



Growth and Photosynthesis Responses of Safflower Cultivars to Water Stress at two Developmental Stages

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ABSTRACT: In a 2-year field study, effect of water deficit at different growth stages was examined on growth, oil, yield, and photosynthesis traits of two safflower cultivars at Shiraz University. Two cultivars were Sina and Isfahan as main-plot; water deficit was imposed at four levels (100%, 80%, 60% and 40% FC) as sub-plot and at two development stages (vegetative and reproductive) as sub sub-plot. The results showed that time and the level of water deficit significantly affected plant height, grain number per plant, seed oil percentage, thousand grain weight, biological yield and grain yield, as well as photosynthesis traits consisted of stomatal conductance, photosynthesis rate and substomatal CO₂ concentration. The reductive effect of water deficit was closely related to its intensity. The highest and the lowest sensitivities to water stress were observed in grain number and thousand grain weight, respectively. According to the cultivars' responses to water deficit, it seems that Sina is more tolerance cultivar. Since there was no significant reduction in grain yield from 100% to 80% field capacity, applying irrigation at 80% field capacity, especially at the vegetative stage of safflower could be recommended for the regions with limited water resources.

Key words: Drought, oil percentage, stomatal conductance.

INTRODUCTION

Among the different abiotic stresses, water deficit is the constraint that induces a highly negative impact on safflower production and growth. In response to water deficit, crops revealed wide range of manner, varying from great sensitivity to high tolerance (Eslam *et al.*, 2010). Water stress influenced growth and yield that depended to species and variety. Moreover, sensitivity to drought varies by development stage. Water deficit stress at any stage of crop growth can cause an irreversible loss in yield potential (Pirasteh-Anosheh *et al.*, 2013; Kazemeini *et al.*, 2014).

All crops response differently in different growth stages to changing water status of the soil under deficit irrigation. It means that plants are more sensitive to water deficit at one or more stages than the other stages (Istanbulluoglu *et al.*, 2009). These sensitive stages are during flowering and boll formation stages in cotton, during vegetative growth of soybean, flowering and grain filling stages of wheat, vegetative and yielding stages of sunflower and sugar beet (Kirda, 2002).

Cakir (2004) in a 3-year study showed that all vegetative and yield parameters were significantly affected by water deficit in soil profile due to omitted irrigation during the sensitive stages. He indicated that even a single irrigation omission during one of the sensitive growth stages, caused up to 40% grain yield losses during dry years. Kazemeini *et al.* (2009) showed that irrigation levels significantly affected seed yield

and oil percentage. These researchers indicated that deficit irrigation, during the critical growth period should be avoided. Water stress occurring during different growth stages may reduce grain yield to different degrees, and the extent of yield reduction depends not only on the stage of the plant development, but also on the intensity of the stress (Claasen and Shaw, 1970; Pirasteh-Anosheh *et al.*, 2012).

Recently there has been interest in optimizing irrigation application due to water scarcity in Iranian fields, such as safflower field. Safflower, a strongly tap-rooted annual plant from the family Asteraceae, is native to the Middle East. It is resistant to saline conditions and to drought stresses (Bassil and Kaffka, 2002). In contrast, some researchers believe that safflower is a sensitive crop plant to water stress. Safflower is usually grown on dry lands or under dry farming conditions with various levels of water stress (Hamzehzarghani and Kazemeini, 2011).

Although there are well studies about response of crops to water stress, however there is low information about changes in safflower growth and photosynthesis in response of water stress imposed at different growth stages, especially in Iran fields. So, the objective of this study was examination of water deficit effect at vegetative and reproductive stages and in different intensity on growth, oil, photosynthesis, yield and its components. The better understand of sensitivity of the safflower stages to water deficit can be useful to achieve the acceptable production.

MATERIALS AND METHODS

This 2 years study was conducted at Experimental Fields of College of Agriculture, Shiraz University, Shiraz, (1810 m above the sea level with longitude of

52° 35' and latitude 39° 4') during 2010-2011 and 2011-2012 growing seasons. Some physical and chemical properties of experimental site soil are shown in Table 1.

