INNSPUB

Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 12, No. 3, p. 392-399, 2018
http://www.innspub.net

RESEARCH PAPER

OPEN ACCESS

Changes in some morpho-physiological traits of safflower in response to water deficit and nano-fertilizers

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Article published on March 30, 2018

Key words: Capitol, Chlorophyll, Green cover, Leaf temperature, Safflower, Water deficit

Abstract

A split plot experiment based on randomized complete block design with three replications was arranged in 2017, to assess the effects of foliar application of nano-fertilizers (SiO_2 and Mn_2O_3) on some morpho-physiological traits of safflower (*Carthamus tinctorius* L.) under different irrigation intervals (I1, I2, I3 and I4: Irrigation after 70, 100, 130 and 160 mm evaporation from class A pan, respectively). Leaf temperature was increased, but other morpho-physiological traits of safflower were decreased by decreasing water availability, particularly by moderate and severe stresses. Foliar sprays of nano-Mn_2O_3 and especially nano-SiO_2 reduced leaf temperature, while enhanced leaf water content, chlorophyll content index and ground green cover, leading to an increase in plant height, branches per plant and capitols per plant under normal and limited irrigation conditions. These results indicated that foliar sprays of the nano-fertilizers could be a useful method for improving safflower performance under adverse environmental conditions.

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Introduction

Safflower (*Carthamus tinctorius* L.) is an important oilseed crop that can somewhat tolerate environmental stresses such as drought (Dwiedi *et al.*, 2005). Safflower is a day-neutral, long day plant (Johnston *et al.*, 2002) and is grown in different seasons (spring/summer/fall) and warmer temperatures (Johnston *et al.*, 2002). Although safflower is a drought tolerant plant, severe water deficit can potentially decrease physiological performance and yield of this crop (Mohammadi *et al.*, 2016).

Drought is considered as one of the main environmental factors that cause osmotic stress and inversely influence plant performance as well as global crop production (Saruhan Gu̇ler *et al.*, 2012). Water deficit occurs when water potential in the rhizosphere is sufficiently negative to reduce water availability to sub-optimal levels for plant growth and development (Boyer, 1982).

Numerous physiological and metabolic responses occurring during drought help plants to overcome injuries caused by osmotic stress (Bray *et al.*, 2000). Water content and water potential of plant tissues are considered as the physiologically appropriate integrators of drought effects.

Thus, relative water content (RWC) has been recommended as a suitable screening tool for drought-tolerant crops (Terzi *et al.*, 2013, Mohammadi *et al.*, 2016). Leaf temperature is another easily measured physiological parameter, which allows an indirect way to estimate plant transpiration and it is well correlated with water availability (Blum 1989).

All green plants can contribute to primary production by photosynthetic activity in leaves that play a major role in this process. Therefore, adequate leaf area should be critical to plant regeneration for a constant primary production. Ideally, leaf area index varies among crops, depending on the morphological and anatomical structure of the plants (Nelson and Sommers, 1995). Reduced canopy photosynthetic rates under water stress is attributed to decreased leaf expansion, leaf area, leaf age of the canopy and increased senescence rates (Marani *et al.*, 1971).

Foliar nutrition is an option when nutrient deficiencies cannot be corrected by applications of nutrients to the soil (Sarkar *et al.*, 2007; Cakmak, 2008). In open-field conditions, where the factors that influence the uptake of the nutrients are variable, foliar fertilization is a privilege. Among the micronutrients, Mn^{2+} nutrition can improve drought tolerance of plants (Khan *et al.*, 2003). Exogenous application of Mn^{2+} increases photosynthesis, net assimilation, and relative growth and yield (Lidon and Teixerira, 2000; Sultana *et al.*, 2001).

Agricultural applications of beneficial nanoparticles (NPs) are currently interesting fields of research (Karunakaran *et al.*, 2013). The NPs interact with plants causing many morphological and physiological changes, depending on the properties of particles. Among the NPs, nano-silicon has gained greater consideration during recent years. Silicon is plentiful in soils and the second most common element on earth after oxygen and has been recognized as a beneficial nutrient for plant growth and development (Wainwright, 1997; Siddiqui *et al.*, 2015).

