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FB, OA: supervised the research and designed the project; SMTTL: performed the experiments, collected the data, and wrote the first draft of manuscript; BA, AB: analyzed and interpreted the data, and edited the manuscript; all the authors proofread and finalized the manuscript

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ORIGINAL RESEARCH PAPER

Influence of cut-off irrigation on seed quality and physiological indices of various safflower (*Carthamus tinctorius* L.) genotypes

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Abstract

Safflower (*Carthamus tinctorius* L.) is an oilseed crop adapted to arid and semiarid regions. In this study, an experiment was performed to evaluate the effects of water deficiency on plant height, 1,000-grain weight, seed yield, harvest index, relative water content (RWC), oil yield, and oil content in 15 safflower genotypes. A split-plot randomized complete blocks design was arranged with three replications. Safflower plants were grown under normal irrigation and water deficit conditions in Sarvestan, Fars Province, Iran during 2016 and 2017 growing seasons. Combined analysis results indicated that water deficit stress had negative effects on all measured indices. Average seed yield declined by 65.91% (2,337.91 to 796.79 kg ha^{-1}) due to water deficit stress. Genotype also had a significant effect on evaluated indices, and the interaction between genotype and irrigation significantly influenced all indices except plant height. Under both conditions, highest RWC, seed yield, and oil yield were observed in Dincer and PI-537598 genotypes. Maximum plant height and 1,000-seed weight in both irrigation conditions were observed in the Dincer genotype. In the normal irrigation condition, maximum harvest index and oil content were observed in the CW-74 genotype. Thus, Dincer and PI-537598 were classified as the best genotypes (based on seed yield, RWC, and oil yield) under both normal irrigation and water deficit stress conditions.

Keywords

safflower; oil yield; water deficit stress

Introduction

Water deficiency is the most critical problem influencing the growth and yield of cultivated crops in arid and semi-arid zones such as Iran. The annual precipitation in Iran is only 250 mm [1] and the mean rainfall is less than one third of the global mean precipitation [2].

Adaptation to water stress can be induced in plants by altering diverse morphological, physiological, biochemical, and molecular characteristics. Drought resistance can be defined as the capacity of crops to develop, grow, bloom, and yield seeds in water-limiting conditions [3]. Water preservation during droughts is pivotal to the survival of crops susceptible to water stress [4].

Full irrigation procedures could maximize yield in agricultural production; however, a better strategy may be to optimize irrigation management or water quantity which is accompanied by a diminish in yield [5]. Previous research indicates that in water deficit conditions, leaves of various crops become depleted in relative water content [6,7].

Safflower (*Carthamus tinctorius* L.) is one of the world's oldest cultivated oilseed crops, though it is less abundant than other oil crops such as soybean, sunflower, and peanut. Safflower is not cultivated at a large scale worldwide, and its cultivation is not very intensive [4]. The content of oil in oilseeds is affected by the seed development process and abiotic environmental stress [8]. Safflower plants are able to overcome abiotic stress conditions, including high temperatures, drought, and salinity [9]. However, significant depletion in seed yield had been observed in safflower plants experiencing extreme water deficiency [10]. Water use efficiency in safflower is remarkably stable, with only small depletions in crop yield occurring in drought conditions [10]. Thus, these characteristics may facilitate the cultivation of safflower in areas with soil and climatic limitations. Genotypic variation of seed oil content has been measured in safflower; however, environmental conditions also affect oil content [11,12]. Furthermore, it has been demonstrated that agronomic practices have important effects on oil percentage and oil yield in different safflower cultivars [13]. Thus, the aim of this study was to investigate the effect of water deficit stress on plant height, 1,000-grain weight, seed yield, harvest index, relative water content (RWC), oil yield, and oil content of different spring safflower genotypes grown in Iran.

Material and methods

The field experiment was conducted in Sarvestan area, located 90 km from Shiraz, Iran (southeast of Fars Province). The experiment was conducted at an altitude of 1,557 m above sea level with coordinates 29.57° N, 53.28° E during the 2016 and 2017 growing seasons. The mean rainfall over a ten-year period was 245 mm. Plots consisted of five planting lines (6 m long) with a spacing of 30 cm between lines and 10 cm between seeds. Fifteen safflower genotypes (Kino-77, Dincer, Sina, Goldasht, PI-537636-s, Soffeh, CW-74, CW-4440, Hartman, Lesaf, PI-250537, Gilla, PI-537598, Faraman, and IL-111) were planted in February and grown in a split-plot arranged in a randomized complete block design with three replications. The main plot consisted of two irrigation treatments: cut off irrigation and normal irrigation. Safflower genotypes were arranged in subplots. The duration between planting and harvesting was 6 months. In the control group, normal irrigation was applied for 6 months until the crops were harvested. In the cut off irrigation group, crops were grown under full irrigation for 3 months (until the heading-bud stage), after which the plants were no longer watered. This stress continued for 3 months until the end of experiment. All crops were harvested in early August. The experiment was performed in 2 consecutive years. Treflan herbicide (2.5 L ha^{-1}) was applied to control weeds. According to soil analysis, 300 kg ha^{-1} urea, 100 kg ha^{-1} super phosphate, and 50 kg ha^{-1} potassium sulfate were applied to the soil as fertilizer (Tab. 1).

