

## Effect of auxin foliar application on seed yield and fatty acids composition of two safflower genotypes under late-season drought

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**Abstract:** To assess the response of two safflower genotypes to auxin foliar application under late-season drought stress using a factorial split-plot experiment with the randomised complete block design, a two-year experiment (2016–2017 and 2017–2018) was conducted in Iran. The watering regime as specified in two levels including, regular irrigation and drought stress in main plots and two safflower genotypes and auxin foliar application in two levels including non-foliar application (control) and auxin foliar application at a concentration of 30 ppm were categorised in subplots. Drought stress at the seed filling stage reduced the safflower seed and oil yield. Moreover, a significant drought-induced decrease in linoleic acid, palmitic acid and behenic acid content, as well as an increase in oleic acids, was observed in two safflower genotypes. Among two safflower genotypes, the early maturing genotype less affected by drought and Goldasht had higher seed yield than the Padideh by 814 kg/ha, respectively. Auxin foliar application alleviates the adverse effects of drought, which led to an 18% increase in seed yield and components. However, the auxin application had no effect on the safflower seed oil content but palmitic acid content was affected by auxin foliar application. Generally, safflower along with auxin application can be recommended to develop safflower cultivation in semi-arid areas.

**Keywords:** early maturity; *Carthamus tinctorius* L.; growth regulator; polyunsaturated fatty acid; spineless

Plant growth and development are critically influenced by unpredictable abiotic factors. To survive fluctuating changes in their environments, plants have had to develop robust adaptive mechanisms (Bielach et al. 2017). Since then, auxin and cytokinin have been recognised as the main regulators of plant development (Benjamins and Scheres 2008, Werner and Schmülling 2009). Indole acetic acid, as one of the key auxins occurring naturally in plants, is known for its beneficial effects on the growth of plants subjected to stress conditions. Auxin is a very potent hormone and it has many effects regulating various physiological processes including cell elongation and senescence

(death) and shedding of leaves (Ludwig-Müller 2011, Simon and Petrášek 2011). The abortion of flowers as well as flower and seed development are regulated and promoted by auxin during the reproductive stages (Figueiredo et al. 2016).

In many regions of the world, including Iran, drought stress is one of the most important factors responsible for decreasing agricultural crop yields (Fathi and Tari 2016). Drought stress occurs frequently during the grain filling stage, which accelerates the leaf senescence process in the plant. As a result, the grains remain incompletely filled, which ultimately leads to agricultural yield losses worldwide (Abid et al. 2017).

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Safflower (*Carthamus tinctorius* L.) has been grown commercially as one of the oldest oil-seed crops. Safflower is mainly used for edible oil (rich in linoleic and/or oleic acids), and natural dyes as a source of yellow and red dyes for food and clothing (Weiss 2000). The oil contents of safflower seeds ranged from 23.08% to 36.51%. Fatty acids in safflower oil are mostly contained linoleic acid (72.5–77.4%), oleic acid (13.5–17.6%), stearic acid (1.5–2.4%) and palmitic acid (5.7–7.9%) (Joshani et al. 2019). The major fatty acid of safflower oil is linoleic acid, which accounted for 55.1–77.0% in oils, with a mean value of 70.66% (Matthaus et al. 2015).

Safflower is more drought tolerant than other oil-seed crops. So, it is suitable for the newly reclaimed soils where other oil-seed crops are difficult to grow (Hamza 2015). But, drought during the flowering and seed-filling stages significantly decreased seed yield in spring safflower (Soheili-Movahhed et al. 2019). On the other hand, the safflower seed yield is generally lower than that of other oil-seed crops; so, it is necessary to increase safflower yield. Information regarding the effect of growth regulators on the growth and yield of safflower are very limited (Soheili-Movahhed et al. 2019). Typically, auxin products are to be applied in the early vegetative stages. Study on the effect of growth hormones on foliage has largely focused on applications that occur near the flowering stage, due to the important roles of auxin in seed growth. Hazrati et al. (2020) found that the highest

safflower seed yield was obtained in the auxin foliar application. In addition, leaf foliar application of auxin in safflower cultivars increased linoleic acid and stearic acid of fatty acids in comparison with non-use of auxin (Hazrati et al. 2020). Therefore, the aim of this study was to evaluate the possible positive effect of auxin foliar application on drought tolerance in two spring and winter safflower cultivars.

