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# Effect of Salinity Stress and Exogenously Applied Methyl Jasmonate on Growth and Physiological Traits of Two *Carthamus tinctorius* Varieties

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**Abstract**
Salinity stress is one of the main limiting factors for optimum agricultural productivity of safflower, *Carthamus tinctorius* L., in arid and semi-arid regions. It could leads to significant changes in plant biochemical, physiological, and growth traits. Salinity induced endogenous rise in jasmonic acid and its methyl esters (MeJA) has been reported. In the present study, effects of salinity stress (6 and 12 ds $m^{-1}$) and the exogenous application of MeJA (0.1 and 0.5 mM) on the leaf number, shoot fresh weight, shoot length, chlorophyll a/b, soluble sugar, proline, and malondialdehyde (MDA) contents were investigated in two safflower varieties (Isfahan and IL111). Salinity stress negatively affected the growth of both varieties. Lipid peroxidation was not observed in Isfahan variety, but it significantly increased in the salinity resistant safflower, IL111. Soluble sugar and proline as the important osmoprotectants and free radical scavengers were elevated by salinity stress. Exogenous application of MeJA to the salinity stress-imposed plants slightly improved the growth due to inductions in the rate of photosynthesis; however, MeJA application impaired the growth of non-stressed plants because of induction of stomatal closure and as a result reduced photosynthesis.

**Keywords**: *Carthamus tinctorius*; Growth; Methyl jasmonate; Salinity stress; Physiology

## Introduction

Nowadays, salinity stress is considered as a serious concern for plant cultivation in arid and semi-arid regions of the world. High salt concentration can cause hyperosmotic stress and nutritional imbalance in plant cells leading to oxidative stress and inhibit the plant growth (Kang et al., 2005; Zhou et al., 2018). The scarcity of good quality water as well as the competition between domestic, industrial, and agricultural water consumptions encourage farmers to use saline water (Electrical conductivity in the range of 3-6 ds $m^{-1}$) for plant irrigation (Gholami Zali, 2018). Salinization of land is progressively increasing throughout the world. Excessive salt concentrations can transform fertile and productive lands to barren ones (Shahbaz and Ashraf, 2013). A saline soil environment could decrease water availability and uptake, cause ion toxicity associated with the excessive uptake of toxic ion, and create mineral deficiency to an extent that may adversely affect plant growth and crop production yield (Navarro et al., 2002).

Safflower, *Carthamus tinctorius* L. is a plant species cultivated in semi-arid

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regions. It is a member of Asteraceae family, an oil seed crop, and a source of coloring agent in food industry (Ekin, 2005). Safflower's flowers are also well known for their medicinal properties (Ekin, 2005).

The deleterious effects of salinity on plant growth are attributed to decrease in osmotic potential of the growing medium that is usually accompanied with the specific ion toxicity and nutrient deficiency as well (Fariduddin et al., 2013). Osmotic stressed plants showed a progressive reduction of relative water content (RWC) and stomatal closure, which both limit metabolic productivity, plant growth, and yield (Zhou et al., 2018). To maintain the cell volume and resist against dehydration, the plants accumulate solutes in their cells. This phenomenon, known as osmotic adjustment, has been observed in plants' stem, leaves, roots, and fruits (Rao et al., 2006; Rahdari and Hoseini, 2012). To trigger this physiological response and improve plant tolerance, the exogenous addition of signaling molecules such as jasmonic acid (JA) is a well-known mitigation strategy. The JA and methyl jasmonate (MeJA), collectively referred to as jasmonates, are naturally occurring plant growth regulators that are widely distributed in the plant kingdom, and are known to regulate various aspects of plant development and responses to environmental stresses (Droby et al., 1999). MeJA affects a wide diversity of physiological and biochemical processes (Gao et al., 2004). In general, the JA inhibits stomatal opening, cell division, plant growth, photosynthetic activities, flower bud formation, seed germination, and embryogenesis. In addition, JA promotes the induction/promotion of leaf senescence and petiole abscission, fruit ripening, and chlorophyll and carotenoid biosynthesis (Yeh et al., 1995). As an example of successful role of JA in induction of stress tolerance, application of MeJA (10 or 20 µM as foliar spray) has been applied on six rice varieties for mitigating of salt stress (9 ds $m^{-1}$) (Mahmud, 2017).

In the present study, MeJA was exogenously applied to safflower seedlings to investigate its regulatory role on tolerance mechanism of safflower cultivated under saline conditions. Vegetative growth parameters, contents of osmolytes, and the photosynthetic pigments were measured in both control and MeJA-treated plants in order to discover the underlying physiological responses.

