WALIA journal 31(S1): 104-109, 2015
Available online at www.Waliaj.com
ISSN 1026-3861
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Improving physiological performance of safflower under salt stress by application of salicylic acid and jasmonic acid

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Abstract: A greenhouse experiment with factorial arrangement based on randomized complete block design with three replications was conducted in 2014 to evaluate the effects of salicylic acid (1 mM) and jasmonic acid (0.5 mM) on some physiological trairs of safflower plant under salt stress (control, 4, 8, and 12 dS/m). Leaf chlorophyll content index (CCI), photosystem II efficiency (Fv/Fm), relative water content (RWC), leaf area index (LAI) and grain yield per plant decreased with increasing salinity. The CCI, Fv/Fm, RWC and LAI were significantly higher for plants treated with jasmonic acid and salicylic acid under saline and non-saline conditions, compared with control. These superiorities in physiological performance of hormone treated plants led to significant advantage in grain yield of safflower under different salinity treatments. Therefore, salicylic acid and jasmonic acid can be used to promote growth and development of safflower under favorable and unfavorable environmental conditions, which ultimately can enhance grain yield.

Key words: *Chlorophyll content; Jasmonic acid; Safflower; Salicylic acid; Salinity*

1. Introduction

Salinity is the most devastating environmental stress that causes a reduction in plant growth and productivity. The increasing global population is putting a strain on food production in such a way that there is now higher demand for foods and this will force the use of saline soil and water for agricultural production (Ashraf, 2009). Reduced plant growth under salinity is a consequence of several physiological responses including modification of plant water status, photosynthetic efficiency and carbon allocation and utilization (Abdul Jaleel *et al*., 2007). Inhibited plant growth may be caused by decreased turgidity from high concentrations of salts in the soil under water deficit conditions (Kim and Lee, 2001). One viable strategy of overcoming the salt-induced injurious effects on plant growth is the exogenous application of osmo-protectants, growth regulators and stress signaling molecules (Farooq *et al*., 2010). Survival under these stressful conditions depends on the plant's ability to perceive the stimulus, generate and transmit signals and instigate biochemical changes that adjust the metabolism accordingly (Hasegawa *et al*., 2000).

Salicylic acid (SA) plays an important role in the defense response to pathogen attack and biotic and abiotic stresses in plant species (Shi *et al*., 2006). Many studies report the role of SA in inducing stress tolerance in plants. For example, SA has been found to induce drought tolerance in wheat (Singh and Usha, 2003), salinity tolerance in barley (El-Tayeb, 2005), heat tolerance in mustard (Dat *et al*., 1998), chilling tolerance in maize (Janda *et al*., 1999) and heavy metal stress tolerance in barley (Metwally *et al*., 2003). Jasmonic acid (JA) is another naturally occurring plant growth regulator which can affect many morphological, physiological and biochemical processes in plants (Norastehnia *et al*., 2007). Foliar application of JA modulates several physiological responses, leading to improved resistance against abiotic stresses (Walia *et al*., 2007). JA application to the stressed plants reduces the amount of lipid peroxidation and stimulates the synthesis of antioxidant enzymes, enhancing the content and yield of artemisinin as well (Aftab *et al*., 2011).

Safflower (*Carthamus tinctorius* L.) is a tap-rooted multipurpose crop which can tolerate environmental stresses including salinity and water stress (Lovelli *et al*., 2007). It is one of the most important oilseed cultivated plants used for edible oil production in the world (Dwivedi *et al*., 2005). The importance of oil crops such as safflower has increased in recent years, especially with the interest in the vegetable oil for the human consumption (Dordas and Sioulas, 2008). Generally, safflower is cultivated on marginal lands that are mostly affected by water and salt stresses (Dordas and Sioulas, 2008). Its cultivation on such soils could only be made profitable by applying chemicals or plant growth regulators exogenously. Thus, this research was aimed to investigate the effect of exogenous application of salicylic acid and jasmonic acid on some physiological traits and grain and oil yields of safflower under salt stress.