Tab. 1 Physicochemical properties of the research station soil.

Sodium absorption ratio		3.4
Magnesium	mEq/L	57.94
Potassium		1.99
Calcium		11.06
Chlorine		38
Sodium		21.05
pH		7.5
Soil depth	cm	0–45
Structure		Sandy loam
Salinity	dS/m	9.6
Sand	%	52
Silt		39.2
Clay		8.8
Field capacity	gr/cm	16.48
Bulk density		1.228
Sampling depth	cm	0–45

Five plants per plot were randomly selected to estimate mean plant height. To evaluate 1,000-seed weight, 100 seed subsamples in four replicates were randomly selected from harvested crops of each plot; these samples were then weighed and the mean weight was multiplied by 10 to estimate 1,000-seed weight. The weight of total harvested seeds in two central rows in each plot was used to calculate the seed yield per unit area.

Harvest index was calculated by the following equation: *Harvest index* (%) = (*Economic yield*/*Biological yield*) × 100.

In order to calculate of RWC, the same size (1 × 1 cm) of fresh leaves was weighed (FW), floated in distilled water for 8 h, blot-dried, and weighed again to measure turgid weight (TW). Leaves were then dried in an oven for 24 h at 80°C (DW). Finally, RWC was calculated using the following formula: $RWC = (FW - DW) / (TW - DW) \times 100$ [14].

Oil content was measured using a time-domain nuclear magnetic resonance (TD-NMR), spectrometer (SLK-SG-200, Spinlock Magnetic Resonance Solutions, Malagueño, Córdoba, Argentina). The oil yield (kg ha^{-1}) was calculated by multiplying the grain yield and oil content.

Statistical analysis

All data were tested for homogeneity of variance and normality of residuals using Bartlett tests and the Ryan–Joiner method, respectively. Irrigation treatment and genotype interactions across the 2 years of this study were compared with a combined ANOVA using PROC GLM in SAS 9.2 software (SAS Institute, Cary, NC, USA). Means were separated by applying the Duncan's multiple range test when the *F* test was significant at $p \leq 0.05$.

Results

Combined analysis

Results of the combined analysis of variance for plant height, 1,000-seed weight, seed yield, harvest index, RWC, oil yield, and oil content are presented in Tab. 2. As there were no significant differences between years for most tested indices (except harvest index and RWC), combined means for both years were used for comparisons.

Results showed that genotype had a significant effect on all evaluated indices. The effect of different water levels also had a significant effect on all indices ($p < 0.01$). Results of the combined ANOVA indicated that the interaction between water deficit stress and genotype had a significant effect on all indices except plant height (Tab. 2). None of the evaluated indices were affected by the interaction between year and irrigation/genotype or the three-way interaction of Year × Genotype × Irrigation (Tab. 2).

Plant height

Plant height significantly decreased in the water deficit stress condition ($p < 0.01$) (Tab. 2). The highest mean plant height was observed in normal irrigation (Fig. 1). The highest mean plant heights in normal irrigation were observed in Dincer (76.21 cm) and PI-250537 (73.51 cm) and lowest mean plant heights were observed in Sina (60.56 cm) and IL-111 (61.50 cm). However, in stress conditions the Dincer genotype was tallest (37.12 cm) and Gilla, Sina, IL-111, and Kino-77 genotypes (22.53, 24.37, 24.75, and 25.87 cm, respectively) were shortest (Fig. 1). This indicates that water deficit stress affected plant height by reducing growth rates. Plant height was significantly different in normal irrigation (control) and water deficit stress treatments (Tab. 2).

Tab. 2 Combined analysis of variance indices of 15 safflower genotypes grown under normal irrigation and water deficit stress conditions in growing seasons of 2016 and 2017.

