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Growth and yield parameters of safflower (*Carthamus tinctorius*) as influenced by foliar methanol application under well-watered and water deficit conditions

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Abstract

Safflower (*Carthamus tinctorius* L.) response to foliar spraying of methanol in well-watered and water deficit stress conditions was studied in a split plot experiment with randomized complete block design. Two treatments of full irrigation (control) and interrupted irrigation at flowering stage were compared in main plots and four levels of methanol [0, 10, 20 and 30% (v/v)] in subplots. Results indicated that interrupted irrigation at flowering stage significantly decreased plant height, leaf chlorophyll, seeds number and weight as compared with the control. Interaction effect of irrigation and methanol showed that the highest rate of seed yield under well-watered conditions was obtained at 10% methanol; higher doses of methanol decreased seed yield. Under water stress conditions at flowering stage, seed yield was significantly increased with increasing the methanol dose to 30%, in comparison with 0% methanol or control treatment. This experiment showed that foliar application of methanol in higher doses under drought stress conditions at flowering stage may increase growth and yield of safflower, whereas under fully watered conditions the application of lower doses of methanol may be more effective in yield improvement than use of higher doses.

Key words: drought stress, flowering stage, foliar application, methanol, safflower.

Introduction

Water deficit is the most critical limiting factor for growth and yield of crop plants in arid and semiarid regions around the world. Iran is a country situated in the zone of arid and semiarid regions of the Earth (Modarres and Silva 2007) with low annual precipitation of 250 mm, which is less than one third of the global mean precipitation (Gheiby, Noorafshan 2013). The country has been constantly subjected to drought. Therefore the most convenient strategy under such conditions is a proper water management for increasing crop productivity and achieving stability in crop production system. During drought stress conditions stomatal closure takes place leading to reduction of intercellular CO_2 concentration in leaves and subsequent decrease in photosynthesis rate (Chaves et al. 2002).

Methanol spray is a method that increases CO_2 fixation in plants and methanol may act as a carbon source for plants (Nonomura, Benson 1992). Methanol is the simplest alcohol and can be produced through anaerobic metabolism by some bacteria. Moreover, methanol is emitted from leaves of C_3 plants (Fall, Benson 1996). There are many reports about ability of methanol foliar application to improve growth and productivity of many plant species. Nonomura and Benson (1992) reported that foliar spraying of methanol increased growth and yield of various C_3 plants. Rowe et al. (1994) found that foliar application of methanol and ethanol on tomato plants improved plant growth traits, while root application of these alcohols led to severe plant damage. Hernandez et al. (2000) observed significant improvement in vegetative growth traits and floral development in methanol treated sunflower plants grown under greenhouse conditions, whereas methanol treated plants grown in the field did not show remarkable improvement. Zbiec et al. (2003) reported considerable increase in productivity of different crops including tomato, bean, sugar beet, oil seed rape due to methanol treatment. In a study of winter wheat, photosynthesis rate, stomatal conductance and intercellular CO_2 concentration were increased by methanol application (Zheng et al. 2008). Plant biomass and SPAD chlorophyll content were increased by foliar spraying of soybean plants with 15% (v/v) methanol (Saadpanah et al. 2013). Also, stomatal conductance, photosynthesis rate and growth of broad bean were significantly improved by foliar application of methanol (Zhao et al. 2014).

The decline in intercellular CO_2 is a key limiting factor in photosynthesis under drought stress conditions. Plant productivity may be promoted by increasing the availability of CO_2 in leaves through applying a carbon source. Methanol is considered as a CO_2 source because it is quickly oxidized successively to formaldehyde, formic acid and finally to CO_2 in leaf tissues (Fall, Benson 1996). Therefore, foliar methanol application on drought-stressed plants may be useful in improvement of plant growth and productivity. This study was aimed to examine the effects of methanol spraying on drought-stressed and well-watered safflower plants under field conditions.

