulating the plant response to several abiotic stresses including drought (Senaratna et al., 2000; Yazdanpanah et al., 2011). Thus, the present study was conducted to assess if the exogenous application of SA could ameliorate the adverse effect of drought stress on lettuce plants.

MATERIALS AND METHODS

Plant material and treatments

This study was carried out in layout of 3×3 factorial experiment in randomized complete block design (RCBD) with drought stress levels and SA concentrations as main factors in the greenhouse of agricultural faculty of Ilam University at 2009-2010. Physical and chemical properties of soil in experiment were presented in Table 1. Seeds of lettuce were obtained from Pakan Bazr Co. (Isfahan, Iran) and cultured in bed to obtain seedlings for experiment. When the seedling had 2-4 true leaves, the seedling of similar size were selected and transferred into plastic pots with 20 cm height, and 23 cm diameter which were filled with about 7 kg of 1:1:1 mixture of fine sand, leaf mould and garden soil. When 4-6 true leaves of seedling were expanded, SA was sprayed in ratio 0, 0.75 and 1.5 mM until both sides of the leaves were completely wet. 72 h after foliar spray, all plants were subjected to three levels of drought stress including stress-free conditions (irrigation within the field capacity (FC)), mild stress (30% of FC) and severe stress (30% of FC) until end of experiment.

Table 1. Soil analysis result for physical and chemical Characteristics.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Humidity (%)</th>
<th>Soil</th>
<th>Sand (%)</th>
<th>Silt (%)</th>
<th>Clay (%)</th>
<th>pH</th>
<th>EC (ds/m)</th>
<th>N (%)</th>
<th>P (ppm)</th>
<th>K (ppm)</th>
<th>OC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Value</td>
<td>32</td>
<td>Loam-silty</td>
<td>22</td>
<td>77</td>
<td>11</td>
<td>7.3</td>
<td>0.7</td>
<td>0.04</td>
<td>37</td>
<td>333</td>
<td>0.42</td>
</tr>
</tbody>
</table>

Plant mass and leaf area determination

All plants were cut at soil surface and fresh and dry weights (dried at 105 °C for 24 h) of plants were determined and Leaf area were measured using a Leaf area meter.

Photosynthetic pigments

0.25 g from young and full-developed leaves were homogenized with 5ml of acetone (80%) using pestle and mortar and centrifuged at 3,000 rpm. The absorbance of supernatant was measured with a UV/visible spectrophotometer at 470, 663 and 645nm and chlorophyll and carotenoids contents were calculated using the equations proposed by Lichtenthaler and Wellurn (1983) given below:

\[ \text{Chlorophyll a (µg/ml)} = 12.21(A_{663}) -2.81(A_{646}) \]
\[ \text{Chlorophyll b (µg/ml)} = 20.13(A_{646}) -5.03(A_{663}) \]
\[ \text{Carotenoids (µg/ml)} = (1000A_{470}-3.27[chl a]-104[chl b])/227 \]

Relative water content

Discs (1cm in diameter) from middle portion of fully developed leaf were randomly taken from chosen plants of each replicate. Discs were weighed (FW) and then immediately floated on distilled water for 5 h in the dark. Turgid weights (TW) of leaf discs were obtained after drying excess surface water with paper towels. Dry weights (DW) of discs were measured after drying at 75 °C for 48 h. Relative water content (RWC) was calculated using the following formula (Korkmaz et al., 2010):

\[ \text{RWC} = \left( \frac{\text{FW-DW}}{\text{TW-DW}} \right) \times 100 \]

Electrolyte leakage (EL)

In order to assess membrane permeability, EL was determined according method described by Korkmaz et al. (2010). Leaf discs (1cm in diameter) from randomly chosen plants per replicate were taken from the middle portion of fully developed leaf and washed with distilled water to remove surface contamination. The discs were placed in individual vials containing 10 ml of distilled water. After incubating the samples at room temperature on a shaker (150 rpm) for 24 h, the electrical conductivity (EC) of the bathing solution (EC1) was determined. The same samples were then placed in an autoclave at 121 °C for 20 min and a second reading...
(EC2) was determined after cooling the solution to room temperature. The EL was calculated as EC1/EC2 and expressed as percent.

