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Oil Content and Fatty Acid Composition in Seeds of Three Safflower Species

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Abstract Seeds of six safflower (*C. tinctorius* L.) genotypes and 19 accessions of two wild species were analyzed for oil and fatty acid composition. Oil content ranged from 29.20 to 34.00, 20.04 to 30.80 and 15.30 to 20.80% in *C. tinctorius*, *C. oxyacantha* Bieb. and *C. lanatus* L., respectively. The main fatty acids of oleic, linoleic, palmitic and stearic acids composed 96–99% of the total fatty acids in all species. The sum of myristic, palmitoleic, arachidic, and behenic fatty acids in oil of the species ranged from 0.43 to 0.57%. The oleic acid in seed oil of *C. tinctorius*, *C. oxyacantha* and *C. lanatus* ranged from 12.24 to 15.43, 14.11 to 19.28 and 16.70 to 19.77%, respectively. The corresponding ranges for linoleic acid were 71.05 to 76.12, 63.90 to 75.43 and 62.47 to 71.08%. Palmitic acid in seed oil varied from 5.48 to 7.59% in C. *tinctorius*, 6.09 to 8.33% in *C. oxyacantha* and 7.44 to 8.78% in *C. lanatus*. The stearic acid of the seed oil showed a variation of 1.72 to 2.86, 2.50 to 4.87 and 3.14 to 4.79% in genotypes of these species, respectively. The fatty acids composition of oil among the cultivated and wild species were not considerably different, indicating that seed oil of the wild safflower is possibly suitable for human consumption and industrial purposes.

Keywords Safflower · Wild safflower · Seed oil · Fatty acid

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Introduction

Oilseeds are important sources of vegetable oils. The suitability of a vegetable oil for a particular use such as nutritional, industrial or pharmaceutical is determined by its fatty acid composition which is highly variable depending on the plant species. This has encouraged researchers to look for new sources of oil or new fatty acid compositions in different plant species. Genetic variation for fatty acid composition is essential for genetic improvement of the oil quality and developing new cultivars [1, 2]. A large number of potential plants have been analyzed for oil content and fatty acid profiles and some have been identified and are cultivated as the new oil seed crops [3]. For nutritional purposes, genetic improvement of already established oil crops has resulted in new genotypes with improved oil quality such as the low erucic acid oil cultivars of *Brassica* species [4] or new flax cultivars with low linolenic acid in the oil [5, 6].

The genus *Carthamus* from the Compositae family comprises 16 recognized species [7]. *C. tinctorius* is the only cultivated species of this genus, but the others are either wild or weeds. *Carthamus oxyacantha* as one of the wild species is widespread in Turkey, subtropical regions of western Iraq, Iran, northwest India, throughout Kazakhstan, Turkmenistan, and Uzbekistan [8]. This species is important because it is assumed to be the ancient male parent of cultivated safflower [9] and these two species have been successfully crossed with each other [10]. Nevertheless, it seems that *C. palestinus* Eig. species has the most likely affinity to cultivated safflower [11]. The *C. lanatus*, a winter growing annual plant is the other species with the second highest distribution in Iran after *C. oxyacantha* [12]. Crosses between *C. lanatus* L. ($n = 22$) and cultivated safflower ($n = 12$) have not

been successful at producing viable offspring, but with the use of embryo rescue and chromosome doubling techniques, *C. lanatus* could be a potential source of biotic stress resistance genes in safflower breeding programs [13].

The wild species of safflower may be crossed with the cultivated one in order to improve the economically important traits such as seed oil characteristics. There are some reports on the fatty acid composition of safflower [14–16]; however, the oil composition of wild species has not yet been fully determined. The objective of this study was to assay the oil content and fatty acid composition of safflower and the wild accessions of *C. oxyacantha* and *C. lanatus* collected from different regions of Iran and to investigate the variation for these traits among and within these species. This information can be important for improving oil content and fatty acid composition in safflower breeding programs.

