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Influence of Hydro-priming duration on Morpho-physiological traits of milk thistle under water stress

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Abstract

A split plot experiment with three replications was conducted in 2015, to investigate the effects of hydro-priming duration (P_1, P_2 and P_3: 0, 8 and 16 hours, respectively) on some morpho-physiological traits of milk thistle (*Silybum marianum* L.) under different irrigation intervals (I_1, I_2, I_3 and I_4: Irrigation after 70, 100, 130 and 160 mm evaporation from class A pan, respectively). Irrigation levels and hydro-priming treatments were allocated to main and sub plots, respectively. Relative water content (RWC), membrane stability index (MSI), and all morphological traits decreased, but leaf temperature (LT) increased with increasing water stress. The LT reduced as a result of increasing priming duration up to 16 hours. Increasing water limitation led to considerable reduction in plant height, but this was enhanced by hydro-priming under all irrigation treatments. Hydro-priming for 16 hours was the best seed pretreatment to improve field performance of milk thistle in control and water stress conditions.

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Introduction

Medicinal plants have gained a considerable importance in agricultural production, pharmacy and exportation dueto their use as a raw material for the pharmaceutical industry (Abou- Arab and Abou-Donia, 2000). Milk thistle (*Silybum marianum* L.) is an annual plant belonging to the Asteraceae family with large prickly white veined green leaves and a reddish-purple flower that ends in sharp spines. Milk thistle is mainly used as a medicinal plant particularly for curing liver diseases due to the presence of active silymarin compounds (Hassan El-Mallah *et al.,* 2003). This plant natively grows in the Mediterranean and is widespread in other regions in the world including Iran (Ghavami and Ramin, 2007).

In a large part of the agricultural areas in the world, water deficit is an important factor limiting growth and productivity of the crops (Borsani *et al.,* 2001). Water deficit occurs when water potentials in the rhizosp here are sufficiently negative to reduce water availability to sub-optimal levels for plant growth and development (Boyer, 1982). To survive against the stress, plants have involved a number of morphological, physiological and biochemical responses. Under drought stress, plant leaves are dehydrated, and photosynthesis is decreased. The decrease in photosynthesis of dehydrated leaves is usually caused by a decrease in stomatal conductance and transpiration. As a consequence of the reduction in transpiration rate, leaf temperature increases and the effects of drought and heat stresses frequently combine to scorch leaves (Ghassemi-Golezani *et al.,* 2014a). Ghassemi-Golezani and Mardfar (2008) indicated thatdrought during vegetative stage had the greatest impact on plant height and biomass, while during the reproductive growth had an effect on crop productivity (Costa-Franca *et al.,* 2000).

Seed hydro-priming as a simple method can be used to reduce the harmful effects of water stress on plants (Ghassemi-Golezani *et al.,* 2008). The beneficial effects of hydro-priming have been reported for many field crops such as chickpea (Ghassemi-Golezani *et al.,* 2012), pinto bean (Ghassemi-Golezani *et al.,* 2010a,b), mung bean (Ghassemi-Golezani *et al.,* 2014b), borage (Ghassemi-Golezani *et al.,* 2013a), lentil (Ghassemi-Golezani *et al.,* 2013b), maize (Pareraand Cantliffe, 1994), sugar beet (Sadeghianand Yavari, 2004), andbarley (Ghassemi-Golezaniand Abdurrahmani, 2012). Since hydro-priming can improve field performance and yield of medicinal plants under stressful conditions (Ghassemi-Golezani *et al.,* 2012). However, the field responses of milk thistle to different hydro-priming durations, particularly under stressful conditions, were not documented, but it seems that hydro-priming can reduce the harmful effects of water stress and improve physiological conditions and field performance of milk thistle. Thus, this research was carried out to evaluate the effects of different hydro-priming durations on some morpho-physiological traits of milk thistle under different irrigation treatments.

Materials and methods

This experiment was conducted in 2015 at the Research Farm of the Faculty of Agriculture, University of Tabriz, Iran (Latitude 38°05′ N, Longitude 46°17′ E, Altitude 1360 m above sea level). The climate is characterized by mean annual precipitation of 245.75mm per year, mean annual temperature of 10°C, annual maximum temperature of 16.6°C and mean annual minimum temperature of 4.2°C. The experiment was arranged as split-plot, based on randomized complete block design in three replications, with the irrigation treatments (I_1, I_2, I_3 and I_4: irrigation after 70, 100, 130 and 160 mm evaporation from class A pan, respectively) in main plots and hydro-priming durations of 0 (P_1), 8 (P_2) and 16 (P_3) hours in sub-plots. Seeds of milk thistle (*Silybum marianum* L) were obtained from Pakan Bazr, Esfahan, Iran and Seeds were divided into three sub-samples, one of which was kept as control (non-primed, P_1) and two other samples were soaked in distilled water at 20°C for 8 (P_2) and 16 (P_3) hours and then seeds were dried at 20-25°C for 24 hours.