**Proline content**

Proline content was determined according to the method described by Bates et al. (1973). Fresh leaf material (0.5 g) was homogenized in 10 ml of 3% aqueous sulfosalicylic acid and the homogenate was centrifuged at 10,000 rpm. 2 milliliter of the supernatant was mixed with 2ml of acid ninhydrin and 2ml of glacial acetic acid in a test tube. The mixture was placed in a water bath for 1 h at100 °C. The reaction mixture was extracted with 4ml toluene and the chromophore containing toluene was aspirated, cooled to room temperature, and the absorbance was measured at 520nm with a UV/visible spectrophotometer. Appropriate proline standards were included for the calculation of proline in the samples.

**Malondialdehyde (MDA)**

The MDA content of leaves was measured by Stewart and Bewley method (1980) with some modification. 0.5 g of fresh leaves were cut into small pieces and homogenized by addition of 5 ml phosphate buffer (50 mM) in a nice bath. Then, the homogenate was transferred into a tube and centrifuged at 14,000 g for 30 min at 4°C. 1 ml of supernatant and 1 ml of 0.5% thiobarbituric acid (TBA) in 20% trichloroacetic acid (TCA) solution were added into a new tube. This mixture was incubated at 98°C for 30 min, then cooled and centrifuged at 10,000 g for 10 min. The supernatant was subjected to analysis with the spectrophotometer. The MDA content was calculated from the subtracted absorbance (A532−A600) using the extinction coefficient of 155 m m−1 cm−1.

**Statistical analysis**

Data were analyzed for significant differences using a factorial analysis of variance with drought stress levels and SA concentrations as main factors. Statistical analysis was performed using SAS and MSTATC software programs and the means compared using the Duncan’s Multiple Range Test at p=0.05.

**RESULTS AND DISCUSSION**

**Fresh and dry weight and leaf area**

The statistical analysis showed that drought stress and application of SA had a significant effects on the morphological, physiological and biochemical parameters of the lettuce plants (Table 2). Fresh and dry weight and leaf area of plant significantly affected by drought and SA application in P<0.01, but the interaction effect on mentioned traits was not significant. Simple effects of drought (Table 2) showed that drought stress significantly decreases fresh and dry weight and leaf area of plant. Highest fresh weight (261.54 g), dry weight (22.96 g) and leaf area (91.19 Cm²) were observed under non stress condition (control) and lowest fresh weight (195.72 g), dry weight (9.15 g) and leaf area (49.36 Cm²) achieved in severe stress condition. Water stress is characterized by wilting, closure of stomata and decrease in cell enlargement and growth due to reduction of water content, turgor and total water potential. Cell division, enlargement and differentiation, are the main processes that determine the quality and quantity of plant growth, affected by various internal and external factors, such as water stress (Patel and Golakia 1988). Reduction in fresh and dry weight of Lettuce under drought condition might be associated with suppression of cell expansion and cell growth due to the low turgor pressure and also more leaf senescence under drought stress. The fresh and dry weight reduction under drought condition in various crops has already been reported (Rane et al. 2001; Bhatt et al. 2005). Severe water and salt stress is known to induce ionic stress, which changes in plant water relations, causes reduction in meristem activity as well as cell elongation, premature abscission, senescence of adult leaves, thus reduce the available photosynthetic area (Bhatt et al. 2005; Zahra et al. 2010).

**Table 2. ANOVA for dependent variable for treatment applied, drought, salicylic acid and their interaction for lettuce plant.**

<table>
<thead>
<tr>
<th>Sources of Variation</th>
<th>Fresh weight</th>
<th>Dry weight</th>
<th>Leaf area</th>
<th>RWC</th>
<th>Chl a</th>
<th>Chl b</th>
<th>Chl a+b</th>
<th>Carotenoids</th>
<th>Electrolyte leakage</th>
<th>MDA</th>
<th>Proline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drought (D)</td>
<td>9848.2**</td>
<td>434.6**</td>
<td>4041.9**</td>
<td>1360.3**</td>
<td>2.12**</td>
<td>0.009**</td>
<td>1.98**</td>
<td>0.078*</td>
<td>60.50**</td>
<td>0.403**</td>
<td>3717.80**</td>
</tr>
<tr>
<td>Salicylic acid (SA)</td>
<td>1057.7**</td>
<td>13.0**</td>
<td>362.2**</td>
<td>1.6**</td>
<td>0.01**</td>
<td>0.006**</td>
<td>0.04**</td>
<td>0.007*</td>
<td>6.94**</td>
<td>0.026**</td>
<td>669.23**</td>
</tr>
<tr>
<td>D*SA</td>
<td>35.9**</td>
<td>1.3**</td>
<td>23.0**</td>
<td>3.4**</td>
<td>0.17**</td>
<td>0.052**</td>
<td>0.41**</td>
<td>0.0004**</td>
<td>0.20**</td>
<td>0.021**</td>
<td>29.73**</td>
</tr>
<tr>
<td>Error</td>
<td>24.9</td>
<td>0.9</td>
<td>10.5</td>
<td>2.8</td>
<td>0.05</td>
<td>0.011</td>
<td>0.07</td>
<td>0.0002</td>
<td>0.46</td>
<td>0.003</td>
<td>23.05</td>
</tr>
</tbody>
</table>