## Materials and Methods

### *Culture conditions and treatments*

The seeds of two spineless safflower varieties (*Carthamus tinctorius* IL111 and Isfahan) were obtained from the Oil Seed Section, Seed and Plant Improvement Institute, Karaj, Alborz, Iran. The main difference between these two safflower varieties is their specific growth rates; IL111 reaches to the end of vegetative growth approximately 25 days earlier than the Isfahan variety. The seeds of both varieties were sterilized with sodium hypochlorite (1%) for 5 min and then washed several times by distilled water. The uniform sterilized seeds were germinated by placing them between two layers of filter paper in petri-dishes containing double distilled water. The petri-dishes were kept in darkness at ambient temperature (~25°C) until the emergence of radicles (about 72 h). The germinated seedlings were then transferred to pots containing sand, clay, and humus (2: 1: 1) in a culture room in day/night temperatures of 26/18°C under 16 h photoperiod. When plants had 3 to 4 fully expanded leaves, they were irrigated for two weeks with 0, 6, 12 ds $m^{-1}$ soluble sodium chloride. The plants were also sprayed with 0, 0.1, and 0.5 mM MeJA for one week. After two weeks, the plants were harvested for growth and physiological analysis.

### *Measurement of chlorophyll contents*

The contents of photosynthetic pigments including chlorophyll *a* and *b* (Chl*a* and Chl*b*) were determined according to the method described by Lichtenthaler (1987).

The fresh leaves of both varieties (each one amounting to 0.5 g) were homogenized in acetone (80%) and then centrifuged at 15,000 × g for 30 min. The absorbance of each supernatant was recorded at the wavelengths of 646.8, 663.2, and 470 nm. The contents of Chl*a* and Chl*b* were then calculated by the following empirical correlations (Lichtenthaler, 1987).

$$\text{Chl}a\ (\text{mg mL}^{-1}) = 12.25\ A_{663\cdot2} - 2.79\ A_{646.8} \quad (1)$$

$$\text{Chl}b\ (\text{mg mL}^{-1}) = 21.51 A_{646.8} - 5.10\ A_{663.2} \quad (2)$$

***Determination of osmolytes' contents***

The contents of soluble sugar and proline were measured for both varieties according to the methods of Bates (Bates et al., 1973) and Somogyi- Nelson (Somogyi, 1952), respectively.

For determination of free proline content, 0.5 g fresh weight of either samples was frozen in liquid nitrogen and then grounded to homogenization with 10 mL 3% (w/v) sulfo salicylic acid. The samples were then centrifuged at 15,000 × g for 30 min. The supernatant was then mixed with ninhydrin reagent and acetic acid at 1:1:1 ratio. The samples were subsequently placed in a boiling water bath (100 ºC) for 1 h. Thereafter, the samples were left in ambient condition and then incubated in an ice bath for 5 min. A 2 mL toluene was added to each specimen and quickly shaken with a vortex device until two distinct phases were formed. The absorbance of the upper phase was measured by a spectrophotometer (Unico 2100, USA) at 520 nm. The free proline content was calculated by a standard curve method and expressed as μg g-1 FW.

For determination of soluble sugar contents, 0.2 g fresh weights of roots and shoots were grounded with 15 mL distilled water at room temperature and thereafter was heated to the boiling point. The samples were passed from filter paper. A 2 mL aliquot of the filtrate was then mixed with equal volume of copper-carbonate-tartrate reagent (Somogyi, 1952). The mixture was placed in boiling water (100 ºC) for 8 min. The solution was then cooled at room temperature and then 2 mL chromogenic reagent, phosphomolybdic, (Somogyi, 1952) was added to the mixture. After a few minute hand shake, the solution absorbance was read at 660 nm by the UV/Vis spectrophotometer. The soluble sugar was quantified by a calibration curve method using glucose as standard.

***Lipid peroxidation***

The content of malondialdehyde (MDA), a product of lipid peroxidation, was determined by the method of Heath (Heath and Packer, 1968). In brief, the leaves were homogenized by 1% (w/v) trichloroacetic acid (TCA). The homogenate in TCA was mixed with the thiobarbituric acid at 0.5% (w/v). The mixture was subsequently incubated at 95 °C in a water bath for 30 min and rapidly cooled in an ice bath. The solution absorbance was read at 532 nm. After subtracting the non-specific absorbance at 600 nm, the MDA concentration was calculated using an extinction coefficient of 155 mM $cm^{-1}$.

***Statistical analysis***

All the experiments were done in triplicates unless otherwise specified. The experiments were arranged in a randomized complete block design with five replicates. The data analysis was done by one way analysis of variance using Excel, SPSS, and MSTATC. The set of means were compared using the LSD test at 95% confidence interval.