Table 1: Some soil characteristics of the experimental site.

Year	pH	EC(dS m ⁻¹)	N(%)	K(mg kg ⁻¹)	Texture	C (cm ³ m ⁻³)	PWP(cm ³ m ⁻³)
2010-11	7.56	0.68	0.054	724.38	Silt loam	0.29	0.11
2011-12	7.43	0.79	0.62	734.51	Silt loam	0.28	0.13

This study was arranged in a split split plot experiment based on randomized complete design with three replications. The treatments were consisted of safflower cultivars at two levels: Isfahan (C1) and Sina (C2) as main plot; irrigation regime at four levels: 100 (I1, as control), 80 (I2), 60 (I3) and 40% (I4) field capacity (FC) as sub plot and time of water stress at two levels: vegetative (S1) and reproductive (S2) as sub sub plot. Isfahan was a high yielding cultivar and early maturity, with red flower and without thistle; while Sina was a high yielding cultivar and late maturity, with reddish orange flower and without thistle.

The fertilizers were consisted of 115 kg N in urea (½ at planting time and ½ at stem elongation) and 50 kg P₂O₅ in triple super phosphate. Weeds were controlled handy and chemically by trifluralin (2 L ha⁻¹) as pre-plant and soil incorporated. Each plot was 12 m² (3×4 m). The soil water content was monitored in each plot by using the gravimetric method at 30 cm intervals down to 90 cm. Time-volume technique (Grimes *et al.*, 1987) is an irrigation technique in which irrigation water is applied by polyethylene pipes set in each plot and the time of each plot irrigation is calibrated by a timer and a standard container. Then, irrigation water amount of each plot (measured by gravimetric method) was converted to time (min) and applied. The amount of applied water was measured by time-volume technique. Ten leaves plants with 30cm height were considered as vegetative stage, while 50% flower emergence was considered as reproductive stage. At grain filing stage ten plants were randomly selected and, stomata conductance (gs), photosynthesis rate (A), and

substomatal CO₂ concentration (Ci) were measured using portable photosynthesis system (IGRA model LCA4-ADC, Hoddeson, UK). At physiological maturity, harvesting was done by hand using a 1 m² quadrat in the central of each plot; to determine plant height (Ht), grain number per plant (GN), thousand grain weight (TGW), grain yield (GY), biological yield (BY). For TGW, GY and BY determination harvested safflower plants were oven dried at 70°C for 48 h.

Data were subjected to combined analysis of variance (ANOVA) using software SAS v. 9.1 (SAS Institute, Cary, NC, USA). Furthermore, LSD test was applied to means comparison at 1% probability level. Since the effects of year and its interactions with residues rates and irrigation intervals were not significant, means of two years were used.

RESULT AND DISCUSSION

Water stress reduced plant height (Ht), so that the highest and the lowest height were achieved in plants grown under 100 and 40% field capacity (FC) treatments, respectively (Fig.1a). Effect of water deficit on Ht in vegetative was more compared to reproductive stage. Drought stress reduced vegetative growth duration and so decreased Ht. Reduction of plant height due to water deficit stress might be associated to impact of drought stress on photosynthesis and consequence on assimilates production (Cakir, 2004). Kazemeini *et al.* (2009) also suggested that this reduction could be due to competition of sinks such as shoot and root on source (i.e. photosynthesis production).