2. Material and methods

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A pot experiment with a factorial arrangement on the bases of randomized complete block (RCB) design with three replications was conducted in 2014 (Tabriz, Iran) to investigate the effect of exogenous foliar application of salicylic acid and jasmonic acid on physiological performance of safflower under salt stress. In each plastic pot (20 × 20 cm) containing 1.0 kg of perlite 20 seeds of safflower were sown at a depth of 3cm and then tap water (0.8 dS/m) and saline solutions (4, 8 and 12 dS/m) were added to achieve 100% FC. All pots kept inside a glass greenhouse under natural light. Minimum and maximum temperatures of greenhouse were 25 and 30 ℃, respectively. After germination, plants were thinned to 10 plants per pot. During the growth period, the pots were weighed and the losses were made up with Hoagland solution (EC =1.3 dS/m and pH= 6.5-7). Perlites within the pots were washed every 20 days and non-saline and salinity treatments were reapplied in order to prevent further increase in electrical conductivity (EC), due to adding the Hoagland solution. Salicylic acid (1 mM) and jasmonic acid (0.5 mM) were separately sprayed on plants at two vegetative and one flowering stages.

Photochemical efficiency of photosystem II (Fv/Fm) was measured using a portable chlorophyll fluorometer. Measurements were made after 20 min dark adaptation (Maxwell and Johnson, 2000) from 3 plants. Chlorophyll content index of leaves was measured by a chlorophyll meter (CCM- 200). Relative water content was determined according to Barr and Weatherley (1962). Fresh weight of the youngest fully expanded leaf was recorded within 24 h after excision. Turgid weight was obtained after soaking the leaf for 24 h in distilled water. After that, the leaves were quickly and carefully dried with tissue paper prior to determination of turgid weight. Leaf dry weight was obtained after drying the sample for 48 h at 75°C. Relative water content was calculated as:

RWC = [(fresh weight – dry weight) / (turgid weight – dry weight)] × 100 [1]

Leaf area was measured at the flowering stage using a leaf area meter (ADC-AM300). At maturity, all plants from each pot were harvested. Then grains were detached from the pods and grain yield per plant was determined. Analysis of variance of the data appropriate to the experimental design and comparison of means at p≤0.05 were carried out, using MSTATC software.

3. Results

3.1. Chlorophyll content index (CCI)

The analysis of variance of data showed significant effects of salinity on safflower chlorophyll content index. However, no significant effects of exogenous foliar application of SA and JA on this trait were found. Interaction of salinity × hormone for this trait was not statistically different (Table 1). The lowest chlorophyll content of safflower was recorded for S_4 (12 dS/m), but there was no significant difference in CCI of S_1, S_2 and S_3 (Table 2).

Table 1: Analysis of variance of the data of safflower plants under different salinity treatments and hormonal applications

S.O.V	d.f	MS CCI	Fv/Fm	RWC	LAI	Grain yield
Replication	2	401.73	0.001	27.25	0.016	0.012
Salinity (S)	3	469.93**	0.011**	376.47**	1.166**	0.787**
Hormone (H)	2	117.18^{ns}	0.038**	568.58**	0.283**	0.272**
S×H	6	176.67^{ns}	0.001^{ns}	25.91^{ns}	0.026**	0.011^{ns}
Error	22	90.55	0.001	22.64	0.004	0.009
C.V (%)	-	17.18	4.31	6.42	3.42	6.35

ns ,**: No significant and significant at p≤0.01, respectively

Table 2. Means of chlorophyll content index (CCI), Fv/Fm, relative water content (RWC), leaf area index (LAI) and grain yield for different salt stress and hormonal applications

Treatments	CCI	Fv/Fm	RWC (%)	LAI	Grain yield (g/plant)
Salinity					
S_1	60.9 a	0.799a	83.4 a	2.4 a	1.8 a
S_2	57.7 a	0.761 ab	73.3 b	1.8 b	1.5 b
S_3	58.1 a	0.738 b	70.1 b	1.7 c	1.3 c
S_4	44.7 b	0.718 c	69.4 b	1.6 d	1.1 d
Hormonal application					
Control	51.8 a	0.690 b	66.2 b	1.7 b	1.3 b
SA	57.8 a	0.779 a	76.8 a	2.0 a	1.5 a
JA	56.5 a	0.793 a	79.2 a	2.0 a	1.5 a