## Results

***Effect of salinity stress on growth parameters***

Salinity stress had a negative effect on the growth of safflower aerial parts. The shoot length of the plants cultivated in 6 ds $m^{-1}$ were decreased to half in both varieties compared to control (Fig. 1). Interestingly, the further increase in salinity up to 12 ds $m^{-}$

$^{1}$ had no significant effect on the shoot length of IL111 variety, while the Isfahan variety of safflower showed a significant decrease to one quarter (from 20 to 5 cm) compared to the control (Fig. 1). This indicates the difference in salinity tolerance of the two studied varieties. However, the leaf number was decreased consistently in both safflowers (Fig. 2) so that there was about 30% decrease with an increase in salinity from 0 to 6 ds $m^{-1}$. The increase in salinity from 6 to 12 ds $m^{-1}$ had no significant effect on leaf number. The shoot fresh weight was decreased about 62% in both varieties with an increase in salinity from 0 to 6 ds $m^{-1}$; while there was no more decrease in the fresh weights with the increase in salinity up to 12 ds $m^{-1}$ (Fig. 3).

***Effect of MeJA on growth parameters***

The spray of MeJA on both safflower varieties cultivated under normal growth conditions negatively affected their growth. The spray of either 0.1 or 0.5 mM MeJA had the same impairing effect on both varieties so that the shoot length, leaf number, and shoot fresh weights were decreased about 65%, 42%, and 57% respectively (Fig. 1-3). The increase in MeJA dosage from 0.1 to 0.5 mM caused no significant changes on the plant growth parameters.

The application of MeJA to the safflower under moderate salinity of 6 ds $m^{-1}$ improved the plant growth. The shoot length of safflower Isfahan showed an increase from 10 to 17 cm upon treatment with both 0.1 and 0.5 mM MeJA (Fig. 1). The safflower IL111 only showed an increase of about 25% in shoot length at the higher dosage of 0.5 mM MeJA and the lower dosage had no significant improvement on the shoot length. The leaf number of safflower IL111 showed no significant improvement upon MeJA treatment, while the safflower Isfahan showed a 23% increase upon treatment with 0.1 mM MeJA and no significant change at higher dosage of 0.5 mM MeJA (Fig. 2). The fresh weight of safflower Isfahan increased about two fold upon treatment with both concentrations of 0.1 and 0.5 mM MeJA (Fig. 3). The safflower IL111 only showed about 54% improvement in fresh weight upon treatment with 0.5 mM MeJA.

The application of MeJA to the safflower under high salinity level (12 ds $m^{-1}$) caused no significant improvement in growth parameters. It also slightly impaired the shoot length of safflower IL111 (Fig. 1).

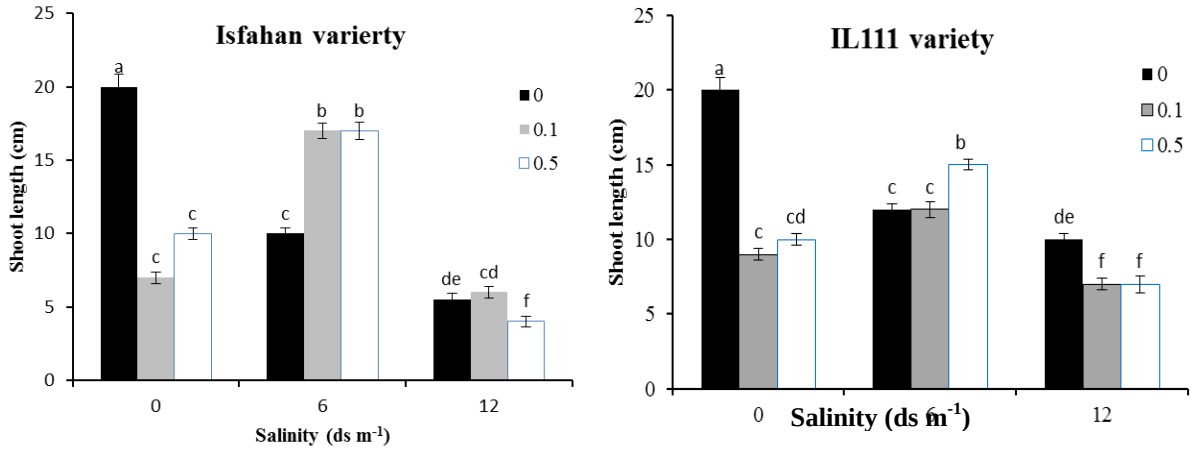


**Fig. 1. Effect of salinity and Methyl Jasmonate (MeJA) on shoot length of safflower varieties (Isfahan and IL111). The shoot length of both varieties were measured in the plants that were exposed to three different salinity levels (0, 6, and 12 ds $m^{-1}$) under treatment with three MeJA concentrations (0, 0.1, 0.5 mM)**