## MATERIAL AND METHODS

**Experimental design and procedure.** In this research, two safflower genotypes were cultivated in a field for two growing seasons (2016–2017 and 2017–2018) under two different watering regimes. Field experiments were conducted at the experimental field of seed and plant improvement institute (SPII) in Alborz province, Iran (35°49'12"N, 51°06'33"E, 1 321 m a.s.l.) (Figure 1). A split-plot factorial test was conducted in a complete randomised blocks design with three replications in which a combined analysis over two genotypes, two auxins foliar applications and two irrigation treatments were carried out. The irrigation was performed in two levels: regular watering (control) and the cut off watering from the seed-filling stage in main plots and foliar auxin applications including 0 (control) and 3 000 mg/L (30 ppm) auxin (0.5% surfactant-containing solution) and two safflower genotypes including Goldasht (early maturity and spineless with red dye flower),

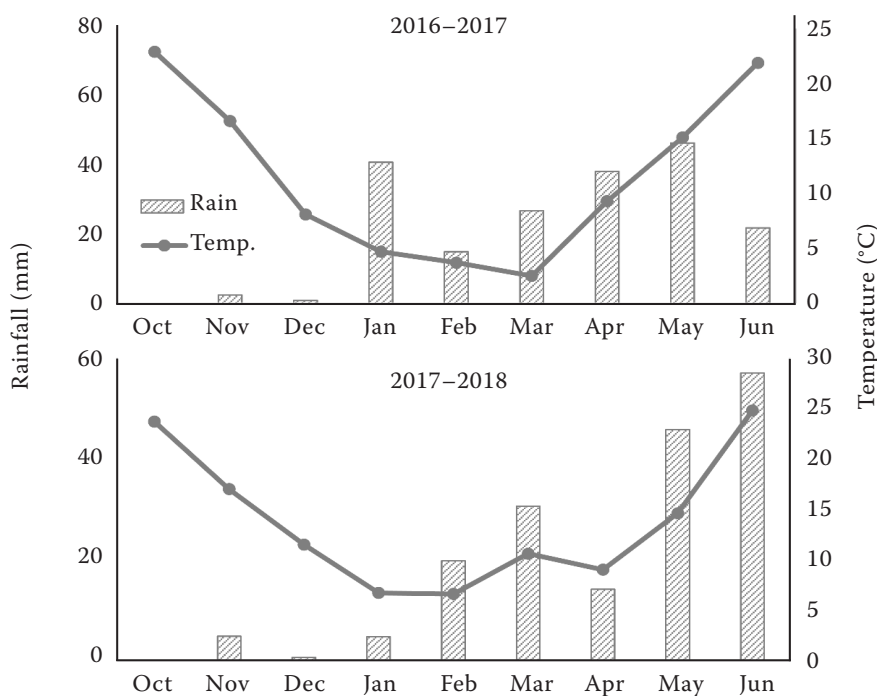


Figure 1. Local weather data (average monthly temperatures and sum of monthly precipitation) of experimental site during 2016–2018

Table 1. Physicochemical characteristics of the soil sample

Soil depth (cm)	Sand	Clay (%)	Silt	pH	EC (dS/m)	OC (%)	N	P (ppm)	K
0–30	24	27	49	7.24	2.22	0.58	0.06	12.6	256

EC – electrical conductivity; OC – organic carbon

and Padidehh (late maturity and spinny with orange dye flower) in subplots. Auxin (indole-3-acetic acid) was prepared from Barafshan company (Iran) and ethanol was used as a solvent to prepare the indole acetic acid solution. The foliar application was done in one step in 50% flowering, according to the BBCH scale, code 65 (Flemmer et al. 2015). Safflower plants sprayed with distilled water are considered as control.

