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Hydro-priming effects on safflower under water limitation: Some physiological traits, grain and oil yields

Saeid Ghassemi[1], Iraj Yaghoubian*[1], Yasser Yaghoubian[2]

[1]*Department of Plant Eco-Physiology, Faculty of Agriculture, University of Tabriz, Tabriz, Iran.*

[2]*Genetics and Agricultural Biotechnology Institute of Tabarestan, Sari Agricultural Sciences and Natural Resources University, Sari, Iran.*

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Abstract

This experiment was conducted to evaluate the effects of hydro-priming durations on some physiological traits and yield of safflower (*Carthamus tinctorius* L.) under different irrigation treatments. The results indicated that leaf water content (LWC), membrane stability index (MSI), chlorophyll content index (CCI), maximum quantum yield of the PSII (Fv/Fm), grain yield, oil percentage and yield decreased, but leaf temperature (LT) increased with increasing water stress. LT declined, but LWC, CCI, grain yield, oil percentage and yield enhanced as a result of hydro-priming treatments. Hydro-priming had no significant effect on MSI and Fv/Fm.

***Corresponding Author:** Iraj Yaghoubian ✉ yaghobian.i@gmail.com

Introduction

Safflower (*Carthamus tinctorius* L.) is an annual plant from the Compositae family. This plant is one of the most important oil grain cultivated plants used for edible oil production in the world (Dwivedi *et al.*, 2005), and at the last century, has been cultivated mainly for the oil extracted from its grains (Corleto *et al.*, 1997). The oil content of its grain varies from 35 to 50 % (Camas *et al.*, 2007). Safflower commonly grows in semi-arid areas with temperate climates in many regions of the world and is used as industrial oils, spice and birdseed (Johnston *et al.*, 2002).

Environmental stresses such as drought may limit safflower performance in the field. Water deficit is a major constraint which reduces the productivity of crops. 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. Some of the deleterious effects of environmental stresses such as water limitation on crop performance may be also overcome by seed priming (Ghassemi-Golezani *et al.*, 2008).

Seed priming is a pre-germination treatment in which seeds are held at a water potential that allows imbibition, but prevents radicle extension (Bradford, 1986), and then seeds are dried back to the original moisture level (McDonald, 2000). Common priming techniques include osmopriming (soaking seeds in osmotic solutions such as polyethylene glycol), halo-priming (soaking seeds in salt solutions) and hydro-priming (soaking seeds in water). The beneficial effects of priming have been associated with various biochemical, cellular and molecular events including synthesis of RNA and proteins (Bray *et al.*, 1989; Dell' Aquilaand Bewley, 1989). Priming also restores activities of enzymes involved in the cell detoxifying mechanisms such as super oxide dismutase, catalase and glutathione reductase in aged seeds (Bailly *et al.*, 1997). These effects can lead to better stand establishment, more vigorous plants, better drought tolerance, earlier flowering, earlier harvest and higher seed yield (Harris *et al.*, 2001; 2002).

However, the field responses of safflower to different hydro-priming durations, particularly under stressful conditions, were not documented. Based on various beneficial effects of seed priming, this research was carried out to evaluate the influence of hydro-priming durations on some physiological traits and oil yield of safflower under water stress.

Materials and methods

A split plot experiment (using RCB design) with three replications was conducted in 2014 at a farm of the Hashtrood, East Azerbaijan, Iran (Latitude 37°28′ N, Longitude 46°52′ E, Altitude 1643 m above sea level) to evaluate the effects of hydro-priming durations on physiological performance, grain and oil yields of safflower (*Carthamus tinctorius* L.) under water stress. The climate is characterized by mean annual precipitation of 304.05 mm per year and mean annual temperature of 10°C. Irrigation treatments (I_1, I_2, I_3, I_4: irrigation after 60, 90, 120 and 150 mm evaporation from class A pan, respectively) were located in main plots and hydro-priming durations (0, 8 and 16 hours) were allocated to sub plots.