These seeds were treated with 3.3g/kg Benomyl and then were sown by hand in 3 cm depth of a sandy loam soil on 7 May 2015. Each plot consisted of 6 rows of 3m length; spaced 25cm apart.

All plots were irrigated immediately after sowing. After seedling establishment, irrigations were carried out according to the treatments. Weeds were frequently controlled by hand during crop growth and development. All physiological traits were measured at flowering stage:

Physiological traits

Relative Water Content (RWC)

Five young fully expanded leaves were selected from each plot at 11-12 AM local time and were placed in plastic bags within an ice tank and immediately transferred to the laboratory. Ten leaf disks of uniform size were provided and the fresh weight of the disks was recorded (Fw).

Then, leaf disks were put in distilled water for 24 h at 4°C in the darkness. After soaking, water on the leaf surface was quickly and carefully dehumidified with tissue paper prior to the determination of turgid weight (Tw). Finally, dry weight was obtained after drying the leaf disks for 24 h at 80°C (Dw). The value of RWC was determined using the following equation:

$$RWC = [(Fw - Dw)/(Tw - Dw)] \times 100$$

Where Fw, Dw and Tw are fresh weight, dry weight and turgid weight, respectively (Smart and Bingham 1974).

Leaf temperature (LT)

Just before irrigation, leaf temperature (°C)was measured by an infrared thermometer (TES-1327) in upper, middle and lower leaves of a plant from each plot. 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 (Deshmukh *et al.,* 1991). 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 electrical conductivities were measured by a conductivity meter. The MSI was calculated as:

$$MSI = (EC40°C / EC100°C) \times 100$$

Morphological traits

At maturity, plants in 1 m2 of each plot were harvested and stem height, stem diameter, branches per plant and capitol diameter were determined.

Statistical analysis

Analysis of variance of the data was performed, using MSTAT-C and Gen Stat 12. Means of each trait were compared according to Duncan multiple range test at $p \leq 0.05$. Excel software was used to draw Fig.s.

Result and discussion

Physiological traits

Leaf temperatures of milk thistle (LT) weresign-ificantly influenced by irrigation and hydro-priming treatments ($P \leq 0.01$) (Table 1). LT was enhanced as water deficit increased (Fig. 1a). When a plant is exposed to water deficit conditions, it tends to close the stomata for decreasing transpiration and then it cannot cool efficiently, which may lead to increasing leaf temperature and reduction of photosynthetic production (Duffková, 2006).

Rising leaf temperature due to water deficit could be the result of decreasing RWC (Fig. 2a) and stomata closure. LT of plants from P_2 and P_3 seeds were 13.22 and 25.42% lower than that of control (P_1), respectively (Fig. 1b).

Hosseinzadeh-Mahootchi *et al.* (2013) also reported that increasing hydro-priming duration caused a reduction in LT of chickpea plants. Plants from hydro-primed seed lots have lower leaf temperature compared with those from unprimed seed lot, mainly due to rapid seedling emergence and enhanced root growth (Ghassemi-Golezani *et al.,* 2012).

Table 1. Analysis of variance of physiological traits of milk thistle affected by water supply and hydro-priming duration.

Source of Variation	MS			
	Df	LT	RWC	MSI
Replication	2	1.00	10.11	16.78
Irrigation (I)	3	465.954^{**}	253.85^{**}	469.70^{**}
Error	6	5.148	19.63	14.04
Hydro-priming (P)	2	117.250^{**}	38.53^{ns}	32.19^{ns}
I × P	6	2.176^{ns}	3.16^{ns}	9.79^{ns}
Error	16	3.778	18.75	17.22
CV (%)	-	9.1	5.1	7.3

ns, * and **: No significant and significant at p≤0.05 and p≤0.01, respectively.