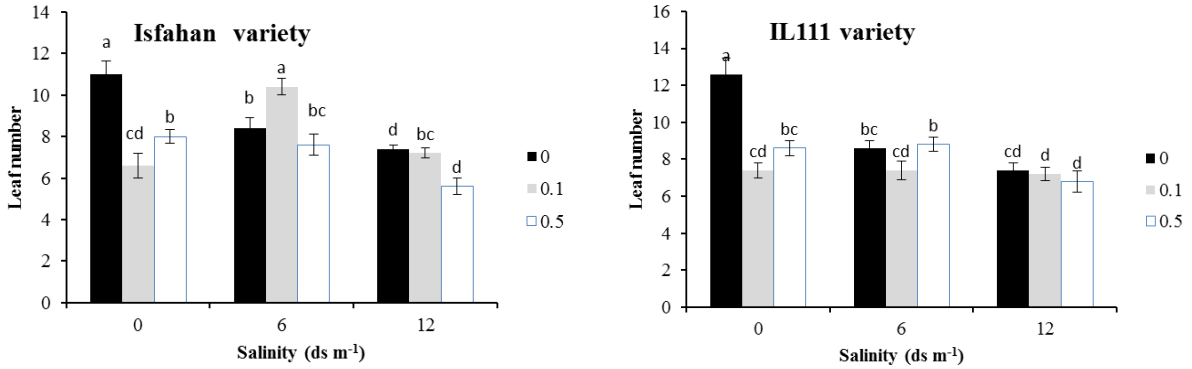


**Fig. 2. Effect of salinity and Methyl Jasmonate (MeJA) on leaf number of safflower varieties (Isfahan and IL111). The leaf number of both varieties were measured in the plants that were exposed to three different salinity levels (0, 6, and 12 ds $m^{-1}$) under treatment with three MeJA concentrations (0, 0.1, 0.5 mM)**

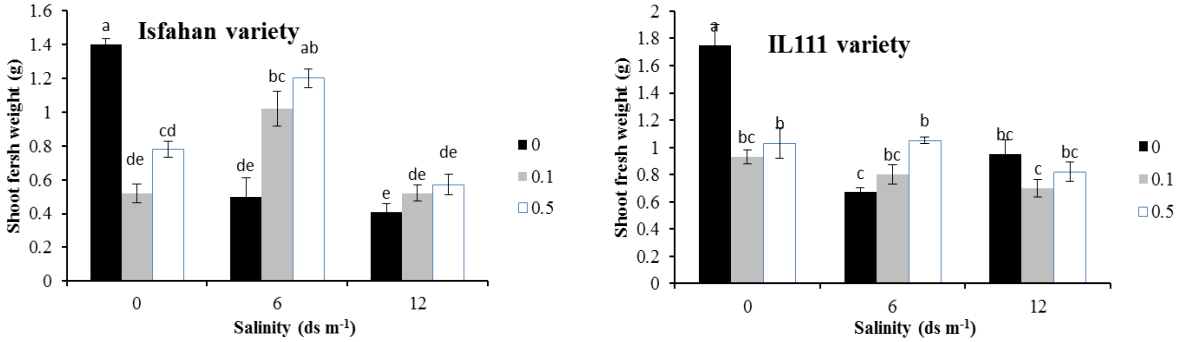


**Fig. 3. Effect of salinity and Methyl Jasmonate (MeJA) on shoot fresh weight of safflower varieties (Isfahan and IL111). The shoot fresh weight of both varieties were measured in the plants that were exposed to three different salinity levels (0, 6, and 12 ds m-1) under treatment with three MeJA concentrations (0, 0.1, 0.5 mM)**

***Effect of salinity and MeJA on MDA content***

The salinity stress did not significantly increase the MDA content of safflower Isfahan, but instead resulted in 2.5 fold higher MDA content of safflower IL111 compared to control (Fig. 4). The application of MeJA (either 0.1 or 0.5 mM) caused no significant changes in the MDA content of safflower IL111 under normal growth conditions, and only slightly reduced it under moderate salinity level (6 ds $m^{-1}$) (Fig. 4). The application of MeJA (0.5 mM) significantly decreased the MDA content of safflower Isfahan at control and moderate salinity conditions; while at high salinity level (12 ds $m^{-1}$) it has no mitigating effect (Fig. 4).

***Effect of salinity and MeJA on osmolytes***

The salinity stress led to a significant increase in the proline content of both safflower varieties so that the safflower Isfahan showed five times higher proline content under both salinity concentrations compared to the control plants (Fig. 5). The safflower IL111 showed a continuous increase in the proline content from about 1.5 to 6.0 mg g $FW^{-1}$ with the increase in salinity from 6 to 12 ds $m^{-1}$ (Fig. 5). The MeJA treatment on both varieties increased the proline content with the highest increase associated with the 0.1 mM MeJA at high salinity level (12 ds $m^{-1}$). The highest dosage of MeJA (0.5 mM) led to higher proline content than the salinity stressed ones only in Isfahan variety (Fig. 5).