Experimental Procedures

Plant Materials

The plant materials included 15 accessions of *C. oxyacantha*, four accessions of *C. lanatus* and six genotypes of *C. tinctorius*. The accessions originated from 11 provinces of Iran. In each accession of wild safflower, the heads of 30–50 plants were collected and their seeds were bulked into a single sample. The genotypes of the cultivated safflower were two landraces of Kouseh (Isfahan province) and Arak 2811 (Arak province), two breeding lines of C_{111} and C_{4110} and two varieties from Canada, Saffire and AC-Stirling.

Oil Extraction

Seeds of the genotypes were dried at 40 °C for 4 h, using a ventilated oven, up to a moisture content of about 5%, and were then ground with a blender. Ten grams of ground seeds were extracted for oil, using petroleum ether for 6 h in a Soxhlet system according to the AOCS method [17] and then oil content percentage was calculated for each sample.

Fatty Acid Profiling

The oil sample of each accession was converted to its fatty acid methyl esters (FAME) according to the AOCS method [17]. Samples of 350 mg oil were treated with 7 ml of 0.5 M (mol l^{-1}) sodium methylate in methanol and heated at its boiling temperature for 10 min and then 5 ml of Boron tri-fluoride in methanol was added and heated again for 2 min. After that, 6 ml of GC-grade hexane was mixed in and heated for 2 min. Finally, 50 ml saturated saline water was added and samples were vigorously shaken for 1 min. at room temperature. The upper phase was taken and used for gas chromatography. The methyl esters of the fatty acids (0.5 μl) were analyzed in a Chrompack CP 9001 series gas chromatograph (Chrompack, Middelburg, The Netherlands) equipped with a flame ionizing detector (FID) and a fused silica capillary column (CP-Sil 88, 50 m × 0.25 mm i.d.; film thickness = 0.2 μm). This process was operated at an oven temperature of 120 °C, which was then raised to 220 °C at a rate of 3.5 °C min^{-1} and then kept at 220 °C for 15 min. The injector and detector temperatures were 250 °C. The carrier gas was nitrogen at a flow rate of 4.93 ml min^{-1} and split ratio was 21.28 ml min^{-1}. Peak identification was performed by comparing the relative retention times with those of a commercial standard mixture of FAME. The fatty acid content of myristic (C14:0), palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0), oleic (C18:1), linoleic (C18:2), linolenic (C18:3), arachidic (C20:0) and behenic (C22:0) were determined using a computing integrator and showed as the percentage of the oil.

The data were subjected to analysis of variance (ANOVA) using GLM procedure of SAS statistical program [18]. The species were considered as treatments and the accessions within each species as replications. Treatment means were separated using the least significant difference (LSD) test ($P < 0.05$).

Results and Discussion

Oil Content

The results showed that there were significant differences among three species for seed oil content (Table 1) and the average of this for *C. tinctorius* (31.46 ± 1.87%) was significantly higher than that of the *C. oxyacantha* (25.34 ± 2.98%) and *C. lanatus* (17.45 ± 2.36%) (Table 2). The seed oil content among the accessions ranged from 29.20 to 34.00% in *C. tinctorius*, 20.04 to 30.80% in *C. oxyacantha* and 15.30 to 20.80% in *C. lanatus* (Table 3). In the accessions of cultivated safflower (*C. tinctorius*), the highest seed oil content (34.00%) was found in the Canadian cultivar AC-Stirling, and the lowest (29.20%) was obtained for the Kouseh landrace (Table 3). Among the *C. oxyacantha* accessions, the highest seed oil content was found in the Isfahan1 and the lowest was obtained from the Lorestan accession. The seed oil content of accession Kohgiluyeh in *C. lanatus* was considerably higher than the other three accessions (Table 3). The lower seed oil content in the wild species than the case in the cultivated safflower was not far from our expectations and