Each plot had 6 rows of 3 m length, spaced 25 cm apart. Before sowing, seeds were divided into three sub-samples, one of which was kept as control (non-primed, P_0) and two other samples were soaked in distilled water at 20°C for 8 (P_1) and 16 (P_2) hours and then dried back to initial moisture content at room temperature of 22±2°C. Seeds were treated with Benomyl at a rate of 2 g/kg before sowing. The seeds were then sown by hand on 1 May 2014 in 4 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. All physiological traits were measured at flowering stage:

Physiological traits

Leaf water content (LWC)

Leaves from three plants in each plot were cut, weighed and then dried in an oven for 48 h at 75°C and reweighed. Leaf water content (LWC) was determined as:

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

Where FW is fresh weight and DW is dry weight.

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)

Membrane stability index (MSI) was determined according to Deshmukh *et al.* (1991). Leaf samples were cut into 10 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 10°C for 10 min and their respective electric conductivities were measured by a conductivity meter. The MSI was calculated as:

$MSI = (EC_{40°C} / EC_{100°C}) \times 100$.

Chlorophyll content index (CCI)

Chlorophyll content index (CCI) was recorded by a chlorophyll meter (CCM-200, Opti- Science, USA) in a plant from each plot. Chlorophyll fluorescence (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.

Grain and oil yields

At maturity, plants in 1 m^2 of the middle part of each plot were harvested and grain yield per unit area was determined. Oil was extracted from 10 g grains of each plot in petroleum ether for 5 hours using a soxhlet system according to the AOCS method (AOCS, 1993). Oil content was determined as a percentage for each sample and then oil yield per unit area was calculated as:

Oil yield = Grain yield × Oil percentage

Statistical analysis

Analysis of variance appropriate to the experimental design was conducted, using Gen Stat 12 software. Means of each trait were compared according to Duncan multiple range test at $p \leq 0.05$. Excel software was used to draw Figs.

Results and discussion

Analysis of variance indicated that irrigation treatments and hydro-priming durations significantly influenced leaf water content (LWC), leaf temperature (LT) and chlorophyll content index (CCI). However-membrane stability index (MSI) and chlorophyll fluorescence (Fv/Fm) were significantly affected by irrigation treatments and hydro-priming had no significant effect on this traits (Table 1).

Table 1. Analysis of variance of the effects of hydro-priming durations on some physiological traits of safflower under different irrigation treatments.

Source of Variation	Df	Mean Square				
		LWC	LT	MSI	CCI	FV/FM
Replication	2	9.36	0.583	15.19	33.08	0.000686
Irrigation (I)	3	550.15**	469.852**	523.95**	334.67**	0.042921**
Error	6	8.40	11.102	13.01	18.08	0.002427
Hydro-priming (P)	2	99.36*	145.083**	46.86^{ns}	88.08*	0.003803^{ns}
I × P	6	26.40^{ns}	3.713^{ns}	16.12^{ns}	1.97^{ns}	0.000410^{ns}
Error	16	23.43	3.764	15.76	15.83	0.001871
CV (%)	-	5.8	9.2	7.0	9.6	5.9

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

With decreasing water availability the LWC was decreased and that may depend on plant vigour reduction (Liu *et al.*, 2002).

The highest LWC was observed in P_2, However, there was no significant difference between P_1 and P_2 (Table 2).

The difference in relative water content may be resulted from the difference in cell wall elasticity (Johnson *et al.*, 1984). It has been reported that high relative water content is a mechanism of drought resistance (rather than drought escape) and it is believed that high relative water content is the result of higher osmotic regulation of tissue with lower elasticity (Ritchie *et al.*, 1990). Many important physiological and morphological processes, such as LWC, leaf enlargement, stomatal opening, and associated leaf photosynthesis are directly affected by the reduction of leaf turgor potential which accompanies the loss of water from leaf tissue (Jones and Turner, 1978). Thus, pre-hydration of seeds improved the uniformity of seedling emergence (Kibiteand Harker, 1991), and early achievement of maximum ground cover are essential for the efficient use of resources like water and light (Ghassemi Golezani *et al.*, 2008).

Table 2. Means of safflower physiological traits influenced by irrigation and hydro-priming durations.