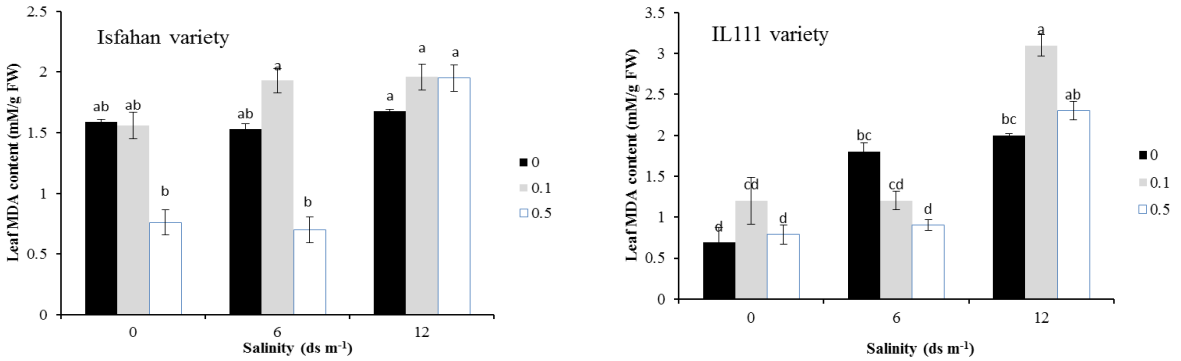


**Fig. 4. Effect of salinity and Methyl Jasmonate (MeJA) on malondialdehyde (MDA) content of safflower varieties (Isfahan and IL111) upon treatment with three different salinity levels of 0, 6, and 12 ds $m^{-1}$ and three MeJA concentrations of 0, 0.1, 0.5 mM**

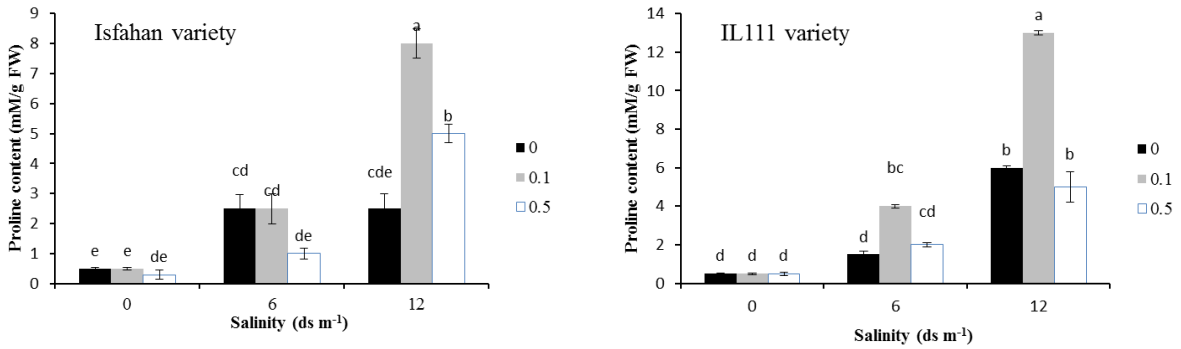


**Fig. 5. Effect of salinity and Methyl Jasmonate (MeJA) on proline content of safflower varieties (Isfahan and IL111). The proline contents of of both varieties were measured in the plants that were exposed to three different salinity levels (0, 6, and 12 ds $m^{-1}$) under treatment with three MeJA concentrations (0, 0.1, 0.5 mM)**

The leaf sugar content of safflower Isfahan was not significantly changed at moderate salinity, but there was about 40% increase in leaf sugar content by imposing high salinity level (12 ds $m^{-1}$) compared to sugar content of non-stressed plants (Fig. 6A). The application of MeJA to non-stressed plants and to the ones under moderate salinity level (6 ds $m^{-1}$) caused 44-100% increase in the sugar content compared to their respective controls (Fig. 6A and 6B). The highest increase was related to the IL111 variety treated with 0.1 M MeJA at non-stressed conditions and also 0.5 mM MeJA at moderate salinity level (6 ds $m^{-1}$). The MeJA application to both plant varieties at high salinity level (12ds $m^{-1}$) caused no significant changes compared to the stressed ones without MeJA application (Fig. 6A and 6B). The root sugar contents of safflower Isfahan was not changed significantly, but that of IL111 variety was increased up to 73% (Fig. 6C and 6D). The application of MeJA (0.5 mM) to the safflower Isfahan at normal growth conditions caused a significant decrease in their sugar content. The MeJA (0.1 mM) applied to the stressed plants (12 ds $m^{-1}$) resulted in about 53% increase compared to the stressed ones only; while in IL111 variety, it had no significant effect (Fig. 6C and 6D).