Table 1 Analysis of variance for oil content and main fatty acids in three species of safflower

Source of variation	*df*	Oil content	C16:0	C18:0	C18:1	C18:2	C18:3	U/T[a]
Between species	2	236.32**	2.57**	2.42**	20.22**	36.54*	0.079**	0.00094**
Within species	22	7.24	0.41	0.35	2.85	8.02	0.008	0.00014

* And ** significant at 0.05 and 0.01 levels of probability, respectively

[a] The ratio of unsaturated to total fatty acids

Table 2 Mean (±SD) of oil content and main fatty acids for three species of safflower

Species	Oil content (%)	C16:0	C18:0	C18:1	C18:2	C18:3	Total	U/T[a]
C. tinctorius	31.46 ± 1.87[a]*	6.48 ± 0.87[b]	2.30 ± 0.38[b]	14.17 ± 1.12[b]	73.87 ± 1.89[a]	0.37 ± 0.15[a]	96.82 ± 0.51	0.91 ± 0.01[a]
C. oxyacantha	25.34 ± 2.98[b]	7.28 ± 0.55[a]	3.16 ± 0.60[a]	17.08 ± 1.37[a]	70.61 ± 2.76[b]	0.19 ± 0.06[b]	98.33 ± 0.99	0.89 ± 0.01[b]
C. lanatus	17.45 ± 2.36[c]	7.90 ± 0.60[a]	3.62 ± 0.78[a]	17.35 ± 1.64[a]	68.68 ± 4.16[b]	0.18 ± 0.06[b]	97.73 ± 1.33	0.88 ± 0.01[b]

* In each column, means with the same superscript letter were not significantly different, using the LSD test ($P < 0.05$)

[a] The ratio of unsaturated to total fatty acids

Table 3 Oil content and fatty acid composition in different accessions of three safflower species

				Fatty acid composition in the oil (%)							
Species	Genotypes	Province	Oil content (%)	C16:0	C18:0	C18:1	C18:2	C18:3	Total	Others[a]	U/T[b]
C. tinctorius	C_{4110}	Isfahan	32.00	5.70	1.72	15.14	73.64	0.28	96.20	0.43	0.92
	C_{111}	Isfahan	30.20	5.48	2.18	13.95	76.12	0.21	97.73	0.49	0.92
	Kouseh	Isfahan	29.20	6.06	2.35	12.24	75.77	0.44	96.42	0.49	0.91
	Arak 2811	Arak	33.10	7.59	2.50	14.09	72.78	0.23	96.96	0.45	0.90
	Saffire	Canada	30.26	6.77	2.19	14.17	73.86	0.56	96.99	0.44	0.91
	AC-Stirling	Canada	34.00	7.30	2.86	15.43	71.05	0.52	96.64	0.52	0.90
C. oxyacantha	Isfahan1	Isfahan	30.80	7.73	3.01	18.79	66.65	0.13	96.18	0.45	0.89
	Isfahan2	Isfahan	29.80	6.09	2.74	14.11	75.43	0.12	98.37	0.43	0.91
	Tehran	Tehran	28.00	7.30	2.80	16.80	70.20	0.14	97.10	0.45	0.90
	Chahar-Mahal 1	Chahar-Mahal	22.00	7.30	2.80	18.00	71.00	0.16	99.10	0.42	0.90
	Chahar-Mahal 2	Chahar-Mahal	28.00	7.57	3.60	14.72	73.79	0.18	98.47	0.52	0.90
	Kohgiluyeh	Kohgiluyeh	26.00	7.33	3.03	16.78	71.57	0.16	98.71	0.43	0.90
	Fars1	Fars	26.00	7.89	3.04	16.00	71.47	0.30	98.40	0.45	0.89
	Fars2	Fars	25.00	7.40	2.90	17.00	70.80	0.25	98.10	0.47	0.90
	Hamedan	Hamedan	25.90	7.60	3.50	17.20	69.60	0.21	97.90	0.48	0.89
	Lorestan	Lorestan	20.04	7.26	3.68	18.12	68.58	0.18	97.64	0.44	0.89
	Ilam	Ilam	21.40	7.28	2.76	17.93	70.50	0.13	97.68	0.50	0.89
	Kermanshah	Kermanshah	24.70	6.88	3.62	16.89	71.47	0.33	98.86	0.53	0.89
	Kordestan1	Kordestan	24.70	6.73	2.62	17.35	71.62	0.17	98.32	0.46	0.91
	Kordestan2	Kordestan	24.00	6.60	2.50	17.30	72.50	0.18	98.90	0.45	0.91
	Arak	Arak	23.80	8.33	4.87	19.28	63.90	0.18	96.38	0.43	0.86
C. lanatus	Lorestan	Lorestan	15.30	8.78	4.79	19.77	62.47	0.15	97.81	0.49	0.86
	Kohgiluyeh	Kohgiluyeh	20.80	7.58	3.34	16.12	70.18	0.26	97.22	0.43	0.89
	Khorasan	Khorasan	17.20	7.44	3.14	16.70	71.08	0.12	98.36	0.47	0.89
	Kordestan	Kordestan	16.50	7.80	3.20	16.80	71.00	0.19	98.80	0.57	0.89