Treatments	LWC (%)	LT (°c)	MSI (%)	CCI	FV/FM
Irrigation					
I_1	90.44[a]	13.67[d]	64.11[a]	47.67[a]	0.8078[a]
I_2	86.67[b]	17.56[c]	62.44[a]	44.11[ab]	0.7600[ab]
I_3	82.11[c]	22.33[b]	53.33[b]	40.11[b]	0.7056[b]
I_4	72.33[d]	30.44[a]	48.00[c]	33.44[c]	0.6478[c]
Hydro-priming					
P_0	80.50[b]	24.67[a]	55.17[a]	38.25[b]	0.7100[a]
P_1	82.08[ab]	20.58[b]	56.67[a]	43.33[a]	0.7375[a]
P_2	86.08[a]	17.75[c]	59.08[a]	42.42[a]	0.7433[a]

Different letters in each column indicate significant difference at $p \leq 0.05$. I_1, I_2, I_3, I_4: irrigation after 60, 90, 120 and 150 mm evaporation from class A pan, respectively. P_0, P_1 and P_2: non-primed and hydro-primed seeds for 8 and 16 h, respectively.

With decreasing water supply, LT significantly increased. In contrast, hydro-priming significantly reduced LT of safflower plants. This effect may associated with decreasing relative water content (Table 2) and consequently reduction in stomatal conductance and transpiration (Siddique *et al.*, 2000).During drought stress, leaves are subjected to both heat and water deficiency stress (Clarke *et al.*, 1993). As a consequence of the reduction in transpiration rates of leaves, leaf temperature increases. Also, MSI was reduced by decreasing water availability. However, MSI under I_1 and I_2 was statistically similar. Hydro-priming had no significant effects on membrane stability (Table 2). Reduction in MSI under water stress related to membrane lipids peroxidation, membrane damage and ion leakage (Chadordooz-Jeddi *et al.*, 2015). Thus, under water deficit, cell membrane subjects to changes such as penetrability and decrease in sustainability (Blokhina *et al.*, 2003).

With increasing water stress CCI of safflower was decreased. The lowest CCI was recorded for I_4. Hydro-priming increased the CCI, however, there was no significant difference between P_1 and P_2 (Table 2). Chlorophyll is the main pigment of photosynthesis in plants. To some extent, the chlorophyll content can reflect the photosynthesis rate of plant and it is strongly influenced by environmental factors (Qiu *et al.*, 2007). The reduction of CCI was probably related with the enhanced activity of the chlorophyllase (Reddy and Vora, 1986) and inducing the destruction of chloroplast structure and the instability of pigment protein complex (Singhand Dubey, 1995). Water stress also decreased CCI in *Avena* species (Pandey and Baig, 2015). Beneficial effect of hydro-priming on CCI of plants from P_1 and P_2 seed lots could be attributed to early improvements in rate and uniformity of seed germination and seedling emergence (Ghassemi Golezani *et al.*, 2010).

Rapid emergence of seedlings could lead to the production of vigorous plants with high CCI (Ghassemi Golezani *et al.*, 2008).

Maximum quantum yield of photo system II (Fv/Fm) decreased as a result of water stress. Difference between I_1 and I_2 and also I_2 and I_3 treatments was not significant for this trait. Fv/Fm was not affected by hydro-priming durations (Table 2). Drought stress affects photosystem efficiency and decreases quantum yield of photosystem II (Ahmed *et al.*, 2002; Pandey and Baig, 2015). This result may have been sustained by a decline in initial fluorescence values (F_o) and increase in maximum fluorescence values (Fm) under water stress. Changes in F_o value under water stress affect the probability of energy transfer from light harvesting complex to the PSII reaction center (Lotfi *et al.*, 2015). Reduction in Fv/Fm under water stress indicates that occurrence of chronic photo-inhibition due to photo-inactivation of PSII probably associated with the degradation of D_1 protein (Giardi *et al.*, 1996).

Grain yield, oil percentage and oil yield of safflower were significantly affected by irrigation and hydro-priming, but the interaction of irrigation × hydro-priming was not significant for these traits (Table 3).

Table 3. Analysis of variance of grain and oil yield of safflower under irrigation and hydro-priming durations.

Source of Variation	Df	Mean Square		
		Grain yield	Oil percentage	Oil yield
Replication	2	588.3	1.687	123.1
Irrigation (I)	3	39235.7**	168.290**	9632.2**
Error	6	1193.0	1.170	169.8
Hydro-priming (P)	2	49345.7**	83.928**	9468.3**
I × P	6	337.8ns	4.262ns	43.8ns
Error	16	800.8	5.111	171.3
CV (%)	-	9.1	6.9	12.5

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

The highest and the lowest grain yields per unit area were obtained from irrigations after 60 and 150 mm evaporation, respectively. However, there was no significant difference between I_1 and I_2 treatments (Fig. 1a).