Materials and methods

Field trials of this experiment were conducted during spring and summer 2013 at the experimental research farm of Islamic Azad University, Sanandaj Branch (35°10' N; 46°59' E; altitude 1393 m) west of Iran. The long-term annual rainfall and mean temperature of the region are 471 mm and 13.35 °C respectively. The main soil physicochemical properties were: sand 41.5%, silt 24%, clay 34.5%, electrical conductivity 0.79 dS m^{-1}, pH 7.6, organic carbon 0.91%, total N 0.08% and available P and K 7.67 and 310 mg kg^{-1} respectively. Fertilizers at rates of 80 kg N ha^{-1} in the form of urea and 25 kg P ha^{-1} in the form of superphosphate were incorporated into the soil immediately before sowing operations. The trial was laid out in a split-plot arrangement based on randomized complete block design in three replications. Two treatments of full irrigation (control) and interrupted irrigation at flowering stage were compared in main plots. Four levels of methanol concentration, including 0 (distilled water as control), 10, 20 and 30 % (v/v), were set up in sub-plots.

Seeds of safflower cv. 'Goldasht' (obtained from agricultural research station of Sararood, Kermanshah, Iran) were sown on 7 May 2013. Each sub-plot consisted of four rows, 4 m long with 25 cm row spacing and 5 cm between plants. The well-watered plots (full irrigation) were regularly irrigated to field capacity. Interruption of irrigation was started at the stage of flowering initiation and continued for 16 days; re-watering was again applied as in fully watered plots to allow recovery of stressed plants.

Foliar spraying of safflower plants by methanol and distilled water treatments was done twice during the vegetative development of the plant (first spraying at 2 to 4-leaf stage and the second spraying 14 days later). The amount of sprayed methanol solution and distilled water was about 1 L per plot (250 mL m^{-2}) at each stage of spraying. For each spraying treatment (distilled water and methanol) 2 g L^{-1} glycine was added to the solution to avoid foliar injuries from methanol application (Nonomura and Benson 1992). The plants were harvested on 18 August (103 days after sowing).

Plant height, canopy temperature, leaf chlorophyll content, head number per plant, seed number per head, 1000-seed weight and seed yield were recorded. Mean plant height in each plot was determined from five randomly selected plants per plot. Canopy temperature was measured on the 15^{th} day after termination of irrigation (one day before re-watering) with a non-contact infra-red thermometer (DT-8810, CEM Inc., China) held at an angle of 20 to 30° to the horizontal plane and 50 cm above the plant according to the method described by Amani et al. (1996) and Ayeneh et al. (2002). Mean temperature was determined from three measurements per plot. Mean leaf chlorophyll content was determined as SPAD index from seven SPAD readings on the mid blade of randomly selected leaves in each plot using a portable SPAD chlorophyll meter (Minolta SPAD-502 meter, Tokyo, Japan). Number of fertile heads per plant and seed number per head were determined based on five randomly selected plants from the central two rows of each plot at harvest stage. To estimate 1000-seed weight trait, the mean weight of four randomly selected 100-seed sub-samples from harvested plants of each plot was calculated and multiplied by 10. Seed yield per unit area was determined by weighing the total number of harvested seeds from two central rows of each plot.

The data were subjected to analysis of variance (ANOVA) operations using PROC GLM of the SAS software, version 9.1 (SAS Institute, Cary, NC) and means comparison was done by the least significant difference (LSD) test at probability level of 0.05.

Results

Plant height, canopy temperature and SPAD index

Comparison of plant height means showed that the interruption of irrigation during flowering led to reduction of plant height. Under normal irrigation the mean plant height was about 57 cm; under drought stress height was 41 cm (29% lower). Methanol application had no significant effect on plant height (Table 1).

When irrigation was interrupted at the flowering phase of safflower, mean temperature of canopy in stressed plants was significantly higher (by 17%) than for well-watered plants. Foliar application of methanol in conditions of normal irrigation did not affect canopy temperature, but methanol under water deficit slightly decreased canopy temperature (Table 1).

SPAD index of leaf chlorophyll content at the end of the interrupted irrigation period was 19% lower in stressed plants than in normal irrigated plants. Methanol application in normal irrigation conditions had no significant effect on leaf chlorophyll content, while under water deficit stress, foliar spraying with 30% methanol led to a significantly higher SPAD index (51.8) compared to the control (42.6; Table 1).

