Advances in Bioresearch
Adv. Biores., Vol 7 (4) July 2016: 34-40
©2016 Society of Education, India
Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
ICV Value 8.21 [2014]

Advances
in
Bioresearch

ORIGINAL ARTICLE

Salicylic Acid regulate Physiological Performance of milk thistle (*Silybum marianum* L.) under water stress

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ABSTRACT

A field experiment was conducted in 2015 to determine the effect of different irrigation levels (I1, I2, I3 and I4: irrigation after 70, 110,150 and 190 mm evaporation, respectively) and salicylic acid (SA) applications (0 and 1 mM) on some physiological traits of milk thistle. The experiment was arranged as split plot (using RCB design) with three replications, with irrigation levels in main plots and SA treatments in sub plots. Results showed that ground green cover, leaf water content (LWC), chlorophyll content index (CCI) and maximum quantum yield of the PSII (Fv/Fm) decreased, but leaf temperature (LT) increased with increasing water stress. LT declined, but all other traits enhanced as a result of SA application under both well watering and limited irrigation conditions. Effects of water stress and SA on membrane stability index were not significant, indicating that cell membranes of milk thistle remain stable at irrigation intervals up to 190 mm evaporation.

***Keywords**: chlorophyll, ground cover, milk thistle, salicylic acid, water stress*

Received 18/04/2016 Accepted 02/07/2016

How to cite this article:
K Ghassemi-Golezani, S Ghassemi and I Yaghoubian. Salicylic Acid regulate Physiological Performance of milk thistle (*Silybum marianum* L.) under water stress. Adv. Biores. Vol 7 [4] July 2016: 34-40. DOI: 10.15515/abr.0976-4585.7.4.3440

INTRODUCTION

Milk thistle (*Silybum marianum* L.) is an annual plant belonging to the Asteraceae family. Seeds of this plant contain flavonoid substances, which are important in the modern pharmaceutical industry [20]. Silymarin is the pharmacological active component of the fruit, which is composed of an isomeric mixture of the flavonolignans silychristin, silydianin, and the diastereoisomers silybin and isosilybin [29]. Milk thistle is distributed in most temperate areas of the world [6].

Arid and semi-arid regions of the world facing water shortage, therefore using new and more tolerant plant species with higher production is a vital necessity [3]. The most parts of Iran is considered as one of the arid and semiarid areas in the world, thus selecting drought tolerant plants is a key goal for improving crop production in these conditions. 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, membrane disruption, stomatal closure and low photosynthesis. These drought-stressed plants consequently exhibit poor growth and yield [44]. Although many studies have identified the physiological responses of plants to water deficit [12, 16], it is not so evident how the environmental variability and complexity can influence these processes. The responses of plants facing the environmental variability involve the way in which the different plant parts interact with one another, and the different time scales in relation to its development [7].

Breeding, genetic engineering and application of plant growth regulators are some approaches to increase plant tolerance against stresses. Salicylic acid (SA) is one of the growth regulators that participates in regulation of physiological processes of plants in response to environmental stresses [13, 27].Application of SA significantly increased growth parameters, photosynthetic pigments and proline content and decreased lipid peroxidation [9]. K^+ accumulation of leaves, photosynthesis, relative vitality and photosystem II efficiency (Fv/Fm) in mung bean [14]and also leaf chlorophyll content index, Fv/Fm, relative water content and leaf area index were significantly higher for safflower plants treated with SA compared with control [13]. Several studies also supported a major role of SA in modulating the plant response to several abiotic stresses including drought [46].Therefore, SA can be used to promote growth and development of plants under favourable and unfavourable environmental conditions. The ameliorative effect of SA on plant growth under abiotic stress conditions have been related to its role in nutrient uptake, membrane stability, water relations, stomatal regulation, photosynthesis, growth and inhibition of ethylene biosynthesis [2, 43]. This research was carried out to investigate changes in physiological performance of milk thistle in response to water stress and foliar application of SA.