Source of variation	*Df*	Plant height (cm)	Thousand-seed weight (g)	Seed yield (kg ha^{-1})	Harvest index (%)	RWC (%)	Oil yield (kg ha^{-1})	Oil content (%)
Year (y)	1	14.20^{ns}	4.91^{ns}	32,327.30^{ns}	437.09**	286.71**	198.18^{ns}	2.31^{ns}
Error (a)	4	947.90	3.80	1,539,671.90	8.10	4.44	57,676.52	76.04
Irrigation (I)	1	68,338.68**	5,609.13**	106,876,897.80**	1,466.97**	5,878.65**	10,861,438.79**	1,457.19**
Y × I	1	25.50^{ns}	12.22^{ns}	70,051.10^{ns}	19.42^{ns}	18.38^{ns}	248.19^{ns}	3.39^{ns}
Error (b)	4	78.81	20.46	255,513.80	5.79	8.28	15,818.19	6.24
Genotype (G)	14	189.45**	246.35**	433,352.70**	84.83**	192.85**	25,941.65**	76.31**
G × I	14	13.68^{ns}	48.64**	235,427.30**	31.95**	62.34**	11,676.43**	30.89**
G × Y	14	6.72^{ns}	6.52^{ns}	51,712.50^{ns}	4.92^{ns}	7.56^{ns}	284.11^{ns}	10.22^{ns}
G × Y × I	14	8.99^{ns}	1.82^{ns}	55,326.60^{ns}	5.22^{ns}	13.92^{ns}	247.93^{ns}	9.30^{ns}
Error (c)	112	9.97	13.73	31,462.90	3.73	7.90	1,606.13	5.83
CV%		6.52	10.12	11.31	5.92	3.70	9.22	9.00

Significant at * $p < 0.05$; ** $p < 0.01$; ns – nonsignificant.

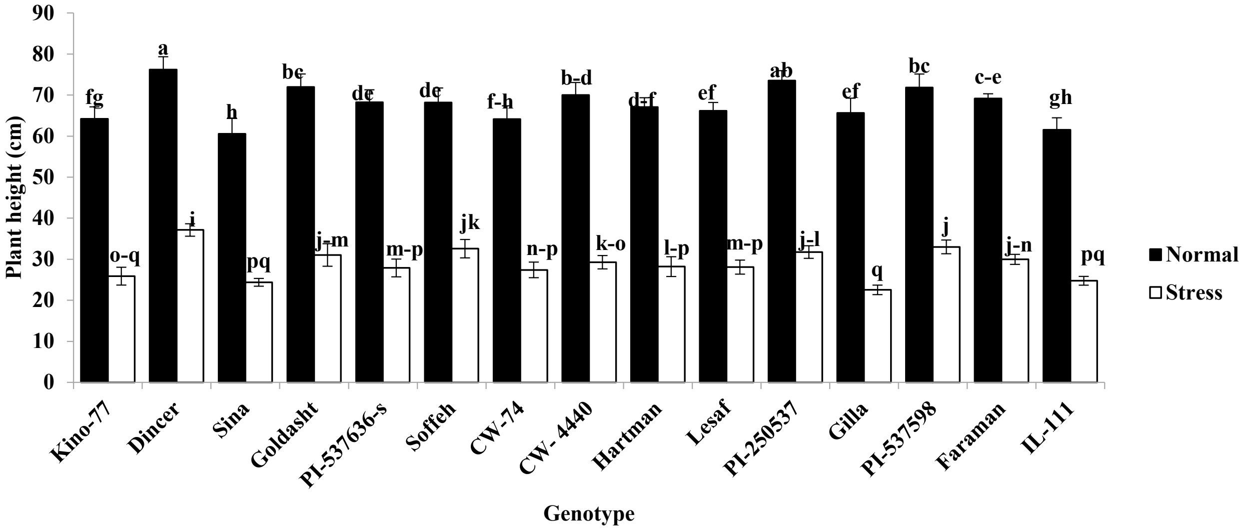

Fig. 1 Interaction effect of irrigation and genotype on plant height. Mean values with the same letters are not significantly different ($p > 0.05$) according to the Duncan test. Data are presented as mean ±*SEM*.