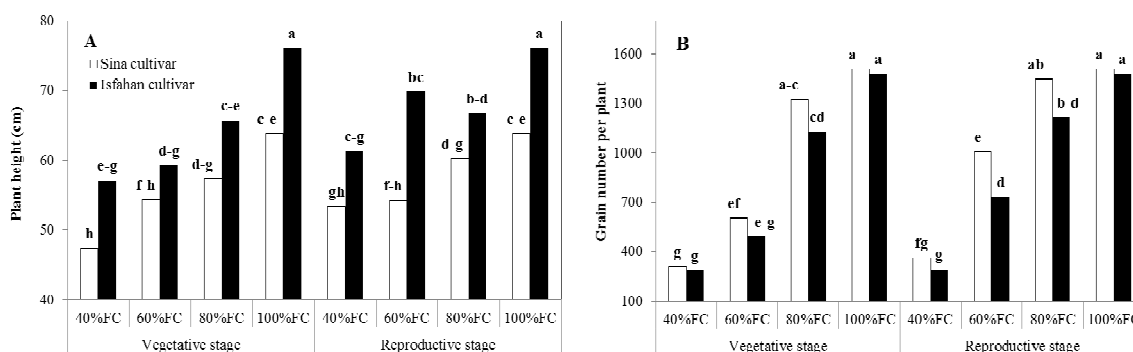


Fig. 1. Effect water deficit at two growth stage on plant height (A) and grain number per plant (B) of two safflower cultivars (means of two years). Column with similar letter had not significant difference (LSD 0.01).

In all water deficit treatments (stages and intensities), plant height of Isfahan was more than Sina cultivar (Fig. 1a) by 14.5%. Differences between two cultivars in reproductive stage treatment were greater than vegetative stage. Overall, the highest plant height was observed in Isfahan cultivar grown under 100% field capacity irrigation (76.1 cm); while the lowest plant height was obtained in Sina cultivar grown under 40% field capacity irrigation at vegetative growth (47.3 cm), as shown in Fig. 1(a). Water deficit may be resulted in shorter internodes, possibly reduced leaf area and decrease in light interception; thereby leading to reduced dry matter production and reduced growth (Yordanov *et al.*, 2000). Grain number per plant (GN) was reduced as affected by drought stress, and this reduction was related with drought intensity and stage (Figure 1b). Water deficit (40% FC) at reproductive stage had higher adverse effect on GN. This impact might be due to changes in capitula per plant, which development of capitula and consequence total grain number per plant are reduced by water stress (Istanbuluoglu *et al.*, 2009). There were no significant differences between grain number per plant of Sina and Isfahan cultivars under without stress conditions (i.e. 100% field capacity) and the highest stress intensity (i.e. 40% field capacity); while, in the other stress treatments (i.e. 60 and 40% field capacity) GN of Sina was more than Isfahan cultivar. This trend was similar in both stages that water deficit was applied. The highest grain number was obtained in Sina cultivar grown under 100% FC conditions (1543.3) (Fig. 1b). In both development stages reduction of irrigation amount from 100% to 80% FC, had no significant and adverse

effect on grain number of Sina cultivar. Our result was consistent with results of Able (1976) that reported reduced grain number of safflower in results of water stress.

Response of thousand grain weight (TGW) to water deficit intensity and stage imposed was lower than other traits such as Ht and GN (Fig. 2a). Irrigation reduction from 100% to 80% FC didn't decreased TGW of both cultivars; also there was no significant difference between 100%, 80% and 60% FC of TGW of Sina cultivar. Drought stress reduces dry matter production and eventually grain weight. Tefera *et al.* (2000) indicated that the impact of water stress at reproductive stage on grain weight can be explained by a reduction in assimilate production during grain filling period. As drought progresses the rate of photorespiration increases and as a result of which loss of CO₂ increases (Mengistu, 2009). Although in all treatments, there was no significant difference between grain weight of Sina and Isfahan cultivar; however, on average TGW of Sina was more than Isfahan cultivar by 7.2%. Overall, the most weighty grains were obtained from Sina cultivar grown under 100% FC irrigation (33.0 g); while, the lightest grains were observed in Isfahan cultivar grown under 40% FC irrigation that imposed in reproductive stage (24.4 g) as shown in Fig. 2(a). Similar attributions have been made by others such as Kazemeini *et al.* (2009) as well as Istanbuluoglu *et al.* (2009). As shown by Istanbuluoglu *et al.* (2009), all plants that were irrigated at vegetative stage produced the lesser TGW compared to those that were irrigated at reproductive stage. They also indicated that the lowest grain weight was recorded in the rainfed treatment.