MATERIALS AND METHODS

A split plot experiment (using RCB design) with three replications was conducted in 2015 at the Research Farm of the Faculty of Agriculture, University of Tabriz, Tabriz, Iran (Latitude 38° 05′N, Longitude 46° 17′E, Altitude 1360 m above sea level) to evaluate physiological performance of milk thistle (*Silybum marianum* L.) under water stress in response to salicylic acid (SA) application. The climate is characterized by mean annual precipitation of 245.75 mm per year and mean annual temperature of 10°C. Irrigation treatments (I_1, I_2, I_3 and I_4: irrigation after 70, 110,150 and 190 mm evaporation from class A pan, respectively) were located in main plots and two levels of SA (0 and 1 mM) were allocated to sub plots.

Each plot had 6 rows of 3 m length, spaced 25 cm apart. Seeds were treated with Benomyl at a rate of 2 g/kg before sowing. The seeds were then sown by hand on 28 May 2015 in 3 cm depth of a sandy loam soil. All plots were irrigated immediately after sowing, but subsequent irrigations were carried out according to the treatments. Weeds were controlled by hand during crop growth and development as required. SA was sprayed on plants at vegetative stage. Following physiological traits were measured at flowering stage:

Ground cover percentage (PGC)

PGC was measured by viewing the canopy through a wooden frame (50 cm × 50 cm), divided into 100 equal sections. The sections were counted when more than half filled with crop green area.

Leaf water content (LWC)

10 g of leaves from a plant in each plot were cut and then dried in an oven for 48 h at 75°C and weighed. LWC was determined as:

$$LWC\ (\%) = ((FW - DW) / FW) \times 100$$

Where FW is fresh weight and DW is dry weight.

Leaf temperature (LT)

Leaf temperature (^{o}C) was measured by an infrared thermometer (TES-1327) in upper, middle and lower leaves of a plant from each plot, just before irrigation. Mean temperature was calculated for each plot.

Membrane stability index (MSI)

MSI was determined by recording the electrical conductivity of leaf leachates in double distilled water at 40°C and 100°C [10]. Leaf samples were cut into 5 discs of uniform size and taken in test tubes containing 100 ml of distilled water in two sets. One set was kept at 40°C for 30 min and another set at 100°C for 10 min and their respective electric conductivities were measured by a conductivity meter.The MSI was calculated as:

$$MSI = (EC_{40^{\circ}C} / EC_{100^{\circ}C}) \times 100$$

Chlorophyll content index (CCI)

CCI was recorded by a chlorophyll meter (CCM-200, Opti- Science, USA) in upper, middle and lower leaves of a plant from each plot. Then mean CCI was calculated for each plot.

Chlorophyll fluorescence

Maximum quantum yield of photosystem II (Fv/Fm) was measured in leaves by a chlorophyll fluorometer (OS-30, OPTISCIENCES, USA) before irrigation of each plot. Dark-adapted leaves (30 min.) were initially exposed to the weak modulate measuring beam, followed by exposure to saturated white light to estimate the Fv/Fm. The quantum yield (Fv/Fm) measures the efficiency of excitation energy capture by open PSII reaction centers, representing the maximum capacity of light-dependent charge separation in PSII [4].

Statistical analysis

Analysis of variance appropriate to the experimental design was conducted, using MSTAT-C software. Means of each trait were compared according to Duncan multiple range test at $p \leq 0.05$. Excel software was used to draw figures.

RESULTS AND DISCUSSION

Percentage ground cover (PGC) of milk thistle was significantly ($P \leq 0.05$) decreased by increasing water stress, with no significant difference between I_1 and I_2 and also between I_3 and I_4 (Figure 1a). Exogenous application of salicylic acid (SA) significantly ($P \leq 0.05$) improved PGC (Figure 1b).