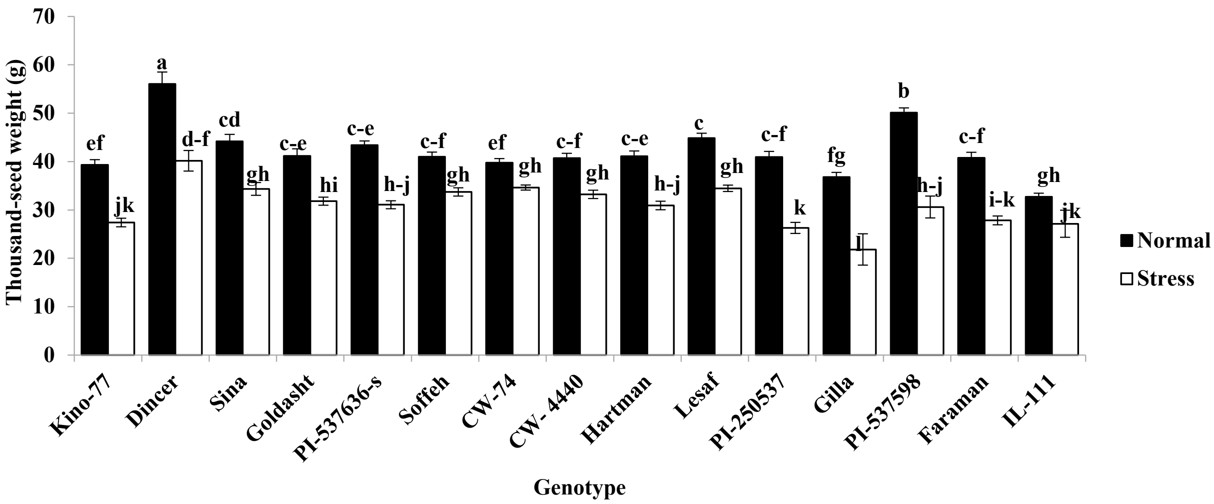

Fig. 2 Interaction effect of irrigation and genotype on 1,000-seed weight. Mean values with the same letters are not significantly different ($p > 0.05$) according to the Duncan test. Data are presented as mean ±*SEM*.

Thousand-seed weight

Irrigation, genotype, and the interaction between irrigation and genotype had significant effects on safflower 1,000-seed weight ($p < 0.01$) (Tab. 2). The highest 1,000-seed weight was obtained under normal irrigation and water deficit stress in Dincer (56.06 and 40.16 g, respectively), while the lowest 1,000-seed weight was observed in IL-111 (32.71 g) with normal irrigation and Gilla (21.83 g) in water deficit stress (Fig. 2).

Seed yield

Seed yield significantly decreased in water deficit stress conditions ($p < 0.01$) (Tab. 2). Maximum yield in the normal irrigation treatment was observed in the Dincer genotype (3,143.23 kg ha^{-1}) and minimum yield was observed in IL-111 (1,873.16 Kg ha^{-1}). Under water deficit stress conditions, Dincer and PI-537598 had higher seed yield (963.38 and 933.07 kg ha^{-1}, respectively) and Gilla, PI-250537, and Lesaf had lower seed yield (644.96, 670.30, and 671.91 kg ha^{-1}, respectively) than other genotypes (Fig. 3).

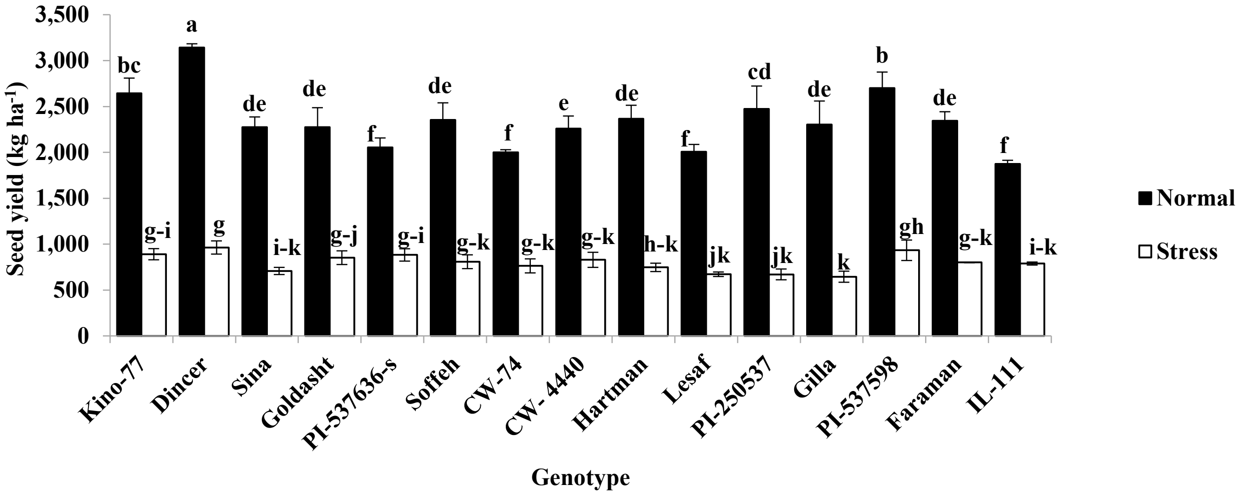

Fig. 3 Interaction effect of irrigation and genotype on seed yield. Mean values with the same letters are not significantly different ($p > 0.05$) according to the Duncan test. Data are presented as mean ±*SEM*.