Before planting, the experimental field was disked and tilled to combine residue and to form a seedbed. Based on the results of the soil tests (Table 1), fertilisers were applied with 150 kg N/ha and 55 kg P/ha in 2016 and 2017. Safflower genotypes were planted on Oct. 12 (2016) and Oct. 13 (2017) respectively using a plot drill (Model Wintersteiger, Ried, Austria). Each experimental plot included six four-meter lines with 30 cm spacing and plant spacing on the line was 5 cm, with two lateral lines as margins.

**Measurements.** Matured plants were harvested in July of 2017 and 2018; then oven-dried at 70 °C. After separating, the number of seeds per plant and thousand seed weight also was measured. Oil content was calculated by nuclear magnetic resonance spectrometer (NMR) at 25 °C (Colnago et al. 2011). Oil content uttered based on a dry weight (DB%), and oil yield was determined as kg/ha. The composition of fatty acid in seed oil was measured in one year of the experiment (2016–2017). The composition of fatty acid in obtained oil was analysed by gas chromatography (Agilent) equipped with an FID and a capillary column (CP-Sil88, 50 m, 0.25 mm, 0.2 µm). Initially, the oil was converted to fatty acid methyl esters (FAME) by hexane and potassium methylate. The initial column temperature was 150 °C, then increased to 240 °C at the rate of 5 °C/min. The injector and detector temperatures were, respectively, 250 °C and 280 °C. Peaks were recognised by comparing retention times with those of a commercial standard FAMEs mixture. The details on the GC running program are provided in Reiahisamani et al. (2018).

**Statistical analysis.** Analyses of variance were carried out by SAS statistical analysis software (v9.1 package, SAS Institute, Cary, USA). The least sig-

nificant difference (*LSD*) test was used to compare treatment means with a probability threshold of 0.05.

## RESULTS AND DISCUSSION

**Seed yield and components.** The combined ANOVA analysis results of safflower seed yield and yield components are shown in Table 2. Drought stress in the seed filling stage significantly reduced the head and seed per plant by 12% and 20%, respectively. Seed yield and thousand seed weight were also affected by drought stress at the seed filling stage and decreased by 14% and 4.7%, respectively (Table 3). In contrast, the auxin foliar application alleviated the drought stress negative impact and increased the head per plant (HPP), seed per plant (SPP), thousand seed weight (TSW), and seed yield (SY) by 10, 18, 3.7, and 18%, respectively. Among the safflower genotypes, the Goldasht genotype had higher yield components than the Padideh. The higher seed yield also was obtained in the Goldasht as 2 269 kg/ha (Table 3). Rastogi et al. (2013) have been reported increasing safflower head per plant by the application of different auxin concentrations in both greenhouse and field conditions. Rastogi et al. (2013) also found that the use of 0.5–1 ppm of auxin is effective in stimulating vegetative growth and increasing seed yield and components. Increased seed and oil yield of safflower by application of growth regulators such as kinetin and salicylic acid has previously been well documented (Zand et al. 2010, Ullah and Bano 2011).

**Oil content and fatty acid composition.** The ANOVA analysis results of safflower oil content and fatty acid composition are shown in Tables 2 and 4. The results showed that the watering regime and auxin foliar application had no significant effect on the safflower seed oil content. Among the genotypes, the Padideh had higher oil content than the Goldasht genotype, by an average of 27.1% (Table 5). The watering regime had a significant effect on oleic acid, linoleic acid, palmitic acid and behenic acid content. Auxin foliar application had a significant effect only on palmitic acid content. The two safflower cultivars

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Table 2. Combined analysis of variance for yield components seed yield and seed oil of safflower genotypes