Drought mainly influences yield by limiting grain number by either influencing the amount of dry matter produced by the time of flowering (particularly in determinate plants) or by directly influencing pollen or ovule function, which leads to decreased grain-set (Prasad *et al.*, 2008).

Hydro-priming improved grain yield per unit area. The highest grain yield was recorded for P_2 (Fig. 1b). The superiority of plants from hydro-primed grains in grain yield per unit area resulted from higher LWC and CCI and lower LT, compared with those from un-primed grains (Table 2).

Beneficial effects of hydro-priming on grain yield were also reported in rice (Farooq *et al.*, 2006) and pinto bean (Ghassemi Golezani *et al.*, 2010).

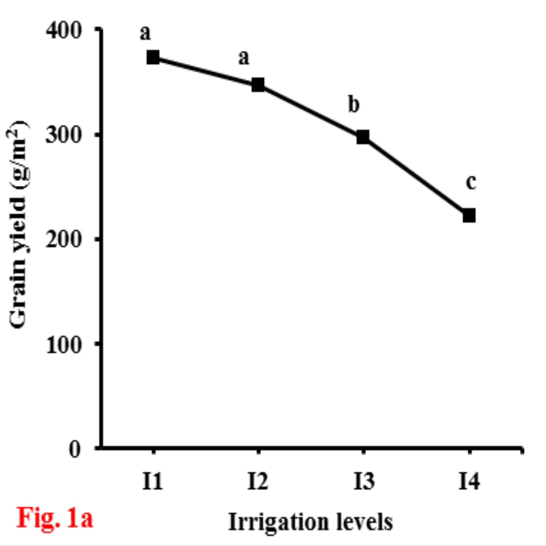


Fig. 1a

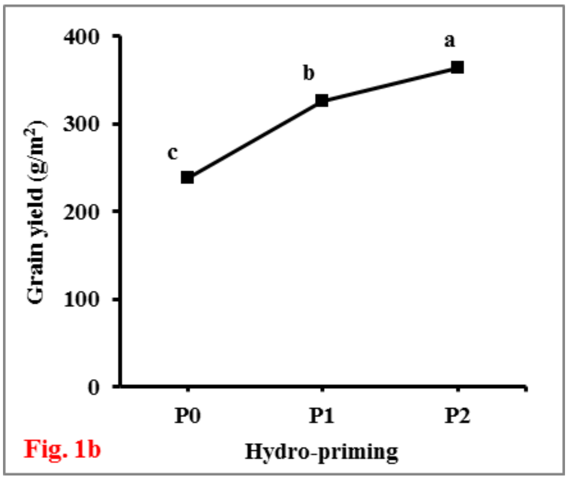


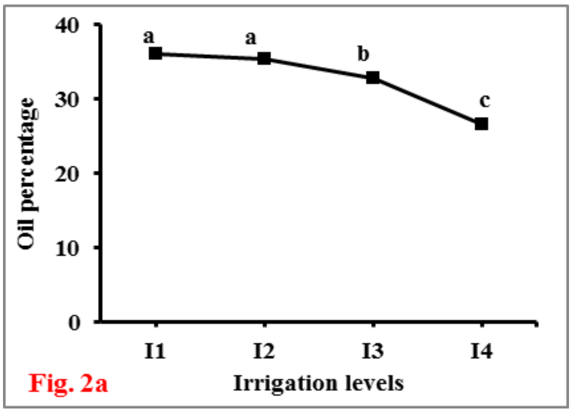
Fig. 1. Changes in grain yield of safflower in response to water stress (a) and hydro-priming (b) I_1, I_2, I_3, I_4: irrigation after 60, 90, 120 and 150 mm evaporation from class A pan, respectively P_0, P_1 and P_2: nonprimed and hydro-primed seeds for 8 and 16 h, respectively.

Oil percentage (Fig. 2a) and oil yield (Fig. 3a) of safflower was similarly decreased as a result of water stress. However, differences in these traits between I_1 and I_2 treatments were not significant.