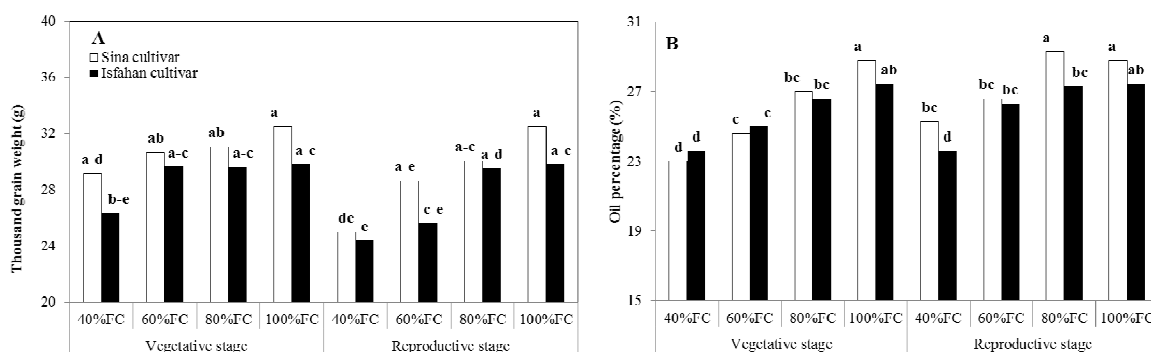


Fig. 2. Effect water deficit at two growth stage on thousand grain weight (A) and oil percentage (B) of two safflower cultivars (means of two years). Column with similar letter had not significant difference (LSD 0.01).

Oil percentage (OP) was decreased under water deficit conditions, and increasing drought intensity increased this reduction (Fig. 2b). Reduction of irrigation amount from 100% to 80%, 60% and 40% FC could decrease OP by 5.4%, 13.7% and 21.3%, respectively. Water deficit imposed at vegetative stage had more adverse effect on OP compared to reproductive stage. Similar results have been observed by Kazi *et al.* (2002); Kazemeini *et al.* (2009) and Eslam *et al.* (2010); who

confirmed that irrigation levels affected the oil percentage. Kazemeini *et al.* (2009) that water deficit stage and intensity could have significant effect on oil percentage. Eslam *et al.* (2010) also showed that water stress during the seed filling stage decreased seed oil percentage, which this reduction associated with drought levels. Except in S1I1 and S2I2 treatments, there were no significant differences between OP of two cultivars (Fig. 2b).

Eslam *et al* (2010) studied the effect of drought stress on OP of safflower cultivars and indicated that there were significant differences in response of oil percentage of 4 genotypes to water deficit. Despite these variations, in their research, there were no significant differences between Isfahan and Sina cultivar in terms of OP. These results also confirmed our findings. Water deficit reduced biological yield (BY) and as irrigation amounts were decreased this reduction was increased (Fig. 3a). So that the highest and the lowest BY were recorded from 100% (9385 kg ha⁻¹) and 40% FC (2686 kg ha⁻¹) treatments, respectively. However, there was no significant difference between BY in 100% and 80% FC treatments (9385 vs. 8190 kg ha⁻¹). Water deficit imposed at vegetative stage had more adverse effect on BY compared at reproductive stage. In drought stress conditions plants allocate more assimilates to root production in compared to shoot. Also reductive effect of water stress on foliage biomass such as stem, leaves

and capitula, could be resulted in reduced BY (Bassil and Kaffka, 2002); as in current study Ht was decreased due to drought stress. These reductions can be related to decreasing in assimilate production, and increasing in photorespiration as well as loss of CO₂ (Tefera *et al.*, 2000). In without water stress (i.e. 100% FC) and light drought stress (i.e. 80% FC) Sina had higher BY compared to Isfahan cultivar. In contrast, at higher water stress levels (60% and 40% FC) two cultivars had no significant difference (Fig. 3a). These trends were similar in both developmental stage treatments. Overall, Sina cultivar grown in 100% FC irrigation had the highest BY; while, the lowest BY was found in 40% FC treatments at two developmental stages and for both cultivars (Fig. 3a). Similar results were found in research of Cakir (2004); Mengistu (2009) and Istanbuluoglu *et al.* (2009). Cakir (2004) indicated that water deficits during the rapid vegetative growth period caused 28-32% loss in dry matter weight.