Sources of variance	df	HPP	SPP	TSW	SY	OP
Year	1	**	**	**	**	ns
Year × rep	4	–	–	–	–	–
Irrigation	1	**	**	**	*	ns
Irrigation × year	1	ns	ns	ns	ns	ns
Error I	4	–	–	–	–	–
Cultivar	1	**	**	**	**	**
Foliar application	1	**	**	**	*	ns
Year × cultivar	1	**	ns	**	**	ns
Year × foliar application	1	ns	ns	ns	ns	ns
Irrigation × cultivar	1	ns	ns	ns	ns	ns
Irrigation × cultivar × year	1	ns	ns	ns	ns	ns
Irrigation × foliar application	1	ns	ns	ns	ns	ns
Year × irrigation × foliar application	1	ns	ns	ns	ns	ns
Foliar application × cultivar	1	ns	ns	ns	ns	ns
Foliar application × cultivar × year	1	ns	ns	ns	ns	ns
Irrigation × cultivar × foliar application	1	ns	ns	ns	ns	ns
Year × irrigation × cultivar × foliar application	1	ns	ns	ns	ns	ns
Error II	24	–	–	–	–	–
CV (%)		6.1	7.5	1.8	23	2.7

ns – non-significant; \* $P < 0.05$ ; \*\* $P < 0.01$ ; HPP – head per plant; SPP – seed per plant; TSW – thousand seed weight; SY – seed yield; OP – oil percent

were significantly different in all fatty acids except palmitoleic acid (Table 4). Interaction effect of irrigation × foliar application on linoleic acid, palmitic acid and linolenic acid was significant, whereas irrigation × cultivar interaction effect, was significant only on arachidic and behenic acids (Table 4).

Change in the fatty acids composition in extracted oil from two safflower genotypes in both normal and stress conditions, auxin foliar application and two genotypes is shown in Table 5. The watering regime and genotypes had a significant effect on

fatty acids composition; so, drought stress at the seed filling stage led to an increase in oleic acid, as well as a decrease in palmitic acid and behenic acid content. In two safflower genotypes, drought stress led to a substantial decrease in linoleic acid content. Analysis of the fatty acid composition of extracted oil from safflower seeds showed that linoleic, palmitic and behenic acid was negatively affected by drought while increasing response in oleic acids content was observed. A similar relation between the fatty acids fraction and the drought stress has been reported for

Table 3. Effect of drought stress and auxin foliar application on seed yield and components of safflower genotypes

Treatment		HPP	SPP	TSW (g)	SY (kg/ha)
Watering regimes	regular watering	24.5 <sup>a</sup>	613 <sup>a</sup>	33.5 <sup>a</sup>	2 008 <sup>a</sup>
	drought	21.6 <sup>b</sup>	486 <sup>b</sup>	31.9 <sup>b</sup>	1 716 <sup>b</sup>
Foliar application	distilled water	22 <sup>b</sup>	503 <sup>b</sup>	32.1 <sup>b</sup>	1 707 <sup>b</sup>
	auxin	24.2 <sup>a</sup>	596 <sup>a</sup>	33.3 <sup>a</sup>	2 017 <sup>a</sup>
Genotype	Padideh	22.2 <sup>b</sup>	505 <sup>b</sup>	24.6 <sup>b</sup>	1 455 <sup>b</sup>
	Goldasht	23.9 <sup>a</sup>	593 <sup>a</sup>	40.8 <sup>a</sup>	2 269 <sup>a</sup>

Values denoted by different letters are significantly different,  $P < 0.05$ . HPP – head per plant; SPP – seed per plant; TSW – thousand seed weight; SY – seed yield