Silicon (Si) in plants, especially in grasses, is equivalent to those of macronutrients such as calcium, magnesium, and phosphorus (Epstein 1999). Si has been shown to be able to promote the growth and development of plants under abiotic and biotic stresses, including water deficit (Hattori and others 2005). Therefore, application of Si may be a way to improve the growth and development, and thus the yield of safflower, especially in arid region.

The most widely reported mechanism is that Si might decrease the oxidative damage in plants subjected to environmental stresses (Saqib and others 2008). Silicon has a positive effect on plants under drought stress. Biel *et al.* (2008) suggest that the protective role of silicon in plants may be connected with accumulation of polysilicic acids inside cells.

Exogenous Si application on maize increased water use efficiency by reducing leaf transpiration and water flow rate in the xylem vessel (Gao and others, 2006). The effect of foliar sprays of nano-silicon and nano-manganese on performance of safflower semiarid regions is poorly documented.

Thus, this research was initiated to generate new information on the efficacy of nanoscale silicon dioxide and manganese dioxide on the growth and development of safflower (*Carthamus tinctorius* L.) under different levels of water supply.

Materials and methods

A field experiment was conducted at the Research Farm of the University of Tabriz, Iran, located at 38.05°N, 46.17° E with an altitude of 1360 m above sea level, mean annual rainfall of 285 mm, mean annual temperature of 10°C, mean annual maximum temperature of 16.6°C and mean annual minimum temperature of 4.2°C.

The experiment was laid out as split plot based on randomized complete block design in three replicates, with irrigation intervals (I_1, I_2, I_3, I_4 for irrigation after 70, 100, 130, and 160 mm evaporation as normal irrigation and mild, moderate and severe deficits, respectively) in main plots, and foliar sprays of nanoparticles (water, SiO_2, Mn_2O_3) in sub-plots. Each plot consisted of 6 rows with 4 m length, spaced 25 cm apart. Seeds were hand sown in about 3-4 cm depth with a density of 80 seeds m^{-2}. Seeds of a spring safflower cultivar (Soffeh) was provided by the Seed and Plant Breeding Research Institute, Karaj, Iran. Nanoparticles of silica (SiO_2) were purchased from the Nanosany, Iran. According to the manufacturer, the particle sizes of nano-SiO_2 ranged from 20 to 30 nm. Specific surface area of nano-sized silica was 180-600 $m^2\,g^{-1}$ and purity was +99%.

The particle sizes of nano-Mn_2O_3 was 30 nm. Foliar sprays of safflower plants with nano-SiO_2 (g/l) and nano-Mn_2O_3 at vegetative (six leaves unfolded) and capitol formation stages.

Physiological traits

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. Leaf water content (LWC) was determined as:

LWC (%) = [(FW - DW) / FW] × 100. Where FW is fresh weight and DW is dry weight. Leaf temperature (°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.

Leaf chlorophyll content index (CCI) was measured by a portable chlorophyll meter (CCM-200, Opti-Sciences, USA). Three plants were marked in each plot and the CCI of the upper, middle and lower leaves of each plant was measured at the flowering stage. Subsequently, the mean CCI for each treatment and replicate was calculated. Ground green cover was measured at flowering stage 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.

Morphological traits

At maturity, 10 plants from each plot were harvested and stem height, branches per plant and capitol number were determined. The mean values of these traits were separately calculated for each plot.

Statistical analysis

Analysis of variance of the data was performed with SAS 9.1 software. Means were compared by Duncan multiple range test at $p \leq 0.05$. Excel software was used to draw Fig.s.

Results and discussion

Analysis of the data (Table 1) showed that water stress and nano-fertilizers had significant effects on leaf temperature, leaf moisture content, green cover percentage, chlorophyll content index and morphological traits such as plant height, branches per plant and capitols per plant. Capitols per plant was also significantly affected by nano-fertilizers. The interaction of Irrigation × nano-fertilizer was only significant for plant height.