** and * represent significant at the 0.01 and 0.05 levels, respectively, and ns represent non-significant.

Foliar spray of SA exhibited significant response to improve growth attributes as compared to unsprayed SA plants. Highest fresh weight (241.02 g), dry weight (16.88 g) and leaf area (74.68 Cm²) were
appeared in 1.5 mM SA treatment, and lowest fresh weight (219.38 g), dry weight (14.49 g) and leaf area (61.99 cm²) observed in control plants. SA-treated plants exhibited an increase in tolerance to water stress. This increase in water stress tolerance was reflected in measured growth criteria. Gutierrez-Coronado et al. (1998) also reported a similar increase in the growth of shoots and roots of soybean plants in response to salicylic acid treatment. Delavari et al. (2010) also indicated that SA increases the leaf area in sweet basil plants, which is agreement of our results. Senaratna et al. (2000) have suggested a similar mechanism to be responsible for SA induced multiple stress tolerance in bean and tomato plants. The ability of SA to increase plant dry mass, ameliorating the adverse affect of water stress, may have significant implications in improving the plant growth and overcoming the yield barrier arising from conditions of limited water availability.

Relative water content (RWC)

Result showed that drought stress had significant effect on leaf relative water content (RWC) in \( p < 0.01 \) but SA effect was not significant. RWC was decreased by increasing drought intensity (Table 3). This reduction in RWC might be resulted in decline of plant growth attributes. RWC in plant had a positive correlation with soil relative water content (Nautiyal et al., 2002). RWC was decreased by increasing evapotranspiration in plants and reducing root growth and activity (Tarumingkeng and Coto, 2003). In addition, Saneoka et al. (2004) showed that RWC in drought stress condition decreased in Agrostis palustris Huds. In this experiment, the enhancement rate of RWC in SA-treated plants was very low. This result was agreement with Shirani Bidabadi (2012) results in banana plant and in contrast with Stevens et al. (2006) report in tomato plants.

Table 3. Effect of drought stress levels on some measured parameters of lettuce plant.

<table>
<thead>
<tr>
<th>Drought stress</th>
<th>Fresh weight (g)</th>
<th>Dry weight (g)</th>
<th>Leaf area (Cm²)</th>
<th>RWC (%)</th>
<th>Chl a (µg/ml)</th>
<th>Chl b (µg/ml)</th>
<th>Chl a+b (µg/ml)</th>
<th>Carotenoids (µg/ml)</th>
<th>Electron leakage (%</th>
<th>MDA (nm/g FW)</th>
<th>Proline (µg/g FW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100% FC)</td>
<td>261.54±</td>
<td>22.96±</td>
<td>91.19±</td>
<td>85.96±</td>
<td>1.566±</td>
<td>0.656±</td>
<td>2.223±</td>
<td>0.401±</td>
<td>31.53±</td>
<td>0.318±</td>
<td>37.827±</td>
</tr>
<tr>
<td>60% FC)</td>
<td>234.46±</td>
<td>14.70±</td>
<td>64.37±</td>
<td>68.96±</td>
<td>1.626±</td>
<td>0.600±</td>
<td>2.237±</td>
<td>0.283±</td>
<td>34.21±</td>
<td>0.488±</td>
<td>65.885±</td>
</tr>
<tr>
<td>30% FC)</td>
<td>195.73±</td>
<td>9.15±</td>
<td>49.36±</td>
<td>62.07±</td>
<td>1.757±</td>
<td>0.655±</td>
<td>1.412±</td>
<td>0.217±</td>
<td>36.72±</td>
<td>0.739±</td>
<td>77.328±</td>
</tr>
</tbody>
</table>

Means with the same letters within rows are not significantly different at \( p < 0.05 \) using Duncan's Multiple Range Test.