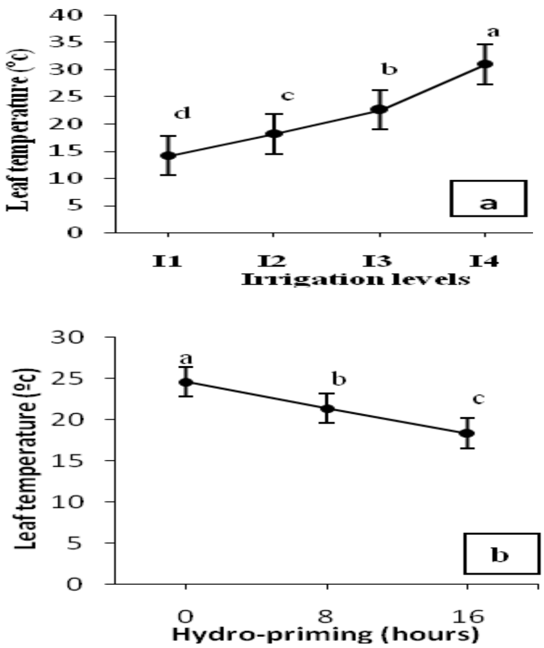

Fig. 1. Changes in leaf temperature of milk thistle in response to water stress (a) and hydro-priming (b) I_1, I_2, I_3, I_4: Irrigation after 70, 100, 130 and 160 mm evaporation from class A pan, respectively. P_1, P_2 and P_3: 0, 8 and 16 hours hydro-priming duration. Different letters indicate significant difference at p≤ 0.05.

Relative water content (RWC) was significantly (P ≤ 0.01) affected by irrigation treatments, but hydro-priming had no significant effect on this trait (p > 0.05). RWC was gradually reduced by enhancing water deficit, with no significant difference between I_1 and I_2, I_2 and I_3 and also between I_3 and I_4. Moderate (I_3) and severe water deficit (I_4) led to 8.75 and 13.68% reduction in the relative water content of milk thistle, respectively (Fig. 2a). The decline in RWC could be attributed to an imbalance between water loss from the leaves due to evapotranspiration in the plant canopy and replenishment by irrigation (Jones, 2007). There is a directive relationship between leaf water content and drought resistance (Merah, 2001). Differences in leaf water content may be resulted from the differences in cell wall elasticity (Johnson *et al.,* 1984). A reduction in RWC of medicinal plants under drought stress has also been observed in *Melissa officinalis* L. (Munné-Bosch and Alegre, 1999), *Borago officinalis* L. (Dastborhan and Ghassemi-Golezani, 2015), *Rosmarinus officinalis* L. (Munné-Bosch *et al.,* 1999) and *Hibiscus rosa-sinensis* (Egilla *et al.,* 2005).

Membrane stability index (MSI) was significantly influenced by irrigation intervals (p ≤ 0.01), but the effect of hydro-priming on this trait was not significant (p > 0.05) (Table 1). MSI of milk thistle leaves significantly decreased under moderate (I_3) and severe (I_4) water deficit (Fig. 2b) as a consequence of membrane lipids peroxidation, membrane damage and ion leakage (Katsuhara *et al.,* 2005). 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 (Moussa and Abdel-Aziz, 2008). Desiccation of plant cells causes cell membrane leakage of ions and electrolytes (Bandurska, 2001). The cell membrane plays an important role in maintaining cell viability, by providing both osmotic and ionic equilibrium between the cellular component and its environment (Bajji *et al.,* 2001). Modifications of lipid composition of plasma membranes are vital in sustaining membrane fluidity, integrity and functionality when confronting external perturbations (Yeilaghi *et al.,* 2012).

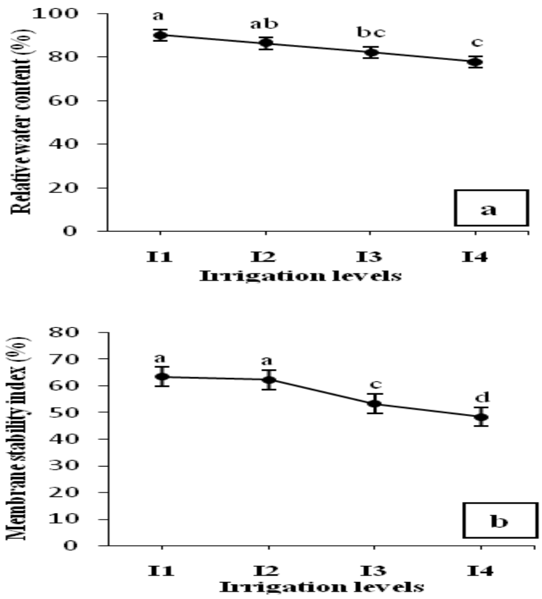

Fig. 2. Changes in Relative water content (a) and Membrane stability index (b) of milk thistle in response to water stress I_1, I_2, I_3, I_4: Irrigation after 70, 100,130 and 160 mm evaporation from class A pan, respectively. Different letters indicate significant difference at $p \leq 0.05$.