Table 3. Analysis of variance for oil fatty acids content of safflower genotypes

Sources of variance	df	OA	LA	PA	PLA	SA	LNA	AA	GA	BA
Rep	2	**	ns	ns	**	**	ns	ns	ns	*
Irrigation	1	**	**	**	ns	ns	ns	ns	ns	**
Error I	2	–	–	–	–	–	–	–	–	–
Cultivar	1	**	**	**	ns	**	**	**	**	**
Foliar application	1	ns	ns	**	ns	ns	ns	ns	ns	ns
Irrigation × cultivar	1	ns	ns	ns	ns	ns	ns	*	ns	**
Irrigation × foliar application	1	ns	**	*	ns	ns	**	ns	ns	ns
Foliar application × cultivar	1	ns	ns	ns	ns	ns	ns	ns	ns	ns
Irrigation × cultivar × foliar application	1	ns	ns	ns	ns	ns	ns	ns	ns	ns
Error II	12	–	–	–	–	–	–	–	–	–
CV (%)		1.3	0.4	0.3	2.6	8.7	8.6	2.5	9.1	12.8

ns – non-significant; \* $P < 0.05$ ; \*\* $P < 0.01$ ; OA – oleic acid; LA – linoleic acid; PA – palmitic acid; PLA – palmitoleic acid; SA – stearic acid; LNA – linolenic acid; AA – arachidic acid; GA – gadoleic acid; BA – behenic acid

safflower (Joshani et al. 2019). Some evidence confirms that concept that membrane lipids augmented by the polyunsaturated fatty acid fraction are better able to conserve the plant's photosynthetic system (Allahverdiev et al. 2001).

The higher oleic acid content was obtained in the Padideh genotype by 17.4%, while the related value for linoleic acid was observed in the Goldasht by 75.5% (Table 5). Generally, safflower oil contains 71–75% linoleic acid and 16–20% oleic acid (Velasco and Fernández-Martínez 2001, Rahmani et al. 2019).

In non-drought stress conditions, auxin foliar application had no significant effect on palmitic acid and linolenic acid but caused a significant reduction in linoleic acid (Figure 2). In contrast, auxin foliar

application had a significant effect on palmitic acid and linolenic acid, under drought stress conditions, and palmitic and linolenic acids content were higher in auxin application compared to distilled water (Figure 2).

In both non-drought stress and drought stress conditions, Padideh had more arachidic and behenic acids than Goldasht cultivar, but in Padideh, drought stress led to a 23% and 34% reduction in arachidic and behenic acids percentage, respectively, compared to normal conditions (Figure 3).

Goldasht genotype had a high unsaturated fatty acids content (90.65%) and the minimum amount of saturated fatty acids (9.35%). In unsaturated fatty acids, the PUFA (polyunsaturated fatty acids) was

Table 5. Effect of drought stress and auxin foliar application on oil percent and fatty acids composition of safflower genotypes

Treatment		OP	OA	LA	PA	PLA	SA	LNA	AA	GA	BA
		(%)									
Watering regimes	regular watering	25.8 <sup>a</sup>	15.4 <sup>b</sup>	74.6 <sup>a</sup>	6.82 <sup>a</sup>	0.059 <sup>a</sup>	1.91 <sup>a</sup>	0.061 <sup>a</sup>	0.17 <sup>a</sup>	0.11 <sup>a</sup>	0.14 <sup>a</sup>
	drought	25.4 <sup>a</sup>	16 <sup>a</sup>	73.4 <sup>b</sup>	6.72 <sup>b</sup>	0.058 <sup>a</sup>	1.91 <sup>a</sup>	0.061 <sup>a</sup>	0.13 <sup>a</sup>	0.11 <sup>a</sup>	0.09 <sup>b</sup>
Foliar application	distilled water	25.5 <sup>a</sup>	15.6 <sup>a</sup>	74.1 <sup>a</sup>	6.75 <sup>b</sup>	0.058 <sup>a</sup>	1.92 <sup>a</sup>	0.060 <sup>a</sup>	0.14 <sup>a</sup>	0.11 <sup>a</sup>	0.11 <sup>a</sup>
	auxin	25.6 <sup>a</sup>	15.8 <sup>a</sup>	73.8 <sup>a</sup>	6.79 <sup>a</sup>	0.059 <sup>a</sup>	1.89 <sup>a</sup>	0.060 <sup>a</sup>	0.15 <sup>a</sup>	0.11 <sup>a</sup>	0.11 <sup>a</sup>
Genotypes	Padideh	27.1 <sup>a</sup>	17.4 <sup>a</sup>	72.4 <sup>b</sup>	5.64 <sup>b</sup>	0.063 <sup>a</sup>	2.36 <sup>a</sup>	0.076 <sup>a</sup>	0.30 <sup>a</sup>	0.14 <sup>a</sup>	0.23 <sup>a</sup>
	Goldasht	24.1 <sup>b</sup>	14.1 <sup>b</sup>	75.5 <sup>a</sup>	7.90 <sup>a</sup>	0.054 <sup>a</sup>	1.45 <sup>b</sup>	0.044 <sup>b</sup>	0.0 <sup>b</sup>	0.08 <sup>b</sup>	0.0 <sup>b</sup>