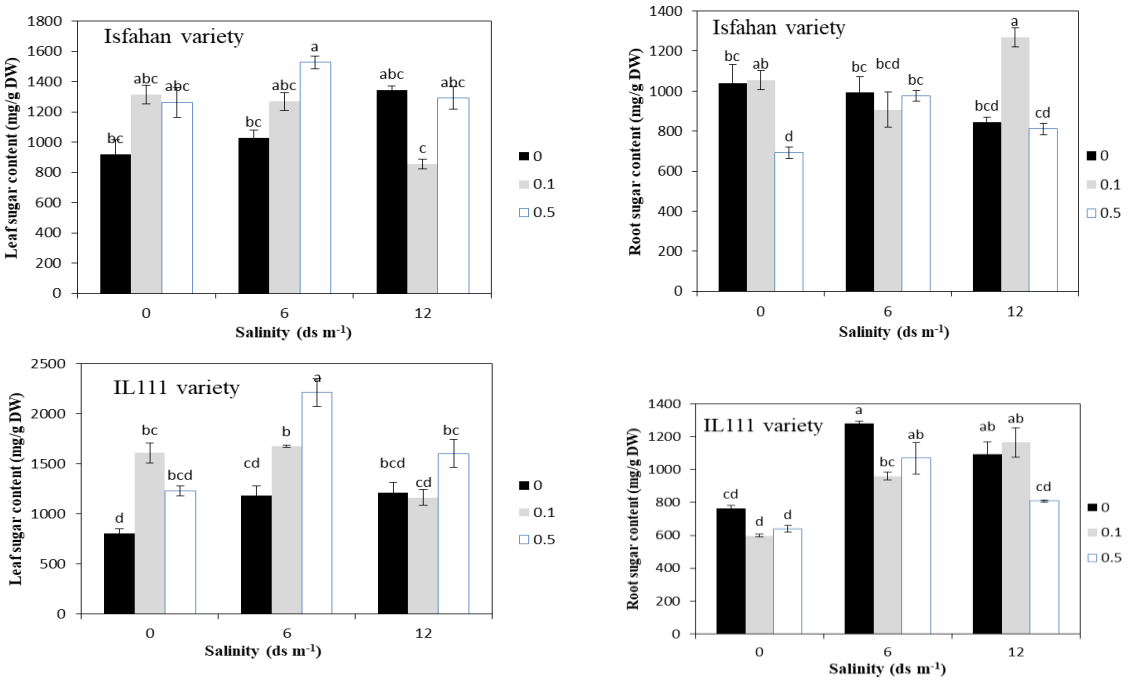


**Fig. 6. Effect of salinity and Methyl Jasmonate (MeJA) on sugar content of safflower varieties (Isfahan and IL111). The sugar contents of both varieties were measured in the plants that were exposed to three different salinity levels (0, 6, and 12 ds m$^{-1}$) under treatment with three MeJA concentrations (0, 0.1, 0.5 mM)**

### *Effect of salinity and MeJA on chlorophyll contents*

Both MeJA treatment and salinity stress resulted in a decrease in the chlorophyll a content of both safflower varieties (Table 1). The chlorophyll b content of safflower IL111 was not changed by MeJA treatment at normal growth conditions. However, the application of MeJA to the safflowers at moderate salinity level (6 ds m$^{-1}$) increased their chlorophyll b content to the level of control plants (~37 mg g FW$^{-1}$). The application of MeJA to the safflower Isfahan under moderate salinity caused about 50% enhancement (from 30 to 45 mg g FW$^{-1}$ for Chlb and from 75 to 120 mg g FW$^{-1}$ for Chla) compared to the Chl contents of stressed plants. The MeJA application had no mitigating effect on the plants at high salinity level (12 ds m$^{-1}$). Overall, the chlorophyll content of safflower Isfahan was higher than that of IL111 variety. Chlorophyll contents were continuously decreased with the increase in salinity levels (Table 1).

All the above results were statistically significant at $p < 0.05$ and the summary of the mean square from the analysis of variances were all presented in Table 2.

**Table 1. Effect of salinity levels (0, 6, and 12 ds m$^{-1}$) and methyl jasmonate (JA) application (0, 0.1, 0.5 mM) on the chlorophyll a and b contents in two safflower varieties (Isfahan and IL111).**

| Treatments | IL111variety | | Isfahan variety | |
|---|---|---|---|---|
| | Chl*a* | Chl*b* | Chl*a* | Chl *b* |
| Control | 175 $^{a}$ | 42 $^{a}$ | 200 $^{a}$ | 75 $^{a}$ |
| EC=6 ds/m | 84 $^{bcd}$ | 20 $^{b}$ | 78 $^{c}$ | 28 $^{c}$ |
| EC=12 ds/m | 56 $^{de}$ | 18 $^{b}$ | 70 $^{c}$ | 35 $^{bc}$ |
| JA 0.1 mM | 101 $^{bc}$ | 35 $^{a}$ | 88 $^{bc}$ | 28 $^{c}$ |
| EC=6 ds/m+ JA 0.1 mM | 109 $^{b}$ | 37 $^{a}$ | 92 $^{bc}$ | 30 $^{c}$ |
| EC=12 ds/m+ JA 01 mM | 43 $^{e}$ | 18 $^{b}$ | 59 $^{c}$ | 21 $^{c}$ |
| JA 0.5 mM | 111 $^{b}$ | 38 $^{a}$ | 77 $^{c}$ | 26 $^{c}$ |
| EC= 6 ds/m+JA 0.5 mM | 95 $^{bc}$ | 30 $^{a}$ | 118 $^{b}$ | 45 $^{b}$ |
| EC=12 ds/m+ JA 0.5 mM | 72 $^{cde}$ | 26 $^{ab}$ | 59 $^{c}$ | 21 $^{c}$ |