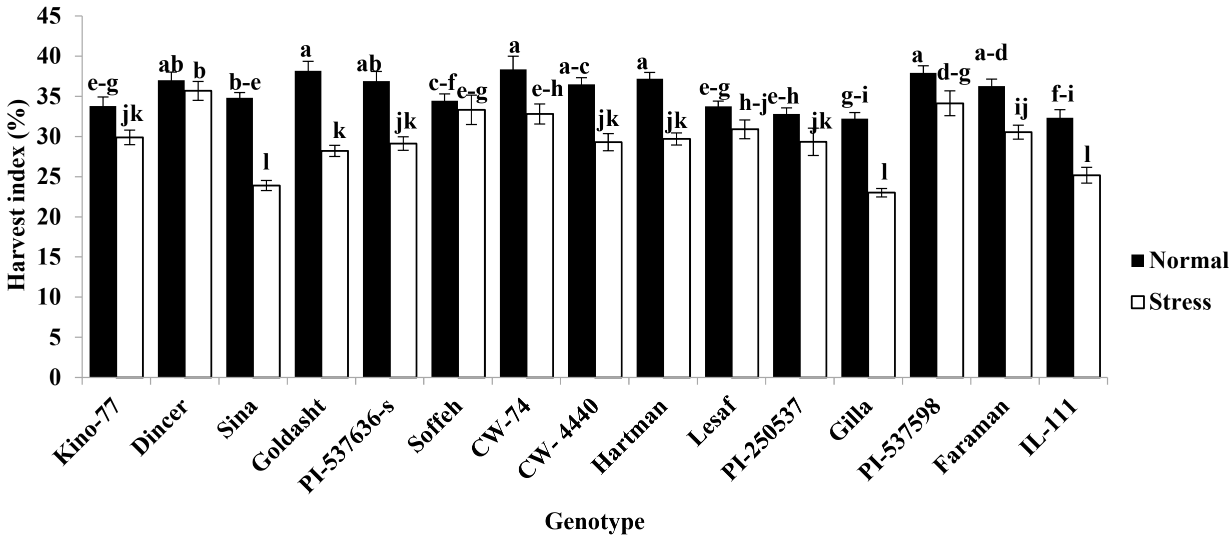

Fig. 4 Interaction effect of irrigation and genotype on harvest index. Mean values with the same letters are not significantly different ($p > 0.05$) according to the Duncan test. Data are presented as mean ±*SEM*.

Harvest index

Harvest index showed significant differences among years, irrigation treatments, genotypes, and irrigation levels ($p < 0.01$) (Tab. 2). Harvest index values of the normal irrigation treatment ranged from 38.35% for the CW-74 genotype to 32.21% for the Gilla genotype. In the water deficit irrigation treatment, harvest index values ranged from 35.67% for the Dincer genotype and 22.99% for the Gilla genotype (Fig. 4). Water deficit stress significantly reduced the harvest index of all studied genotypes except Dincer ($p < 0.01$) (Tab. 2).

RWC

Results showed significant effects of year, genotype, irrigation, and interaction of irrigation and genotype on RWC ($p < 0.01$) (Tab. 2). Highest RWC was observed in the normal irrigation treatment, with 87.07% for Dincer, 85.36% for PI-537598, and