Different letters in each column indicate significant difference at P≤0.05. S1, S2, S3, S4: 0, 4, 8 and 12 dS/m NaCl salinity, respectively
SA: 1 mM salicylic acid and JA: 0.5 mM jasmonic acid

3.2. Efficiency of Photosystem II (Fv/Fm)

Fv/Fm was significantly affected by salinity and foliar application of SA and JA. However, interaction of salinity × foliar application for this trait was not

significant (Table 1). Efficiency of photosystem II significantly decreased with increasing salt stress. The Fv/Fm for JA and SA treated plants was statistically similar, but significantly higher than that for control (Table 2).

3.3. Relative Water Content (RWC)

Salinity and exogenous foliar application of SA and JA had significant effects on relative water content (RWC) of safflower leaves (Table 1). Leaf relative water content was decreased as salt stress increased. However, there was no significant difference between plants under S_2, S_3 and S_4 (Table 2). Exogenous foliar application of JA and SA similarly improved relative water content of safflower leaves, compared with control (Table 2).

3.4. Leaf Area Index (LAI)

Leaf area index of safflower was significantly influenced by salinity and exogenous foliar application of JA and SA (Table 1). Interaction of salinity × hormone for LAI was also significant. Leaf area index of safflower was decreased with increasing salt stress (Table 2). Foliar application of SA and JA similarly enhanced leaf area index of safflower plants (Table 3). This improvement for plants under non-saline condition and 12 dS/m salinity was greater than that under other salinity treatments (Fig 1).

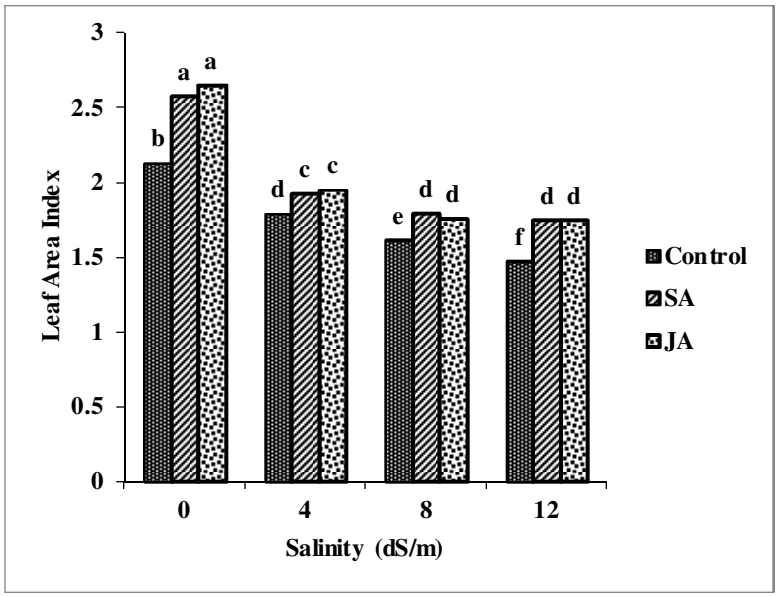

Fig. 1: Leaf area index of safflower plants under salt stress and hormonal applications. Different letters indicate significant difference at P≤0.05. SA: 1 mM salicylic acid and JA: 0.5 mM jasmonic acid

3.5. Grain yield

Grain yield was significantly influenced by salinity and hormonal application (Table 1). Interaction of salinity × hormone for grain yield was not significant (Table 1). Grain yield diminished with increasing salinity. Foliar treatments of plants with SA and JA significantly enhanced grain yield of safflower from 1.3 g/plant to 1.5 g/plant (Table 2).