**Table 2. Summary of mean square from analysis of variance**

| Source | dF | Mean square | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | LN | SL | SFW | MDA | Proline | Chl*a* | Chl*b* | Leaf sugar | Root sugar |
| Replicate | 4 | 2.6 $^{ns}$ | 0.5$^{ns}$ | 18.8 $^{*}$ | 0.12 $^{ns}$ | 0.2 $^{ns}$ | 350 $^{ns}$ | 47 $^{ns}$ | 78011 $^{ns}$ | 1818 $^{ns}$ |
| Variety | 1 | 4.4$^{*}$ | 20$^{***}$ | 902 $^{***}$ | 0.027 $^{ns}$ | 14.7 $^{***}$ | 3280 $^{***}$ | 534 $^{*}$ | 606468 $^{**}$ | 7914 $^{ns}$ |
| EC | 2 | 45 $^{***}$ | 310$^{***}$ | 45431 $^{***}$ | 4.7 $^{***}$ | 198 $^{***}$ | 9424 $^{***}$ | 911 $^{***}$ | 416373 $^{**}$ | 285641 $^{***}$ |
| MJ | 2 | 17 $^{***}$ | 299$^{***}$ | 21626 $^{***}$ | 1.1 $^{***}$ | 35 $^{***}$ | 363 $^{ns}$ | 139 $^{ns}$ | 871381 $^{***}$ | 16315 $^{***}$ |
| Variety × EC | 2 | 5 $^{*}$ | 16$^{**}$ | 1727 $^{***}$ | 1.0 | 15 $^{***}$ | 1463 $^{***}$ | 131 $^{ns}$ | 167637 $^{*}$ | 200018 $^{***}$ |
| Variety × MJ | 2 | 8.7 $^{**}$ | 20$^{***}$ | 24524 $^{***}$ | 1.0 $^{***}$ | 6 $^{*}$ | 6519 $^{***}$ | 1151 $^{***}$ | 198501 $^{*}$ | 76773 $^{**}$ |
| EC × MJ | 4 | 24 $^{***}$ | 99$^{***}$ | 13806 $^{***}$ | 0.5 $^{*}$ | 22 $^{***}$ | 1330 $^{***}$ | 304 $^{*}$ | 428544 $^{***}$ | 120217 $^{***}$ |
| Variety × EC × MJ | 4 | 3.1 $^{*}$ | 10.4$^{**}$ | 75792 $^{***}$ | 0.4 $^{***}$ | 6.8 $^{*}$ | 5363 $^{***}$ | 674 $^{***}$ | 36789 $^{ns}$ | 27592 $^{*}$ |
| Error | 68 | 1.1 | 2.1 | 1.9 | 0.1 $^{***}$ | 1.7 | 218 | 60 | 44542 | 12986 |
| **Total** | **89** | | | | | | | | | |

DF, degrees of freedom; SL, shoot length; SFW, shoot fresh weight; LN, leaf number; MDA, Malondealdehyde; Chl*a* and *b*, chlorophyll *a* and *b*
ns, not -significant
* Significant at p≤0.05.
**Significant at p≤0.01.
***Significant at p≤0.00

## Discussion

In the current study, salinity stress impaired the growth of both varieties (Fig. 1-3). Decrease in shoot length, leaf number, and fresh weight due to salinity stress can be attributed to a reduction in osmotic pressure, nutritional imbalance, ion toxicity, depletion of photosynthetic pigments, and also oxidative stress (Shaki, 2018). Consistent with these observations, the damaging effect of salinity on growth parameters were reported in other plant species such as wheat (Wang, 1999), sugar beet (Ghoulam and Fares, 2001), and safflower (Kaya et al., 2003). The reduced osmotic pressure decreases the turgor pressure as an important factor in cell elongation and divisions (Khulenjani and Salamati, 2018). The high salinity could prevent the absorption of other essential micronutrients accompanied with growth retardation.