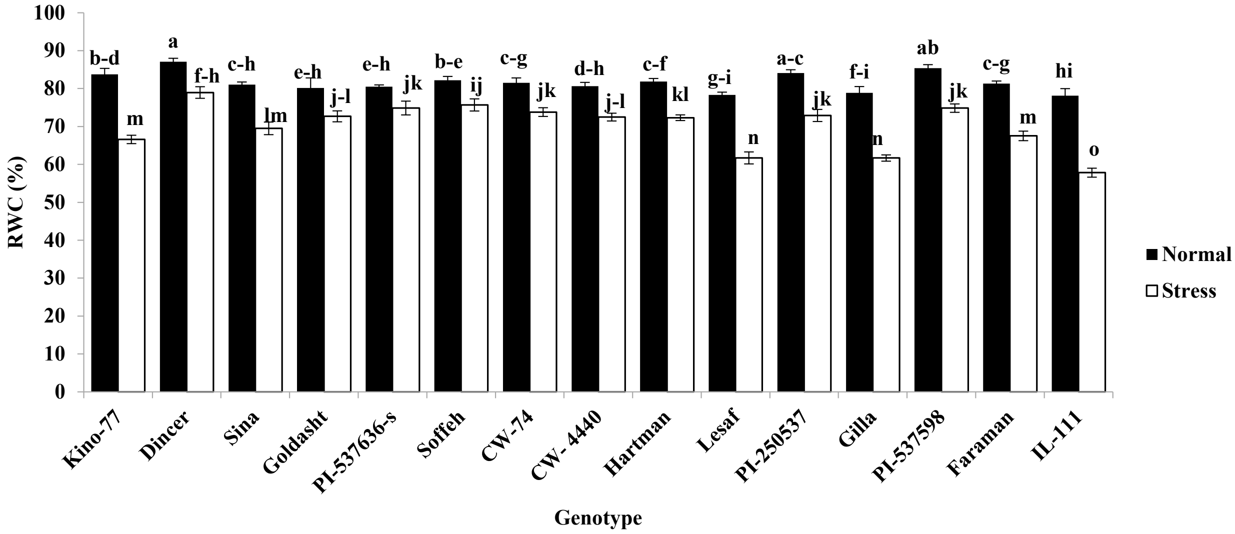

Fig. 5 Interaction effect of irrigation and genotype on relative water content (RWC). Mean values with the same letters are not significantly different ($p > 0.05$) according to the Duncan test. Data are presented as mean ±*SEM*.

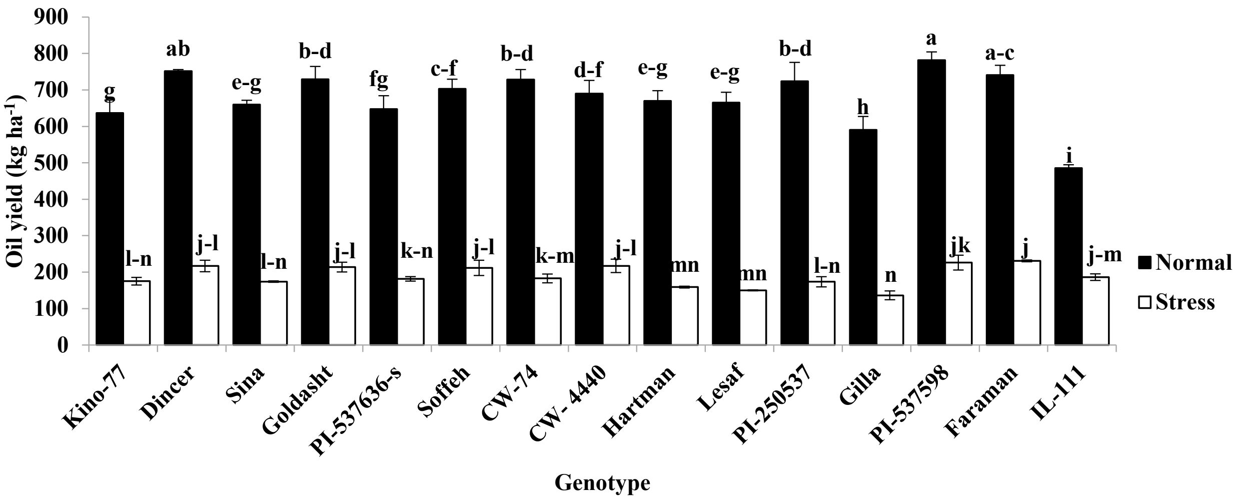

Fig. 6 Interaction effect of irrigation and genotype on oil yield. Mean values with the same letters are not significantly different ($p > 0.05$) according to the Duncan test. Data are presented as mean ±*SEM*.

84.10% for PI-250537 genotypes. Lowest RWC in the normal irrigation treatment was detected in IL-111 (78.10%), Lesaf (78.31%), and Gilla (78.88%) genotypes (Fig. 5). However, a different level of decline in RWC was observed under water deficit stress conditions. The Dincer genotype (78.96% RWC) showed the most potential to retain water during abiotic stress conditions (Fig. 5). Results also showed that water deficit stress resulted in lower RWC than normal irrigation (Fig. 5).

Oil yield and oil content

Significant effects of irrigation, genotype, and the interaction between irrigation and genotype were observed for oil yield and oil content ($p < 0.01$) (Tab. 2). Water deficit stress resulted in decreased oil yield and oil content in all studied safflower genotypes (Fig. 6, Fig. 7). The mean reduction in oil yield was 491.28 kg ha^{-1} (72.2%) and 19.1% for oil content. The highest seed oil yield was obtained from PI-537598 (781.93 kg ha^{-1}), Dincer (752.03 kg ha^{-1}), and Faraman (740.90 kg ha^{-1}) genotypes in the normal irrigation condition (Fig. 6). Additionally, Goldasht, Soffeh, CW-440, and IL-111 produced higher oil yields than other genotypes under water deficit stress conditions.