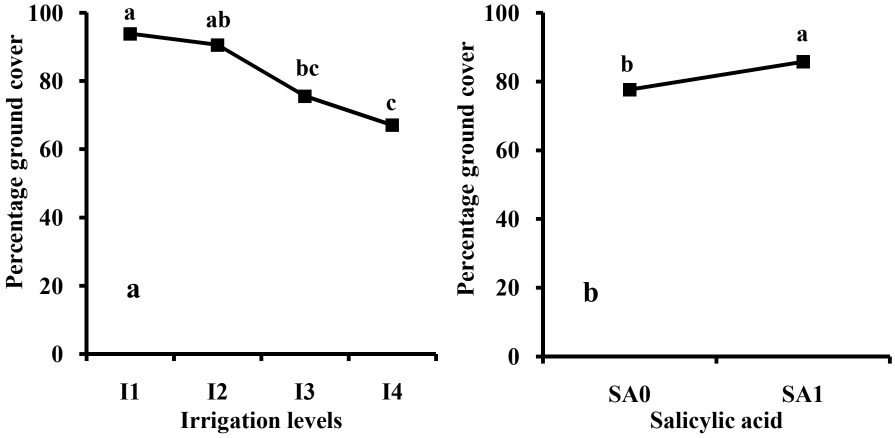

Figure 1. Changes in percentage ground cover of milk thistle in response to water stress (a) and salicylic acid (b). I_1, I_2, I_3, I_4: Irrigation after 70, 110,150 and 190 mm evaporation, respectively. SA_0 and SA_1: 0 and 1 mM salicylic acid

Reduction in percentage ground green cover due to water stress (Figure 1a) can be attributed to competition of plants for water and nutrients[19].Water stress during vegetative stages has the greatest impact on plant height and biomass [15].Percentage and duration of ground cover in soybean[17]and chickpea [12]were also sharply decreased due to water stress at later stages of plant development.SA is considered to be an endogenous growth regulator of phenolic nature that enhanced the leaf area of milk thistle (Figure 1b), as also reported in maize and soybean [26]. Applications of SA also increases number of leaves under stress conditions [1, 49].According to Shakirova [40]the positive effect of SA on growth can be due to its influence on other plant hormones. SA alteres the auxin, cytokinin and ABA balances in plants and increases the growth and yield under both normal and saline conditions.

Leaf water content (LWC) was significantly ($P \leq 0.05$) affected by irrigation treatments and SA. LWC considerably reduced under severe water deficit (I_4), but it was statistically similar under I_1, I_2 and I_3 irrigation treatments (Figure 2a).SA treated plants had moreLWC, compared with control plants (Figure 2b). There is a direct relationship between leaf water content and drought resistance [8, 31].The difference in leaf water content may be resulted from the differences in cell wall elasticity [24]. It has been reported that high leaf water content is a mechanism of drought resistance rather than drought escape and it is believed that high leaf water content is the result of higher osmotic regulation of tissue with lower elasticity [39].Decrease in LWC of plants under drought stress may depend on plant vigour reduction [30].HigherLWC of plants treated with SA may be associated with accumulation of so-called SA-induced proteins that were found in all plant species [36]. Similar results was reported for safflower [13].

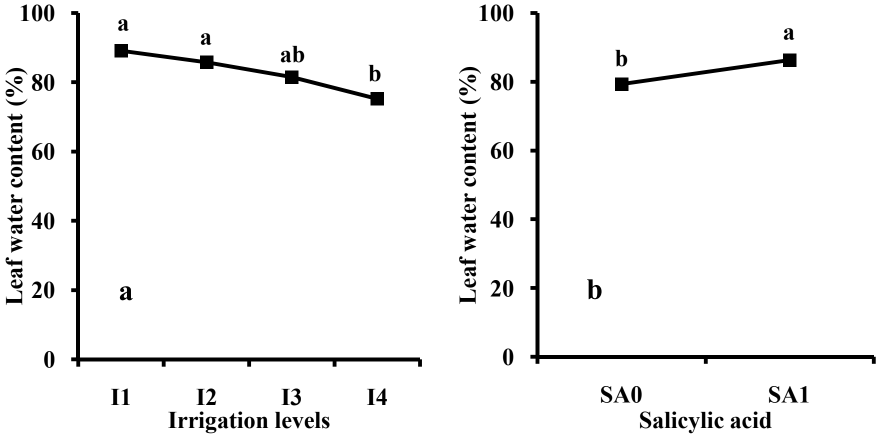

Figure 2. Changes in leaf water content of milk thistle in response to water stress (a) and salicylic acid (b)

I_1, I_2, I_3, I_4: Irrigation after 70, 110,150 and 190 mm evaporation, respectively. SA_0 and SA_1: 0 and 1 mM salicylic acid