Values denoted by different letters are significantly different,  $P < 0.05$ ; OP – oil percent; OA – oleic acid; LA – linoleic acid; PA – palmitic acid; PLA – palmitoleic acid; SA – stearic acid; LNA – linolenic acid; AA – arachidic acid; GA – gadoleic acid; BA – behenic acid



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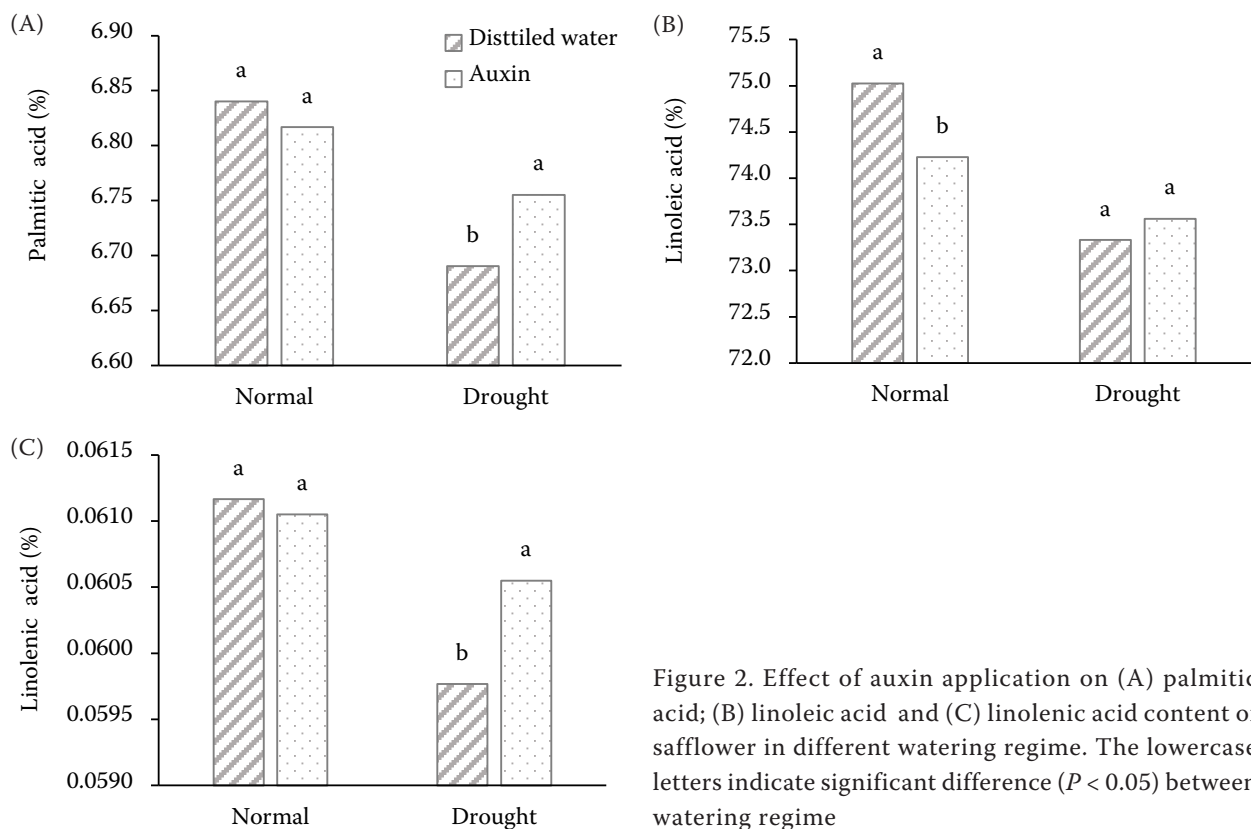