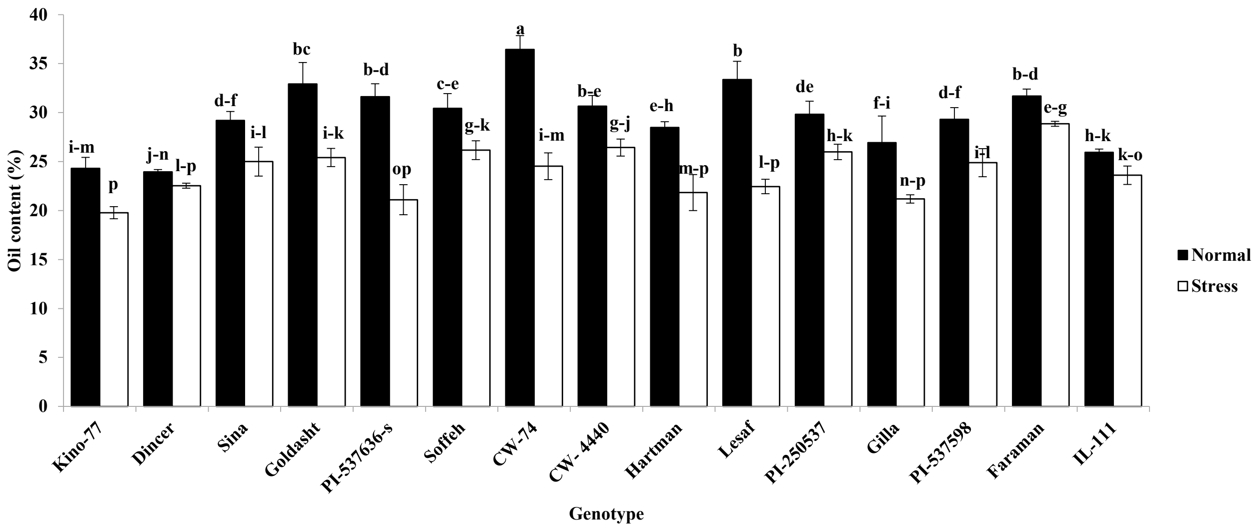

Fig. 7 Interaction effect of irrigation and genotype on oil content. Mean values with the same letters are not significantly different ($p > 0.05$) according to the Duncan test. Data are presented as mean ±*SEM*.

Results showed that the oil content of safflower genotypes ranged from 23.93% to 36.44% in the normal irrigation treatment and 19.77% to 28.85% in the water deficit treatment (Fig. 7). Highest oil content was observed in the CW-74 genotype in the normal irrigation regime, while the Dincer genotype had the lowest decrease in oil content after water deficit stress (Fig. 7). Results showed that the pattern of oil yield variation was different than seed yield among safflower genotypes.

Discussion

Results of this study indicated that water deficit stress may negatively affect plant height, 1,000-grain weight, seed yield, harvest index, RWC, oil yield, and oil content. The interaction between genotype and irrigation treatment was highly significant for 1,000-seed weight, seed yield, harvest index, RWC, oil yield, and oil content.

Water deficit stress drastically decreased plant height. Thus, water deficit stress could reduce safflower height if it occurs during growing stages. This height reduction could be due to an interruption in the photosynthesis process during the water deficit period, leading to a decreased transfer of photo-assimilates to growing sections of crops [15].

Irrigation levels had a significant effect on 1,000-seed weight of safflower. This result is in agreement with Mohammadi et al. [16], which indicated that lower numbers of safflower seeds under water deficit stress may be associated with a diminished transfer of photosynthetic products to seeds through the generative growth stage. Likewise, Mirshekari et al. [17] reported that disrupted irrigation at head-forming and flowering stages of safflower caused reduced 1,000-seed weight during water deficit stress. The authors also suggested that the reduction of photosynthesis and photo-assimilates production under water deficit stress was associated with decreased amounts of photo-assimilates in seeds, resulting in reduced seed weight.

Results of this study also showed that water deficit stress reduced safflower seed yield by 65.9% relative to the normal irrigation treatment. This depletion may be related to cut off irrigation along with stimulation of biological aging and reduction in growth duration and seed filling. Signals may transfer from root to leaf and result in stomatal closing, leading to a reduction in pure photosynthesis [18].