The effects of irrigation treatments and SA on leaf temperature (LT)were significant ($P \leq 0.01$). LT increased as a result of decreasing water availability. However, differences in LT between I_3 and I_4 were not statistically significant (Figure 3a). Application of SA significantly reduced LT of milk thistle plants (Figure 3b). These results showed a negative correlation with LWC (Figure 2).Khan et al.[25]also reported that LWC was lower, whereas LT was higher in stressed faba bean plants, probably due to restricted transpiration cooling induced by stomata closure. For a given reduction in transpiration due to stomatal closure, the increase in LT would depend strongly on environmental factors, particularly the radiation load on the leaf and the heat transfer coefficient of the air. The interactions, although complex, have been analyzed successfully by using the energy-balance approach [37]. Therefore, increasing LT due to water stress is possibly related with decreasing stomatal conductance and transpiration [41], which to some extent was alleviated by SA application [45].

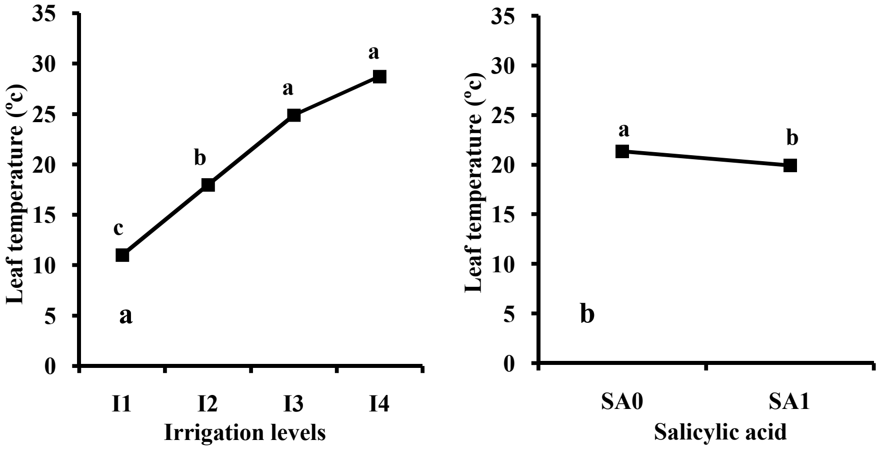

Figure 3. Changes in leaf temperature of milk thistle in response to water stress (a) and salicylic acid (b) I_1, I_2, I_3, I_4: Irrigation after 70, 110,150 and 190 mm evaporation from class A pan, respectively. SA_0 and SA_1: 0 and 1 mM salicylic acid

Irrigation treatments and SA had no significant (P 0.05) effects on membrane stability index (MSI). The degree of cell membrane injury induced by water stress may be easily estimated through measurements of MSI [22]. Under water deficit, cell membrane subjects to changes such as penetrability and decrease in sustainability [5]. The lower membrane stability index reflects the extent of lipid peroxidation, which in turn is a consequence of higher oxidative stress due to water stress conditions [32]. Application of SA ameliorates the impact of abiotic stress through improving antioxidant system necessary to reduce oxidative damage and ion leakage from membranes [48]. However, our results clearly suggest that milk thistle is a drought tolerant plant and irrigation intervals up to 190 mm evaporation had no significant effect on MSI.

Chlorophyll content index (CCI) and maximum quantum yield of photosystem II (Fv/Fm) were significantly affected by irrigation treatments ($P \leq 0.01$) and SA application ($P \leq 0.05$).CCI (Figure 4a) and Fv/Fm (Figure 5a) decreased as a result of water stress, with no significant difference between I_2 and I_3 treatments. However, exogenous application of SA increased these traits (Figures 4b and 5b).

Limited water supply usually causes a reduction in CCI[34]. Chlorophyll loss is a negative consequence of water stress; however, it has been considered as an adaptive feature in plants grown under water deficit [33].The reduction of CCI was probably related with the enhanced activity of the chlorophyllase [38] and inducing the destruction of chloroplast structure and the instability of pigment protein complex [42].Foliar application of SA may contribute to drought tolerance via enhancing antioxidant enzymes activities [40]and inhibiting ethylene synthesis [43]. Kordi et al. [28]reported that SA application with scavenging of ROS may increase chlorophyll content in sweet basil. SA application also improved CCI in wheat [2], cucumber [47]and mung bean [18]under stress.