Yield and yield components

Water deficit at flowering stage resulted in 26% reduction in mean number of fertile heads per plant compared that in irrigation (Table 1). Methanol foliar application under both normal and interrupted irrigation increased number of fertile heads per plant. The highest mean number of 13

Table 1. Interaction effects of irrigation and methanol foliar application on different traits of safflower. Values in each column with the same letters are not significantly different at $P \leq 0.05$ according to LSD test

Irrigation level	Methanol concentration (%)	Plant height (cm)	Canopy temperature (°C)	SPAD index	Number of fertile heads per plant	Number of seeds per head	Weight of 1000 seeds (g)
Normal	0	56.2 a	27.2 c	59.6 ab	8.0 bc	13.6 bc	29.7 abc
	10	58.4 a	25.9 c	58.6 ab	8.7 bc	14.4 b	30.4 abc
	20	58.1 a	26.6 c	55.8 abc	9.7 b	20.9 a	30.8 ab
	30	55.3 a	27.0 c	63.3 a	13.0 a	21.6 a	31.5 a
	Mean	57.0	26.7	59.3	9.9	17.6	30.6
Interrupted	0	40.1 b	32.8 a	42.6 d	6.3 c	8.4 bcd	25.0 c
	10	42.3 b	31.9 ab	48.7 cd	6.7 c	9.5 bcd	25.3 bc
	20	43.7 b	30.4 ab	49.2 cd	7.3 bc	7.2 d	28.7 abc
	30	36.5 b	30.0 b	51.8 bc	9.0 bc	7.9 cd	26.4 abc
	Mean	40.7	31.3	48.1	7.3	8.3	26.3

heads per plant was obtained after treatment with 30% methanol in normal irrigation and the lowest (6.3 heads per plant) after treatment with water in stress conditions (Table 1).

Stress caused by interrupted irrigation reduced the mean number of seeds per head by 53% compared to normal irrigation condition. Moreover, in well-watered plants, with increasing the concentration of methanol, the number of seeds increased from 13.6 seeds (control treatment) to 21.6 seeds per head (30% methanol treatment). Under stress condition, number of seeds was higher by 13% in 10% methanol application than with water (Table 1).

The mean 1000-seed weight was 26.3 g under water stress compared to 30.6 g in normal irrigation condition. Methanol application had no significant effect on 1000-seed weight under normal or water deficit conditions (Table 1).

Seed yield was significantly influenced by the interaction of irrigation and methanol. The highest seed yield under fully watered condition was obtained with application of 10% methanol; and was significantly higher than in the control treatment (distilled water) and even lower with higher methanol doses (Fig. 1). Under water deficit stress conditions, with increasing methanol dose to 30%, an increasing trend in seed yield was observed; and was significantly higher after 30% methanol treatment (Fig. 1).

Discussion

Reduced plant height as the result of water deficit, as observed in this study, is a general response of plants to reduced water availability. Water deficit and drought stress may delay development of plants, leading to plant height reduction (Lu et al. 2012). Plant growth occurs by cell elongation and cell division, which are very sensitive to

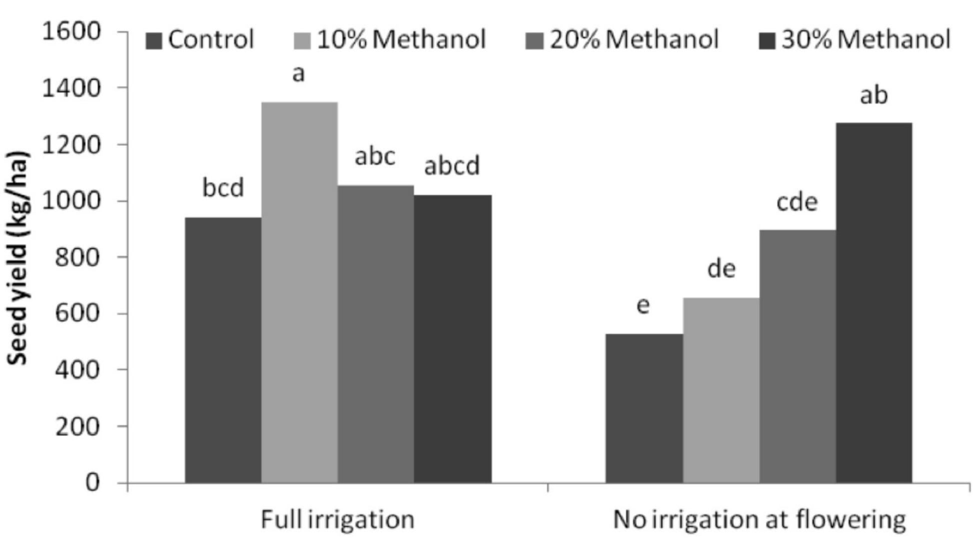

Fig. 1. Interaction effect of irrigation and methanol on seed yield of safflower. Mean values with the same letters are not significantly different at $P \leq 0.05$ according to LSD test.

drought stress. Cell elongation is inhibited by a reduction in turgor pressure resulting from water deficiency. Water deficit also impairs mitosis and cell division. Thereby, disruption of cell elongation and division can explain the observed reduction in plant height and growth (Farooq et al. 2009).