Similarly, Santos et al. [19] reported that water deficit stress had adverse effects on safflower seed yield. Variation in seed yield among irrigation regimes and genotypes was also reported by Singh et al. [20]. It has been reported that higher safflower seed yield is associated with increased heads per crop and seeds per head [21]. Similar to results of this study, Davari [22] reported that water deficit stress reduced the harvest index in canola cultivars. It has been shown that both partial and intensive water deficit stress, especially in the late growing stage, have negative effects on harvest index [23]. Richards et al. [24] also indicated that a high harvest index in the normal irrigation regime can be correlated with high grain yield under water deficit stress. Reynolds et al. [25] showed that a higher harvest index in wheat cultivars may be associated with higher grain yield under normal and abiotic stress conditions. Water deficit stress is known to cause declines in multiple indices such as plant height, seed yield, seed weight, harvest index, number of silique and seeds, and days to maturity [26].

Similar to the results of this study, Hojati et al. [6] reported a significant reduction in RWC after inducing water deficit stress in safflower. RWC is a more reliable criterion for evaluating water status in plant tissues than cell water potential, as RWC is directly associated with cell volume and therefore can better represent the balance between crop water content and transpiration rate [15]. Eslam [27] also showed that RWC can used to select optimal safflower genotypes for water deficit stress conditions. However, RWC decreases when water deficit stress intensity increases due to reduced water potential in leaves [28]. However, stress tolerant safflower genotypes may be able to maintain high RWC levels [4]. Çiçek and Cakirlar [29] suggested that water state plays a pivotal role in drought tolerance of most plants. Any interruption in leaf turgor pressure may result in decreased leaf tissue water, which could suppress most physiological and morphological traits including stomatal opening, photosynthesis, and leaf expansion. During water deficit stress, accumulation of salts and ions around the roots causes negative water potential in saline soil, resulting in decreased leaf stomatal opening. As soluble substance content increases, osmotic potential in the cell may become more negative. Osmotic pressure balance does not respond to water deficit; however, it strongly influences growth rates. Increasing soluble substance content in chloroplasts and vacuoles may lead to structural alterations and membrane leakage [30].

One possible reason for the reduction in oil yield under water deficit stress is reduced seed production and/or seed oil content [31]. Our result was in agreement with Ashrafi and Razmjoo [32], which found that the oil content of safflower and soybean decreased with increased water deficit stress. Oil content can be affected not only by water deficit stress and/or genotype [33], but also the duration of seed development [8]. Our results were in agreement with Premchandra et al. [34], which showed that seed oil content was significantly different among safflower cultivars. Thus, it appears that the variation in oil content among genotypes was related to the interaction between genotype and abiotic environment.

In conclusion, water deficit stress can reduce safflower yield. The Dincer genotype produced the highest yield in normal irrigation and in the water deficit stress treatment. Thus, we suggest that this genotype is an appropriate candidate for cultivation in both normal and water deficit stress conditions.

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Wpływ nawadniania na jakość nasion i wskaźniki fizjologiczne różnych odmian *Carthamus tinctorius* L.

Streszczenie

Krokosz barwierski (*Carthamus tinctorius* L.) jest rośliną oleistą przystosowaną do wzrostu w regionach suchych i półsuchych. Celem przeprowadzonych badań była ocena wpływu deficytu wody na wysokość roślin, wskaźnik plonowania, względną zawartość wody (RWC), masę 1000 nasion, plon nasion, a także plon oleju i zawartość oleju w 15 odmianach *C. tinctorius*. Eksperyment prowadzono metodą losowych bloków w trzech powtórzeniach. Rośliny uprawiano w warunkach optymalnego nawodnienia oraz deficytu wody w Sarvestanie, w prowincji Fars w Iranie w dwóch sezonach wegetacyjnych – 2016 i 2017. Wykazano, że stres deficytu wody miał negatywny wpływ na wszystkie badane parametry. Średni plon nasion obniżył się o 65,91% (z 2337,91 do 796,79 kg ha^{-1}). Wartości badanych parametrów były zależne od odmiany. Stwierdzono również interakcje pomiędzy odmianą i poziomem nawodnienia w przypadku wszystkich badanych parametrów z wyjątkiem wysokości roślin. Niezależnie od poziomu nawodnienia, najwyższą wartość RWC, plonu nasion i oleju stwierdzono w odmianach Dincer i PI-537598. W warunkach normalnego nawadniania, najwyższy wskaźnik plonowania i zawartość oleju stwierdzono w przypadku odmiany CW-74. W oparciu o plon nasion, plon oleju oraz RWC odmiany Dincer i PI-537598 sklasyfikowano jako najlepsze do upraw zarówno w warunkach normalnego poziomu nawadniania, jak i w warunkach suszy.