It also reported that salinity stress causes oxidative stress (Mahmud, 2017). However, in the present study the lipid peroxidation as measured by MDA content was not changed in the safflower Isfahan, but it caused higher MDA content (2.5 fold increase) of safflower IL111 compared to control (Fig. 4). It sounds that the peroxide signaling pathway was activated in the salinity resistant IL111 variety and concomitantly the MDA content was increased. In cooperation with this stress signal, the content of osmolytes (soluble sugar and proline) as non-enzymatic scavengers of reactive oxygen species were increased compared to those of control. The lack of significant increase in the root's sugar content of safflower Isfahan might be because of the absence of peroxide signaling activation in the Isfahan variety (Fig. 6C). The safflower Isfahan also didn't show any significant increase in the leaf sugar content at moderate salinity, possibly because of the same reason (Fig. 6A). The sever salinity stress cause protein reduction in plant cells (Shaki, 2018). This observation could be a possible reason for higher accumulation of soluble sugars at high salinity (Fig. 6A and 6B) as the soluble carbohydrates maintain the structure of proteins possibly thorough interacting with the proteins by their hydroxyl groups. Soluble carbohydrates as hydrophilic groups may substitute for water molecules and prevent protein denaturation and consequent degradation.

The increase in the proline content of safflower Isfahan and also IL111 variety (Fig. 5) can also be attributed to two important enzymes, namely proline carboxylic acid synthase and reductase, which has been shown that their translation

increase under salinity stress (Sairam and Tyagi, 2004). Osmoprotection has been suggested as an important role of proline in plants under salinity stress that leads to osmotic adjustment and scavenging of free radicals. Salinity treatment caused an increase in the proline content of both varieties probably because of an adaptive solution to circumvent the osmotic stress as well as the peroxide accumulation. Increase in proline content has been also reported in barely (El-Tayeb, 2005), soybean (Yoon et al., 2009), and root lentil (Bandeoğlu et al., 2004) under salinity stress.

The salt stress decreases the photosynthetic pigments (Table 1) because of chlorophyll degradation due to both oxidative damage and also chlorophyll denaturation, leading to their consequent degradation similar to protein degradation (Shaki, 2018). This can be a possible reason for the significant decrease in the chlorophyll content of both safflower varieties under salinity stress. Inactivation of peroxide signaling pathway in the safflower Isfahan could also be a possible reason for lower chlorophyll reduction than the IL111 variety under salinity stress (Table 1).

Application of MeJA to the plants under normal growth conditions caused growth reduction in plants. This growth retardation can be attributed to the role of JA in changing the properties of guard cells leading to stomatal closure and reduced photosynthesis. However, there were no significant changes between the plants under salinity stress and the ones treated with MeJA. This observation could be attributed to the dual role of MeJA on photosynthesis. Exogenous application of MeJA could increase the expression of photosynthesis-related genes encoding for example, the small subunit of ribulose 1,5 bisphosphate carboxylate/ oxygenase (Rubisco) as well as increase their translation leading to increased Rubisco and chlorophyll content (Janoudi and Flore, 2003). Similar findings were also reported in several plant species such as barley (Janoudi and Flore, 2003), Arabidopsis (Jung, 2004), rice (Kang et al., 2005; Mahmud et al., 2017) and *Menthapiperita* (Khulenjani and Salamati, 2018). Depending on MeJA dosage and salinity stress, the growth parameters were slightly impaired or improved in two safflower varieties (Fig. 1-3). Consistent with the improvement or retardations in the growth parameters of MeJA-treated plants under salinity stress, the chlorophyll content was also consistently changed (Table 1). The higher leaf sugar content in salt-stressed plants treated with MeJA can also be attributed to higher rate of photosynthesis and also less glucose consumption for the formation of cell wall polysaccharides (Khulenjani, 2015). The lack of photosynthesis in root system is a possible reason for the insignificant changes in the soluble sugar contents of roots (Fig. 6C and 6D). Under high salinity conditions, the proline contents in stressed plants treated with MeJA were significantly increased to recover osmotic adjustment in the plant cells and also to alleviate the oxidative stress. Interestingly, the application of MeJA at high dosage (0.5 mM) resulted in less proline content than the ones treated with the moderate concentration (0.1 mM MeJA) (Fig. 5). It is possibly because of the activation of other enzymes involved in the defensive systems (Mahmud, 2017). The examination of different defensive pathways upon applications of MeJA and salinity stress at different intensities is the other issue of investigations for the future.

## Conclusions

Salinity stress negatively affects the growth of both resistant and non-resistant safflower varieties. To cope with salinity stress, the plants accumulate osmoprotectants (proline and soluble sugar) in their cells. The peroxide accumulation, as measured by lipid peroxidation, as a measure of stress susceptibility did not occur in the non-resistant safflower Isfahan. The exogenous application of MeJA to the plants under normal growth conditions impaired their

growth because of the stomatal closure and reduced photosynthesis; while treatment of the stressed plants with MeJA slightly improved the growth due to improve in the photosynthesis. Depending on salinity level and MeJA dosage, the stimulation of different defensive mechanisms could possibly occur in safflower to circumvent the oxidative damage by maintaining ROS balance.

## References


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