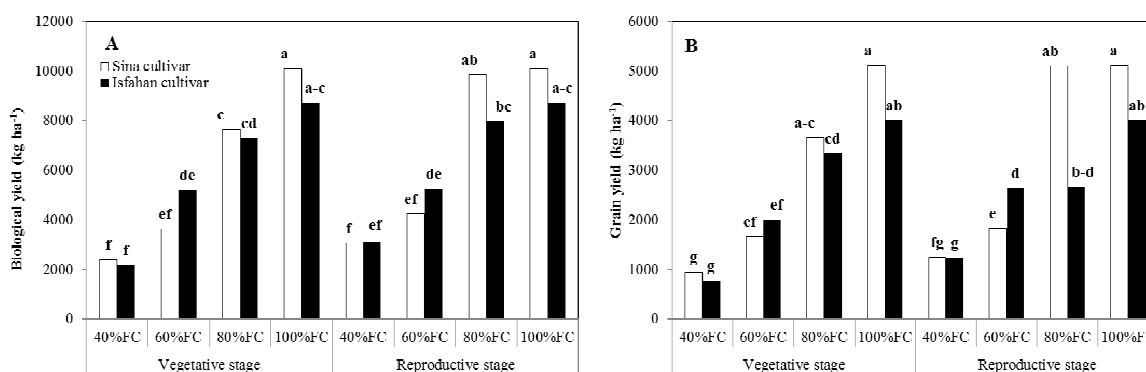


Fig.3. Effect water deficit at two growth stage on biological yield (A) and grain yield (B) of two safflower cultivars (means of two years). Column with similar letter had not significant difference (LSD 0.01).

Drought stress had a clear cut effect on safflower grain yield (GY), as water deficit at 80%, 60% and 40% FC decreased GY by 13.5%, 53.2% and 76.9% compared to without stress (100% FC), respectively. Losses of 15%-25% in grain yield in result of drought stress were reported by NeSmith and Ritchie (1992). Such yield losses have been attributed to reduced photosynthetic rate, altered carbon allocation, and accelerated leaf senescence (Yordanov *et al.*, 2000); our results also indicated that photosynthetic rate was reduced in results of drought stress. Sina cultivar had higher GY in 100% and 80% FC treatments; while there were no significant differences between two cultivars in 60% and 40% FC treatment (Fig. 3b). These trends were similar in both vegetative and reproductive stages. Results of a 3-year field study showed that grain yield was significantly affected by water deficit in any growth stage, and the highest grain yields were observed in the fully irrigated control (Cakir, 2004). Irrigation reduction from 100% to 80% FC caused a significant decrease in GY of Isfahan cultivar; while, this is not observed for Sina cultivar (Fig. 3b). The fact that water stress effects on growth and yield are variety-dependent is well known

(Cakir, 2004). Thus, in our research, Sina might be a more tolerance cultivar than Isfahan cultivar.

Drought stress decreased stomatal conductance (gs), in two development stages and for both Sina and Isfahan cultivars and this reductive effect was closely related to drought intensity (Table 2). Effect of timing and level of water deficit on gs was greater in Sina than Isfahan cultivar; furthermore, response of Isfahan cultivar to four level of drought that imposed in vegetative stage was lower. Although gs was more after irrigation than before, same trends was observed in four levels and two development stages for both cultivars (Table 2). Many studies have given a more quantitative basis to relationships between stomatal opening and leaf water status. Water supply directly affects gs, which it is known that reduced soil moisture decreases stomatal conductance (Pessarakli, 2001).

Response of substomatal CO₂ concentration (Ci) to water deficit treatments was similar to photosynthesis rate (Table 2), so that the highest and the lowest Ci was obtained under 100% and 40% FC conditions, respectively. Steady responses were observed between four levels of water deficit at two development stage for both cultivars, in terms of Ci (Table 2).

Table 2: Effect of water deficit at two developmental stages of photosynthesis traits before and after irrigation in two safflower cultivars.