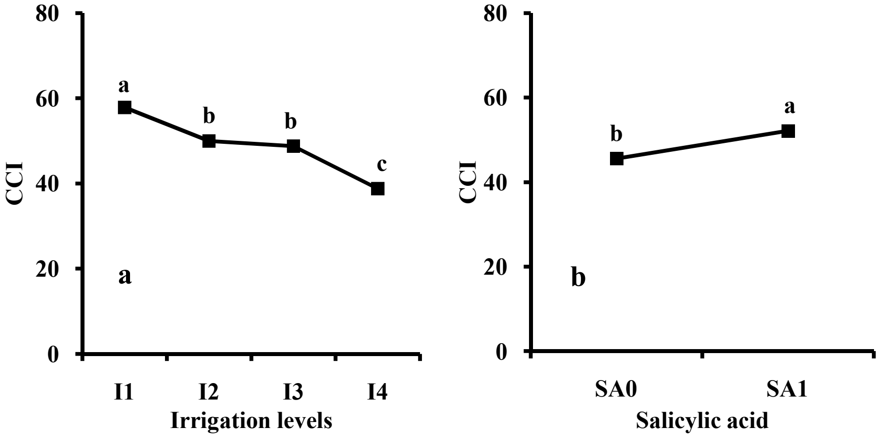

Figure 4. Changes in chlorophyll content index of milk thistle in response to water stress (a) and salicylic acid (b)

I_1, I_2, I_3, I_4: Irrigation after 70, 110,150 and 190 mm evaporation from class A pan, respectively.

SA_0 and SA_1: 0 and 1 mM salicylic acid

Chlorophyll fluorescence analysis is a good index for measuring rapidly the change in photosynthetic metabolism of plants to such environmental stresses as drought [11]. Reduction in photochemical efficiency of the PSII (Fv/Fm) under water stress (Figure 5a) indicates that occurrence of chronic photo-inhibition due to photo-inactivation of PSII probably associated with the degradation of D1 protein [21]. Some other researchers also showed that Fv/Fm reduced as a result of water stress [12, 16]. SA increased the ratio of Fv/Fm (Figure 5b). Exogenous foliar application of SA significantly improved Fv/Fm of safflower [13]and mung bean [14]under normal and stressful conditions. An increase in this ratio as a result of SA application results from photosynthetic electron transport improvement [35]. SA may accelerate the repair and turnover of D1 protein and thus protect photosynthetic system by inducing protein kinase activity and reversible phosphorylation of protein [23].

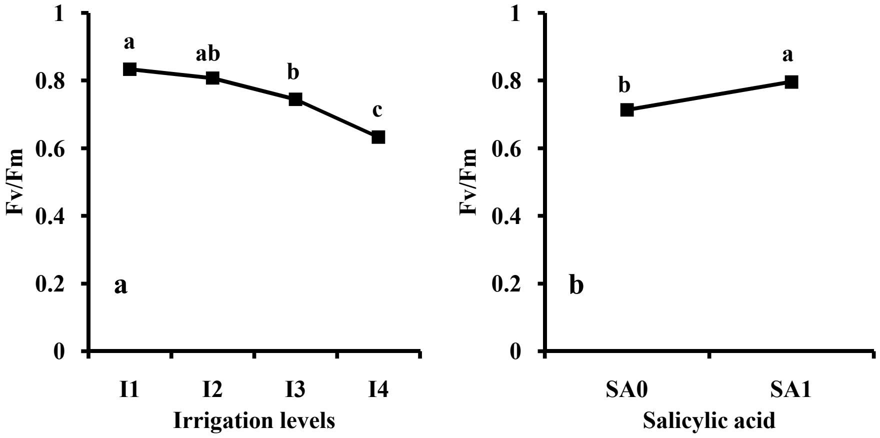

Figure 5. Changes in maximum quantum yield of photosystem II (Fv/Fm) in milk thistle affected by water stress (a) and salicylic acid (b)

I_1, I_2, I_3, I_4: Irrigation after 70, 110,150 and 190 mm evaporation from class A pan, respectively.

SA_0 and SA_1: 0 and 1 mM salicylic acid

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