Increasing canopy temperature during interrupted irrigation can be due to reduced transpiration from leaves. In well-watered plants, the transpiration rate is higher and the transpired water cools the surface of leaves (Keener, Kircher 1983; Gonzalez-Dugo et al. 2005). Reduction of canopy temperature by methanol application in stress conditions may be due to increased stomatal conductance, which consequently results in an increase of transpiration rate and decrease in leaf temperature. Zheng et al. (2008) also observed that leaf temperatures were lower in methanol treated wheat plants than in control plants.

The SPAD chlorophyll index in well-watered plots was about 23% higher than in drought-stressed plots (Table 1). Similarly, Zhang et al. (2007) and Ahmed (2011) observed that the SPAD chlorophyll index was lower in soybean plants under water deficit stress. Leaf chlorophyll content is an important indicator of physiological status in plants (Ling et al. 2011) and the variation in leaf chlorophyll content is considered to be a plant response to environmental stress (Percival et al. 2008).

Reduced number of fertile heads per plant under interrupted irrigation may be due to reduction of flowers fertility rate caused by drought stress. Decreased number of safflower heads per plant arising from water deficiency stress has been also reported by Lovelli et al. (2007). The positive effect of methanol application on number of heads per plant reported here is consistent with the findings of Li et al. (1995), who showed that application of 25 and 50% methanol on soybean plants significantly increased pod number per plant, compared with untreated control plants.

Significant decrease in seed number per head due to interrupted irrigation during the flowering period has also been reported by Mirshekari et al. (2012), Pasban-Eslam (2011) and Kokubun (2011). Water deficit stress during reproductive development of plants can lead to a decrease of photosynthesis rate and consequently reduction of flower fertility, leading to a lower number of seeds per head. The observed increased number of seeds per head after methanol application indicates its promoting effects on head fertility.

Water deficit stress during flowering led to a notable decrease in 1000-seed weight (Table 1). The reduction of photosynthesis and production of assimilates arise due to reduced water availability indicated a decline in allocation of assimilates to seeds and subsequent loss of seed weight. Similarly, Mirshekari et al. (2012) observed that the lowest rates of 1000-seed weight occurred after water stress treatments due to interrupted irrigation at head-forming and flowering stages of safflower.

In this experiment safflower positively responded to higher concentrations of methanol in water deficit conditions. Under drought stress conditions, intercellular CO_2 concentration in the leaf is decreased as a result of limited stomatal conductance. With reducing CO_2 concentration, the photorespiration rate is elevated. In this situation methanol can act as a carbon source for plant photosynthesis. Photorespiration rate in C_3 plants is reduced with spraying of methanol due to promotion of CO_2 assimilation (Fall, Benson 1996). As a consequence, treating plants with methanol can promote net photosynthesis leading to improved yield (Nonomura, Benson 1992). Makhdum et al. (2002) demonstrated that stomatal conductance, net photosynthesis and water use efficiency of cotton were increased by foliar application of methanol. Positive response of safflower to the highest concentration of methanol under water stress conditions in the present study indicated that the deleterious effects of drought stress can be alleviated by methanol application, to increase CO_2 concentration.

In conclusion, growth and yield traits of safflower significantly decreased due to interrupted irrigation at flowering stage in comparison with fully watered status. However, an ameliorative effect was observed after spraying methanol on water-stressed plants. With increasing the concentration of methanol to 30% an ascending tendency in seed yield was observed. Under well-watered conditions the highest seed yield occurred at the lowest concentration (10% methanol). The results suggest that methanol can aid in alleviating the effects of drought stress on safflower plants in field conditions. Recommending an optimal methanol concentration greatly depends on environmental factors such as stress or non-stress conditions, as responses of safflower differ between these conditions.

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