Safflower Cultivar	Development stage	Irrigation regime	g_s ($\text{mol m}^{-2} \text{s}^{-1}$)		A ($\mu \text{mol m}^{-2} \text{s}^{-1}$)		Ci ($\mu \text{mol mol}^{-1}$)	
			Before	After	Before	After	Before	After
Sina	Vegetative stage	100% FC	0.19	0.38	13.99	27.02	238.34	460.0
		80% FC	0.17	0.32	12.56	22.43	213.95	382.0
		60% FC	0.14	0.24	9.90	17.47	168.71	297.5
		40% FC	0.09	0.22	6.44	14.60	109.66	265.6
	Reproductive stage	100% FC	0.14	0.21	10.98	16.15	245.00	360.5
		80% FC	0.12	0.18	9.57	13.49	213.50	301.0
		60% FC	0.11	0.14	8.20	10.46	183.00	233.5
		40% FC	0.08	0.11	6.29	8.87	140.50	198.0
Isfahan	Vegetative stage	100% FC	0.13	0.27	9.53	19.33	162.42	329.2
		80% FC	0.13	0.22	9.20	15.52	157.36	262.7
		60% FC	0.06	0.17	4.97	12.20	85.37	207.8
		40% FC	0.05	0.12	4.09	8.95	80.59	152.4
	Reproductive stage	100% FC	0.10	0.16	7.91	12.05	176.50	269.0
		80% FC	0.09	0.12	7.05	9.32	158.00	208.0
		60% FC	0.07	0.09	5.26	5.70	117.50	163.0
		40% FC	0.04	0.07	3.47	3.35	77.50	119.5
LSD (< 0.01)			0.019	0.22	0.87	2.01	41.50	33.8

g_s : Stomatal conductance, A: Photosynthesis rate, Ci: Substomatal CO_2 concentration, FC: Field capacity.
Each mean is average of two years.

Although in all treatment, Ci was greater after irrigation than before; however, difference between after and before irrigation records were higher in 100% compared to lower field capacities and Sina than Isfahan as well as in vegetative than reproductive stage. It has been known that stomata remain unaffected until the leaf water potential drops to some critical threshold value, then begin to close (Hsiao and Acevedo, 1974; Pirasteh-Anosheh *et al.*, 2013). Drought stress also usually leads to oxidative stress due to stomatal closure that causes the over-reduction of photosynthetic electron chain and high formation of reactive oxygen species in chloroplasts and mitochondria (Liu *et al.*, 2011). Redshaw and Meidner (1972) believed that when water is deficient enough to cause stomatal closure, the increase in stomatal resistance is commonly accompanied by an increase in mesophyll resistance. This reduction in stomatal conductance could be a reason for decreasing of growth and yield under water deficit treatment.

Water deficit stress decreased photosynthesis rate (A), indeed the highest and the lowest A was recorded under 100% and 40% FC conditions, respectively (Table 2). The reductive effect of water stress on A at reproductive stage was more than vegetative stage for both cultivars. Differences between four levels of water deficit at vegetative for Sina were higher; while it was lower at reproductive stage for Isfahan cultivar (Table 2). Except C1S2I3, A was greater after irrigation than before. Differences between after and before irrigation records were the highest in 100%; and were the lowest in 40% FC treatments. Photosynthesis is decreased in every stresses, especially under water and salt stress (Liu, *et al.*, 2011). Changes in biological and grain yield might be a result of drought effects on A, so it was suggested by many researchers (Yordanov *et al.*, 2000; Fiscus *et al.* 2005). The reduction in A would reduce allocation to aboveground biomass and reduce yield. One reason for photosynthesis reduction under water deficit conditions can be its effect on stomatal conductance, which drought stress decreases stomatal conductance and available CO₂ for photosynthesis (Pessarakli, 2001); it means that water stress treatments leads to stomatal closure and limited of gas exchange. Also, there are non-stomatal effects of drought stress in suppressing photosynthesis in addition to the stomatal effect. The basis for these effects may lie in altered transport parameters for CO₂ from the inter-cellular space to the chloroplasts or in altered ability of chloroplasts to photosynthesize. Also it may lie in an increase in respiration in the leaf. As for enzymes in the photosynthetic complex, several of them seem to be rather resistant to water stress (Hsiao and Acevedo, 1974).

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