Table 1. Analysis of variance of the effects of foliar sprays of nano-SiO_2 and nano-MN_2O_3 on some morpho-physiological characters of safflower at different irrigation intervals.

Source	DF	Leaf water content	Leaf temperature	Chlorophyll content index	Ground green cover	Plant height	Branches Per plant	Capitol per plant
block	2	40.79*	0.05	23.28	2.58	9.18	0.09	0.11
Irrigation (I)	3	330.25**	79.13**	1677.65**	289.93**	230.46**	1.49**	0.62
Ea	6	9.30	1.02	10.85	8.62	1.09	0.14	0.43
Nano-fertilzer (NF)	2	39.59*	11.61**	756.90**	60.58**	92.85**	1.67**	0.94*
I × NF	6	9.38	0.90	55.62	6.73	20.42*	0.1	0.12
Eb	16	6.71	1.63	16.60	7.49	4.89	0.18	0.23
CV	-	3.94	5.28	4.60	5.90	3.27	8.59	8.89

*, **: Significant at $p \leq 0.05$ and $p \leq 0.01$, respectively.

The Leaf water content was decreased by increasing irrigation intervals, with no significant difference between I_2 and I_3 and also between I_3 and I_4 (Table 2). The decline in moisture content could be related with an imbalance between water loss from the leaves due to evapotranspiration and water availability (Jones, 2006). Decreasing leaf water content due to water deficit is an indication of decline in turgor pressure in plant cells and causes growth retardation (Kumar and Sharma, 2010). Foliar sprays of nano-fertilizers similarly enhanced leaf moisture content (Table 2), probably as a result of improving root growth and water uptake. Water stress increased leaf temperature, but no significant difference was observed between I_2 and I_3 (Table 2).

Nielsen and Anderson (1989) found that leaf temperature increases at low water potential due to stomata closure under water stress. This may inhibit photosynthesis by limiting the availability of CO_2 within the leaf (Boyer, 1976). Leaf temperature significantly decreased by foliar application of nano-fertilizers, with no significant difference between two nano-fertilizers.

This could be associated with higher water content of nano-fertilizer treated plants (Table 2). Lower leaf temperatures under drought-stress could mitigate the heat stress, reducing respiration as well as the loss of water across the cuticle, thereby improving water use efficiency (Tambussi *et al.*, 2007).

Table 2. Means of some physiological and morphological traits of safflower for different irrigation intervals and nano-fertilizers.

Treatment	Leaf water content	Leaf tempreture	Chlorophyll content index	Ground Green cover	Plant height	Branches Per plant	Capitols Per plant
Irrigation							
I_1	74.34a	21.53 c	100.25a	52.44a	73.75a	5.24a	5.42a
I_2	65.30b	23.34 b	96.48a	48.22b	69.37b	5.24a	5.58a
I_3	63.09bc	23.35 b	88.68b	45.77b	65.13c	4.46b	4.96a
I_4	60.36c	28.42 a	69.59c	38.88c	62.15d	4.63b	5.37a
Foliar spray							
Water	63.70b	25.26a	80.38c	43.75b	64.46b	4.48b	5.05b
Nano-SiO2	66.53a	23.37b	96.19a	47.41a	69.75a	5.18a	5.61a
Nano-Mn2O3	67.08a	23.85b	89.68b	47.83a	68.59a	5.02a	5.33ab

Different letters in each column indicate significant difference at $P \leq 0.05$.

I1, I2, I3, I4: Irrigation after 70, 100, 130 and 160 mm evaporation from class A pan, respectively.

Chlorophyll content index (CCI) was significantly decreased under moderate and severe water deficit (Table 2). Water limitation also reduced CCI in wheat cultivars (Paknejad *et al.*, 2007). Chlorophyll loss also considered as an adaptive feature in plants grown under water deficit (Munne-Bosch *et al.*, 1999).

The reduction in CCI was probably related with the enhanced activity of the chlorophyllase, which induces the destruction of chloroplast structure and the instability of pigment protein complex (singh *et al.*, 1995).