Morphological traits

Morphological traits were significantly affected by irrigation. Effect of hydro-priming was only significant on plant height (Table 2). Increasing water limiting significantly reduced plant height, which was 10.57, 33.37 and 45.81% lower than that under well watering (I_1), depending on severity of stress (Table 3). Under severe water deficiency, cell elongation of higher plants can be inhibited by interruption of water flow from the xylem to the surrounding elongating cells (Nonami, 1998). Drought caused impaired mitosis; cell elongation and expansion resulted in reduced growth and yield traits (Hussain *et al.,* 2008). Reduction in plant height could be associated with the cell enlargement and more leaf senescence in the plants under water stress (Manivannan *et al.,* 2007).

Table 2. Analysis of variance of morphological traits of milk thistle affected by water supply and hydro-priming duration.

		MS			
Source of Variation	Df	Plant height	Stem diameter	Branches per plant	Capitol diameter
Replication	2	85.75	0.01662	3.083	0.3149
Irrigation (I)	3	8328.03^{**}	1.70398^{**}	12.741^{*}	18.4503^{**}
Error	6	13.97	0.00921	1.824	0.1259
Hydro-priming (P)	2	539.58^{**}	0.01154^{ns}	1.083^{ns}	0.0817^{ns}
I × P	6	128.03^{ns}	0.00584^{ns}	0.046^{ns}	0.0166^{ns}
Error	16	74.50	0.01031	2.139	0.2051
CV (%)	-	7.6	8.0	36.6	7.7

ns, * and **: No significant and significant at $p \leq 0.05$ and $p \leq 0.01$, respectively

Table 3. Means of morphological traits of milk thistle affected by water supply.

Treatments	Plant height	Stem diameter	Branches per plant	Capitol diameter
Irrigation				
I_1	145.6^{a}	1.686^{a}	5.111^{a}	7.734^{a}
I_2	130.2^{b}	1.542^{b}	4.889^{ab}	6.067^{b}
I_3	97.0^{c}	1.101^{c}	3.333^{bc}	5.282^{c}
I_4	78.9^{d}	0.729^{d}	2.667^{c}	4.357^{d}

Different letters in each column indicate significant difference at $p \leq 0.05$. I_1, I_2, I_3, I_4: Irrigations after 70, 100, 130 and 160 mm evaporation, respectively.

The highest plant height was obtained for plants from the P_3, followed by P_2 plants. However, there was no significant difference between plants from P_2 and P_3 seed lots (Fig. 3).

Hydro-priming affects DNA and RNA synthesis, alphaamylase activities and better embryo growth. By improving the germination rate, growth consistency, seedling vigor and deployment and plant growth improves. Ghassemi-Golezani *et al.* (2013b) also reported that increasing hydro-priming duration caused a Increase in plant height of lentil plants.

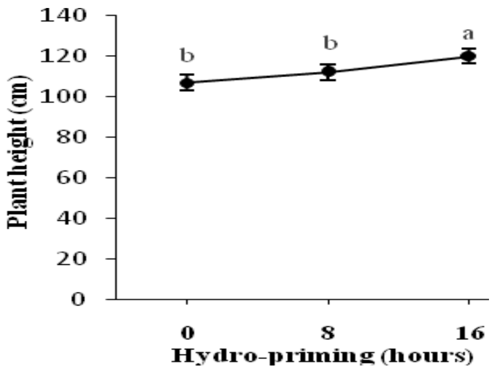

Fig. 3. Changes in Plant height of milk thistle in response to hydro-priming duration P_1, P_2 and P_3: 0, 8 and 16 hours hydro-priming duration. Different letters indicate significant difference at p≤ 0.05.

Stem diameter under water limitation (I_2, I_3 and I_4) was 8.54, 34.69 and 56.76% less than that under normal irrigation, respectively. Significant reductions in stem diameter under water limitation were also reported for other medicinal plants such as Borage (Dastborhan *et al.,* 2013), caraway (Laribi *et al.,* 2009) and chamomile (Razmjoo *et al.,* 2008). An early morphological response to drought stress is the avoidance mechanism through adjustment of plant such as a reduction in stem diameter (Lei *et al.,* 2006).

Branches per plant and capitol diameter significantly reduced with decreasing water availability (Table 2). Minimum and maximum number of branches per plant were observed under I_4 and I_1, respectively. However, there was no significant difference between I_1 and I_2 (Table 3). Capitol diameter was decreased as water supply diminished.

The highest capitol diameter was obtained under well watering (I_1). Capitol diameter under severe water limitation (I_4) was considerably lower than that under other irrigation treatments (Table 3). Reduction in branchs number per plant and capitol diameter due to water stress was also reported for chickpea (Hosseinzadeh-Mahootchi *et al.,* 2013) and sunflower (Goksoy *et al.,* 2004).

Conclusion

In conclusion, the results of the present study indicate that hydro-priming for 16 hours was the best seed pretreatment to improve morpho-physiological traits and field performance of milk thistle in control and water stress conditions.

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