4. Discussion

Chlorophyll is the main pigment of photosynthesis in plants. To some extent, the Chlorophyll content can reflect the photosynthesis rate of plant. It is strongly influenced by environmental factors (Qiu *et al*., 2007). Reduction in CCI under severe salinity (Table 2) can be attributed to a salt-induced weakening of protein-pigment-lipid complex (Strogonove *et al*., 1970) or increased chlorophyllase enzyme activity (Noreen and Ashraf, 2009). The decrease in chlorophyll content under salt stress is commonly reported phenomenon which adversely affects membrane stability (Hajer *et al*., 2006; Ashraf and Bhatti, 2002). Reduction in leaf chlorophyll content index due to severe salinity stress can potentially limit photosynthesis and yield.

The low Fv/Fm values under saline condition (Table 2) may be related with the initial damage occurring in PSII, likely due to low water availability. This reduction in Fv/Fm under salt stress is dependent on damage to reaction centers and reducing electron transport capacity in PSII (Basu *et al*., 1998). Exogenous foliar application of SA and JA significantly improved chlorophyll fluorescence of safflower by increasing PSII efficiency (Table 2). Increasing PSII efficiency may be related with the effects of SA and JA on density of reaction centres per PSII antenna chlorophyll, quantum yield for electron transport and conformational changes in D_1 protein (Bulkhov *et al*., 1999), causing alterations in the properties of PSII electron acceptors (Andréasson *et al*., 1995). Salicylic acid may

accelerate the repair and turnover of D_1 protein and thus protect photosynthetic system by inducing protein kinase activity and reversible phosphorylation of protein (Hui-Jie *et al*., 2011).

One of the early symptoms of salinity stress in plant tissue is the decrease of relative water content (RWC). This reduction of RWC in stressed plants may be associated with a decrease in plant vigor and was observed in many plant species (Halder and Burrage, 2003). The decrease in leaf RWC (Table 2) could be related with ion toxicities, ion imbalance and osmotic stress (Cicek and Cakirlar, 2002). Higher RWC of plants treated with SA and JA may be associated with accumulation of so-called JA-induced and SA-induced proteins that were found in all plant species (Pre *et al*., 2008).

Final leaf size depends on both cell division and cell elongation. Leaf initiation, which is governed by cell division, was shown to be unaffected by salt stress, but leaf extension was found to be a salt-sensitive process (Papp *et al*., 1983). Thus, cell division in leaves appears to be less salt sensitive than cell elongation. On the other hand, cell numbers in leaves were reduced by salinity (Munns and Termaat, 1986). Reduction in leaf area due to salt stress (Table 2) may be resulted from the nutritional imbalance due to an interference of salt ions, such as Na^+ and Cl^- with K^+ involved in both uptake and translocation processes (Errabi *et al*., 2007). Potassium is a major plant macro-nutrient that plays important roles related to stomatal behavior, osmoregulation, enzyme activity, cell expansion, neutralization of non-diffusible negatively charged ions and membrane polarization. Toxic effects of Na^+ are largely due to its ability to compete with K^+ for binding site essential for cellular function (Yildirim *et al*., 2009). Beneficial effects of SA and JA application on leaf area expansion of safflower plants (Figure 1) may be related with enhancing essential nutrients uptake (Mady, 2009), detoxifying super oxide radicals (Joseph et al., 2010), reducing lipid peroxidation (Aftab *et al*., 2011), increasing RWC (Table 2) by augmenting osmo-regulant proline production, improving photosynthetic pigments and consequently enhancing photosynthesis and growth (Ullah *et al*., 2012).

Salt stress considerably reduced grain yield of safflower, due to reductions in leaf chlorophyll content, relative water content and photosystem II efficiency (Table 2). Inhibition of chlorophyll synthesis (Table 2, Delfine *et al*., 1999), reduction of PSII reaction center efficiency (Strasser *et al*., 2000) and decrease in relative water content under salinity (Table 2) can influence leaf area expansion, plant growth (Fig 1, Kalaji and Guo, 2008) and grain yield. The superiorities of SA and JA treated safflower plants in growth and grain yield directly related with enhanced CCI, Fv/Fm, RWC and LAI (Table 2).

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