Photosynthetic pigments (chlorophyll a, b, a+b and carotenoids)

Analysis of variance results of photosynthetic pigments (chlorophyll a, b and carotenoids) are shown in Table 2. Drought stress affected photosynthetic pigments (chlorophyll a, b and carotenoids) and SA affected only carotenoids significantly. The interaction effect of drought and SA on chlorophyll a, b and a+b was significant. Under stress condition, the photosynthetic pigments greatly decreased. Plants under severe drought stress had 52, 36 and 47% less chlorophyll a, a+b and carotenoids, respectively. Drought stress caused changes in the ratio of chlorophyll a and b and carotenoids (Farooq et al., 2009). Chlorophyll content reduction was reported in drought stressed cotton (Massacci et al., 2008) and Catharanthus roseus (Jaleel et al., 2008). Decreases in photosynthetic pigments were due to instability of protein complexes and destruction of chlorophyll by increased activity of chlorophyll degrading enzymes and chlorophyllase under stress condition. In this experiment SA effect on chlorophyll a, b and a+b was not significant, but its effect on carotenoids was significant \( (p<0.5) \). SA treatments increased carotenoids content of the plants. The application of 0.75 and 1.5 mM SA led to 10 and 18% increscent of carotenoids, respectively, in comparion of untreated plants. In bean plants, foliar spray with salicylic acid, increased Chl a, b and carotenoids under normal field conditions (Turkyilmaz et al. 2005). Panceva et al. (1996) showed that long-term treatment (7days) of barley seedlings with SA decreased the rate of photosynthesis (chlorophyll content) whereas short-term treatment (2 hours) did not affect chlorophyll content as compared with untered control plants. Moreover, Khan et al (2003) showed that SA increased photosynthetic rate in corn and soybean. SA regulates physiological and biological processes in plants and can be used as a potential growth regulator to improve plant growth under environmental conditions, but the efficiency of exogenous SA depends on multiple causes such as the species, developmental stage, application method and SA concentration (Borsani et al., 2001). Therefore, effectiveness of SA on chlorophyll content may be due to one of the mentioned reasons.

Electrolyte leakage (EL)

Drought stress and SA treatment was affected EL but their interaction effect was not significant (Table 2). Decrease of FC from 100% to 30% significantly increased 116% of leaves EL. The results showed that the highest (36.72%) and lowest (31.53%) amounts of EL were achieved in severe and non- stress conditions, respectively (Table 3). EL reflects the changes of cell membrane structure under water stress. Its relative conductivity can be used to evaluate the damage on structure and function of cell membranes under stresses. EL enables cell membrane injury to be assessed when plants are subjected to stress. SA reversed the adverse effect of stress and caused a significant decrease in electrolyte leakage. The results showed that highest (35.09%) and lowest (33.35%) amounts of EL were achieved in control and 1.5 mM of SA treatment (Table 4).
The results of the present study are in agreement with Stevens et al. (2006) who determined that SA facilitated the maintenance membrane functions in tomato. This facilitation could be attributed to the induction of antioxidant responses and elevated Ca uptake that protects plants from the oxidative damage by SA (El-Tayeb 2005). These results suggested cell membrane structure of lettuce leaves under drought stress received less damage after pretreatment with SA.

Table 4. Effect of salicylic acid (SA) treatments on some measured parameters of lettuce plant.

<table>
<thead>
<tr>
<th>Traits</th>
<th>SA treatment</th>
<th>Fresh weight (g)</th>
<th>Dry weight (g)</th>
<th>Leaf area (cm²)</th>
<th>RWC (%)</th>
<th>Chl a (µg/ml)</th>
<th>Chl b (µg/ml)</th>
<th>Chl a-b (µg/ml)</th>
<th>Carotenoids (µg/ml)</th>
<th>Electron leakage (%)</th>
<th>MDA (nm/g FW)</th>
<th>Proline (µm/g FW)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 mM</td>
<td>219.383</td>
<td>14.497</td>
<td>61.993</td>
<td>71.854</td>
<td>1.310</td>
<td>0.649</td>
<td>1.959</td>
<td>0.273</td>
<td>35.09</td>
<td>0.574</td>
<td>52.396</td>
</tr>
<tr>
<td></td>
<td>0.75 mM</td>
<td>231.320</td>
<td>15.440</td>
<td>68.262</td>
<td>72.487</td>
<td>1.366</td>
<td>0.657</td>
<td>2.023</td>
<td>0.300</td>
<td>34.02</td>
<td>0.466</td>
<td>59.130</td>
</tr>
<tr>
<td></td>
<td>1.5 mM</td>
<td>241.027</td>
<td>16.888</td>
<td>74.681</td>
<td>72.656</td>
<td>1.273</td>
<td>0.605</td>
<td>1.879</td>
<td>0.328</td>
<td>33.35</td>
<td>0.506</td>
<td>69.513</td>
</tr>
</tbody>
</table>