The low oil percentage due to water deficit (Fig. 2a) may be resulted from the short grain filling duration (Ghassemi-Golezani and Lotfi, 2013). Adequate irrigation during the vegetative stage, leaf development and grain filling stage can increase grain weight and oil storage.

Also, because of decreasing grain yield under water stress (Fig. 1a) oil yield per unit area considerably decreased (Fig. 3a). Hydro-priming significantly increased oil percentage (Fig. 2b) and oil yield (Fig. 3b) of safflower. Hydro-priming results in better growth a plant system protection against tension and increase in oil amount.

Ashrafi and Razmju (2009) in a study on safflower claimed that 6 hours of hydro-priming could improve the hydro-primed seeds physiologic and biochemical characteristics and this leads to increase in oil content of grains.



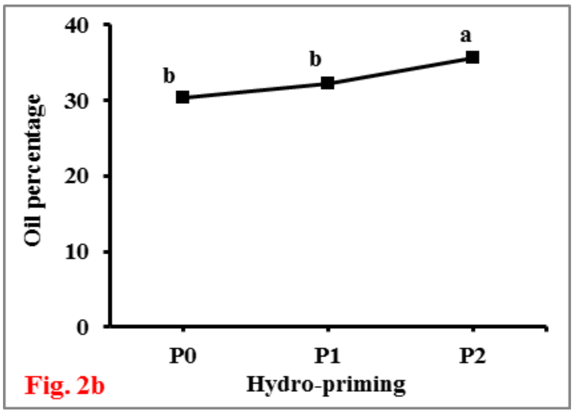
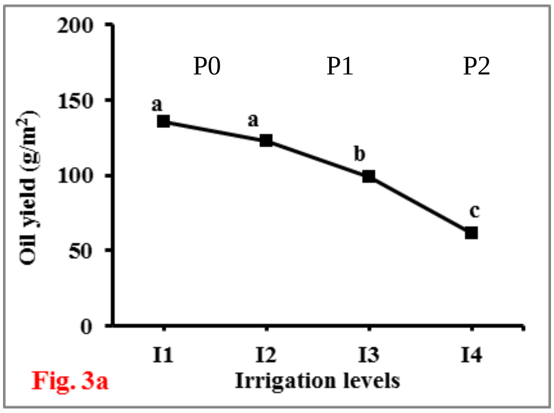


Fig. 2. Changes in oil percentage of safflower in response to water stress (a) and hydro-priming (b) I_1, I_2, I_3, I_4: irrigation after 60, 90, 120 and 150 mm evaporation from class A pan, respectively P_0, P_1 and P_2: non-primed and hydro-primed seeds for 8 and 16 h, respectively.



In general, our results clearly showed that hydro-priming, particularly hydro-priming for 16 hours, have positive and beneficial effect on physiological characters, grain yield and oil production of safflower. Therefore, hydro-priming as a simple and environmentally friendly method can be applied to improve field performance and oil yield of this plant.

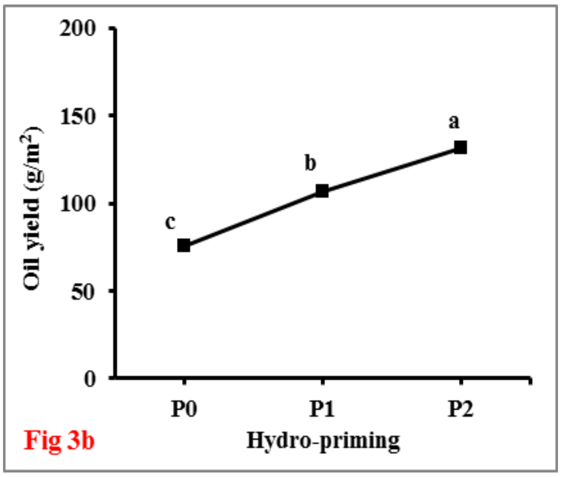


Fig. 3. Changes in oil yield of safflower in response to water stress (a) and hydro-priming (b) I_1, I_2, I_3, I_4: irrigation after 60, 90, 120 and 150 mm evaporation from class A pan, respectively P_0, P_1 and P_2: non-primed and hydro-primed seeds for 8 and 16 h, respectively.

Conclusion

In conclusion, the results of the present study indicate that hydro-priming for 16 hours was the best seed pre-treatment to improve physiological traits and field performance of safflower.

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