The highest CCI was recorded for plants treated with nano-SiO2, followed by nano-Mn2O3, most likely due to higher leaf water content of nano-fertilizer treated plants (Table 2).

The Green cover percentage of safflower decreased with increasing irrigation intervals, but it was statistically similar under I2 and I3 (Table 2). Reduction in percentage ground green cover due to water stress can be attributed to competition of plants for water and nutrients (Ghassemi-Golezani *et al.*, 2010). Percentage and duration of ground cover in soybean (Ghassemi-Golezani *et al.*, 2012) and chickpea (Ghassemi-Golezani *et al.*, 2013) were also sharply decreased as a result of water deficit at later stages of plant development. Drought decreases the leaf area, owing to a loss of turgor and reduces leaf numbers. A reduction in leaf turgor and photosynthesis under water stress suppresses cell expansion and growth, leading to the diminution of leaf area (Anjum *et al.*, 2011) and green cover.

The reduction in leaf area is a mechanism adapted to avoid a higher rate of transpiration and to reduce surfaces for radiation due to water deficit (Hayatu *et al.*, 2014). Increasing green cover by foliar spray of nano-fertilizers (Table 2) can improve light interception, photosynthesis and subsequently crop yield.

Plant height was generally decreased with increasing irrigation intervals (Fig. 1). Reduction of plant height under drought stress was associated with loss of chlorophyll content and green cover due to water limitation (Table 2), leading to less photosynthesis and growth (Cakir, 2004). Kazemeini *et al.* (2009) suggested that this reduction could be due to competition of sinks such as shoot and root for assimilates. The maximum plant height under all irrigation intervals was recorded for nano-SiO2 treated plants, followed by nano-Mn2O3 treated plants. However, this superiority was decreased by increasing water deficit (Fig. 1). Increasing plant height by application of nano-SiO2 was also reported by Janmohammadi *et al.* (2016). In contrast, Le *et al.* (2014) found that nano-SiO2 reduced plant height of cotton.

These results suggest that plant species may respond differentially to nano-fertilizers.

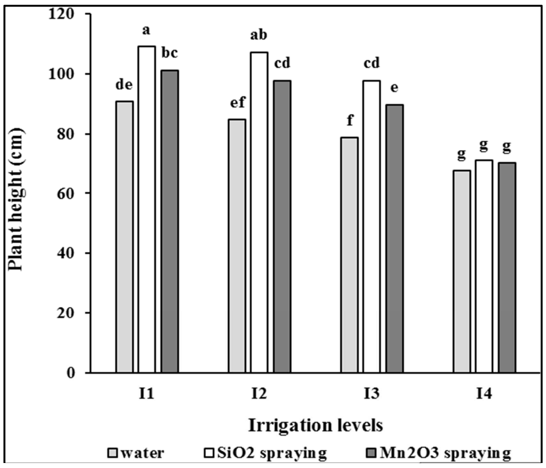

Fig. 1. Means of safflower plant height for interaction of irrigation intervals × nano-fertilizers

I_1, I_2, I_3, I_4: irrigation after 70, 100, 130 and 160 mm evaporation, respectivel Different letters indicate a significant difference at $p \leq 0.05$

The branches per plants were significantly declined under moderate (I_3) and severe (I_4) water deficits, but reduction in capitols per plant due to water stress was not significant (Table 2). Reduction in branches per plant as a consequence of water limitation was also reported for chickpea (Hosseinzadeh-Mahootchi *et al.,* 2013). foliar application of nano-fertilizers significantly increased branches and capitols per plant, which can most likely improve grain yield. Enhancing branches and capitols per plant by foliar sprays of biofertilizers strongly related with the augmentation of ground green cover by these treatments (Table 2).

Conclusions

Water limitation, particularly moderate and severe stresses had negative effects on morpho-physiological traits of safflower. However, foliar sprays of nano-Mn2O3 and especially nano-SiO2 considerably improved leaf water content, chlorophyll content index, ground green cover and consequently plant height, branches per plant and capitols per plant. Therefore, application of these nano-fertilizers could improve field performance of safflower under different levels of water availability.

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