Means with the same letters within rows are not significantly different at p < 0.05 using Duncan's Multiple Range Test.

Malondialdehyde (MDA) content

The MDA content was measured to determine the extent of lipid peroxidation. The analysis of variance on MDA content data revealed that drought stress, SA treatment and their interaction significantly affect MDA content (Table 2). The results showed that the highest (0.739 nm/g FW) and lowest (0.318 nm/g FW) amounts of MDA content was achieved in severe and non-stress condition, respectively. Drought stress enhanced free radicals levels in plants when the damage of membranes was investigated by monitoring MDA content. The data showed that lipid peroxidation in leaves increased as the stress level rose. These results are in agreement with Yazdanpanah et al. (2011) who found water stress increased the lipid peroxidation in the leaves of Satureja hortensis. At the cellular level osmotic stress causes alterations in membrane lipid composition and properties. It has been postulated that low level of the induced leakiness of membrane is caused by lipid peroxidation resulting from uncontrolled ROS increase (Rodrigues-Rosales et al., 1999). Measurement of thiobarbituric acid reacting substances (TBARs) concentration such as MDA is routinely used as an index of lipid peroxidation under stress conditions (Gapinska et al., 2008).

In this experiment when plants were sprayed with SA led to a significant decrease in the level of lipid peroxidation in plants which were under normal and drought stress conditions (Fig. 2). The results showed that the highest (0.57 nm/g FW) and lowest (0.46 nm/g FW) amounts of MDA content was achieved in plants that treated with 0 (control) and 0.75 mM SA concentration, respectively. Present study showed that the amount of MDA in the plant leaves subjected to water stress was reduced in response to SA. Similarly, Yazdanpanah et al. (2011) found a significant decrease in the concentration of MDA of drought stressed plants in response to SA pretreatment. Also Delavari et al. (2010) showed that pretreatment with SA decreased the level of lipid peroxidation induced by oxidative stress in basil plants. Plants treated with SA accumulated less MDA contents compared to control plants under drought stress conditions, suggesting that SA could possess an important role in inducing tolerance to oxidative stress condition in lettuce plants. This is supported by the findings of Agarwal et al. (2005) who mentioned SA treatment of wheat leaves under water stress conditions, resulted low amounts of MDA. Therefore, the lipid peroxidation caused by drought stress was ameliorated by SA treatments.
Proline accumulation
The analysis of variance on proline content revealed that both drought stress and SA had effect on proline content but their interaction effect was not significant (Table 2). The results showed that the highest amounts of proline (77.32 µM/gFW) were achieved in severe stress condition and lowest amount (37.82 µM/gFW) achieved in non-stress condition. There are many reports about proline increasing under drought stress conditions. For example, in basil plants subjected to salt stress, the concentration of proline was increased in the leaves (Delavari et al., 2010). In wheat, an assessment of drought stress effects on proline accumulation in a drought-tolerant and a drought-sensitive cultivar revealed that the rate of proline accumulation and utilization was significantly higher in the drought-tolerant cultivar (Nayyar and Walia, 2003). Many functions have been postulated for proline accumulation in plant tissues. Proline as an amino acid is known to occur widely in higher plants and normally accumulates in large quantities in response to environmental stresses. In addition to its role as an osmolyte for osmotic adjustment, proline contributes to stabilizing sub-cellular structures (e.g. membranes and proteins), scavenging free radicals and buffering cellular redox potential under stress conditions. It may also function as a protein compatible hydrotrope (Srinivas and Balasubramanian, 1995), alleviating cytoplasmic acidosis and maintaining appropriate NADP+/NADPH ratios compatible with metabolism (Hare and Cress, 1997). Also, rapid breakdown of proline upon relief of stress may provide sufficient reducing agents that support mitochondrial oxidative phosphorylation and generation of ATP for recovery from stress condition and repairing of stress-induced damages (Hare and Cress, 1997; Hare et al., 1998). Thus increasing of proline in plants under drought stress decreases adverse effects of stress.