Figure 2. Effect of auxin application on (A) palmitic acid; (B) linoleic acid and (C) linolenic acid content of safflower in different watering regime. The lowercase letters indicate significant difference ( $P < 0.05$ ) between watering regime

found to be dominant with the highest concentration of linoleic acid (75.5%) followed by oleic acid (17.4%). Whereas, palmitic acid showed the highest concentration 7.9% among all the saturated fatty acids. In the present study, the auxin foliar application had no effect on the fatty acid composition of safflower oil except palmitic acid. Palmitic acid content was significantly increased by auxin foliar application.

The outcome of this study showed that drought stress at the seed filling stage impressed the seed

yield and oil content. In contrast, the auxin foliar application reduced the negative effects of drought by improving plant growth. However, the safflower seed oil content did not improve by auxin application. The Goldasht genotype with higher chlorophyll index, osmolytes content as well as higher seed yield was more drought-tolerant than the Padideh genotype; so that can be recommended to develop safflower cultivation and breeding programs in semi-arid areas.

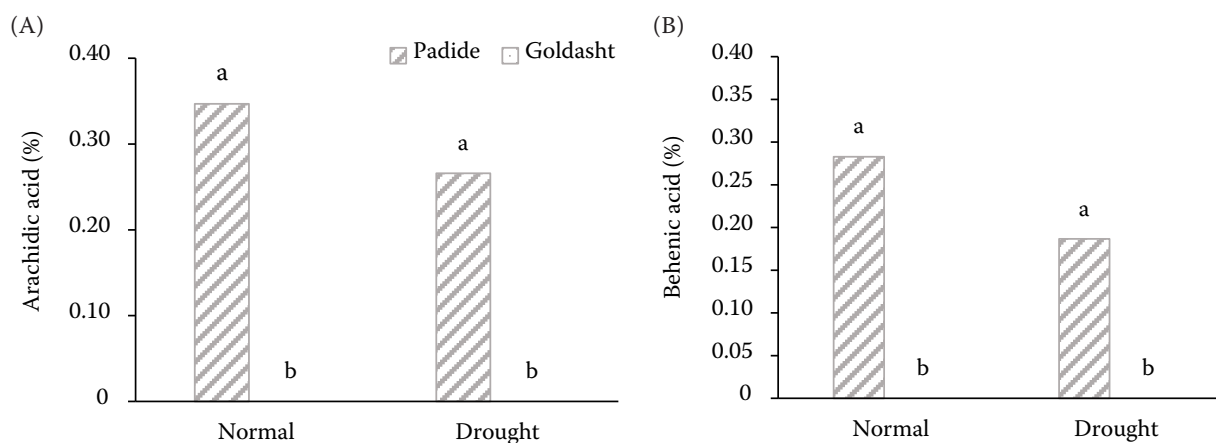


Figure 3. Effect of watering regime on (A) arachidic acid and (B) behenic acid content in two safflower genotypes. The lowercase letters indicate significant difference ( $P < 0.05$ ) between genotypes

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