In this experiment SA treatment increased proline levels in lettuce plants. The data showed that proline content in leaves increased as SA concentrations increased. A highest amount of proline (69.51 µM/gFW) was achieved in application of 1.5 mM SA and lowest amounts (52.39 µM/gFW) were observed in non- treated plants. These results are in agreement with those of Yazdanpanah et al. (2011) who found that SA treatments increased the proline content in the leaves. Similarly, SA increases proline levels of basil plant under salinity stress (Delavari et al., 2010). In the present study, SA induced an accumulation of proline in the leaves under drought stress, when SA was applied an induction occurred in the leaves under stress. Thus, proline can be considered to be one of the important factors involved in SA induced protective mechanism in lettuce leaves in response to drought stress.

CONCLUSION
In summary, results showed that drought stress imposed negative effects on plant growth and productivity of lettuce plant, which were ameliorated by SA treatment. This was associated with the alter of Physiological processes such as increase of photosynthetic pigments and proline content and decrease EL and MDA content of plants by addition of SA. Further, the results indicate that SA can be considered as a potential growth regulator for improving plant growth and yield under limited soil water availability, and it may be recommended in arid and semiarid regions.

REFERENCES
Al I, Li ZH, Xie ZX, Tian XL, Eneji AI, Duan LS. 2008. Caronatine alleviate polyethylene glycol water stress condition and lowest amount (37.82 µM/gFW) were achieved in severe stress condition and lowest amount (37.82 µM/gFW) achieved in non-stress condition. There are many reports about proline increasing under drought stress conditions. For example, in basil plants subjected to salt stress, the concentration of proline was increased in the leaves (Delavari et al., 2010). In wheat, an assessment of drought stress effects on proline accumulation in a drought-tolerant and a drought-sensitive cultivar revealed that the rate of proline accumulation and utilization was significantly higher in the drought-tolerant cultivar (Nayyar and Walia, 2003). Many functions have been postulated for proline accumulation in plant tissues. Proline as an amino acid is known to occur widely in higher plants and normally accumulates in large quantities in response to environmental stresses. In addition to its role as an osmolyte for osmotic adjustment, proline contributes to stabilizing sub-cellular structures (e.g. membranes and proteins), scavenging free radicals and buffering cellular redox potential under stress conditions. It may also function as a protein compatible hydrotrope (Srinivas and Balasubramanian, 1995), alleviating cytoplasmic acidosis and maintaining appropriate NADP+/NADPH ratios compatible with metabolism (Hare and Cress, 1997). Also, rapid breakdown of proline upon relief of stress may provide sufficient reducing agents that support mitochondrial oxidative phosphorylation and generation of ATP for recovery from stress condition and repairing of stress-induced damages (Hare and Cress, 1997; Hare et al., 1998). Thus increasing of proline in plants under drought stress decreases adverse effects of stress.

In this experiment SA treatment increased proline levels in lettuce plants. The data showed that proline content in leaves increased as SA concentrations increased. A highest amount of proline (69.51 µM/gFW) was achieved in application of 1.5 mM SA and lowest amounts (52.39 µM/gFW) were observed in non- treated plants. These results are in agreement with those of Yazdanpanah et al. (2011) who found that SA treatments increased the proline content in the leaves. Similarly, SA increases proline levels of basil plant under salinity stress (Delavari et al., 2010). In the present study, SA induced an accumulation of proline in the leaves under drought stress, when SA was applied an induction occurred in the leaves under stress. Thus, proline can be considered to be one of the important factors involved in SA induced protective mechanism in lettuce leaves in response to drought stress.

CONCLUSION
In summary, results showed that drought stress imposed negative effects on plant growth and productivity of lettuce plant, which were ameliorated by SA treatment. This was associated with the alter of Physiological processes such as increase of photosynthetic pigments and proline content and decrease EL and MDA content of plants by addition of SA. Further, the results indicate that SA can be considered as a potential growth regulator for improving plant growth and yield under limited soil water availability, and it may be recommended in arid and semiarid regions.

REFERENCES


Senaratna T, Touchell D, Bunn E, Dixon K. 2000. Acetyl salicylic acid (Aspirin) and salicylic acid induce multiple stress tolerance in bean and tomato plant. Plant Growth Regulation 30, 157-161.