[a] Sum of myristic (C14:0), palmitoleic (C16:1), arachidic (C20:0) and behenic (C22:0) acids

[b] The ratio of unsaturated to total fatty acids

in agreement with those previously reported [14]. The cultivated safflower has been domesticated for a very long time and grown for oil production, thus it has been subjected to selection for higher seed oil content. Among the cultivated safflower genotypes, the AC-Stirling, a cultivar from Canada which has been genetically improved [19] had the highest seed oil content; however, the landrace Kouseh had the lowest mean of this trait.

There was also considerable variation for oil content in the accessions of wild species. Some accessions in *C. oxyacantha* had higher seed oil content and were in the range of seed oil content observed for cultivated safflower genotypes. These results show the genetic potential of this species to be used directly for oil production or to be used as a source of desirable genes in breeding programs of safflower. The existing variation for seed oil content among the accessions of *C. oxyacantha* in this research furthermore implies that the collection of more accessions may enhance the chance of finding individual plants or populations with higher seed oil content. The self-incompatibility in this species [9] resulting in heterogeneous populations and high genetic variation will provides the possibility of selection and genetic improvement of the traits [20]. The seed oil content in the accessions of *C. lanatus* was low indicating that this species may have lower genetic potential for seed oil content. Nevertheless, this species may be a good source of genes for improving the other traits of safflower.

Fatty Acid Profile

The fatty acid compositions for two wild species of *C. oxyacantha* and *C. lanatus* were almost the same and their differences were not significant; however, cultivated safflower had slightly lower palmitic, stearic, oleic acids, but a higher content of linoleic, linolenic acids as well as a higher ratio of unsaturated fatty acids (Table 2). These five fatty acids composed 96.82 ± 0.51, 98.33 ± 0.99 and $97.73 \pm 1.33\%$ of the seed oil in the species of *C. tinctorius*, *C. oxyacantha* and *C. lanatus*, respectively (Table 2). The other fatty acids including myristic (C14:0), palmitoleic (C16:1), arachidic (C20:0) and behenic (C22:0) were in minute quantities and in total ranged from 0.43 to 0.57% (Table 3).

Averaged over the accessions (Table 2), the main fatty acids in seed oil of three species were the unsaturated fatty acids of linoleic (68.68–73.87%) and oleic (14.17–17.35%), and the saturated fatty acids of palmitic (6.48–7.90%) and stearic (2.30–3.62%). The highly unsaturated fatty acid of linolenic acid (C18:3) content was very low (less than 0.37%) and the proportions of unsaturated to total fatty acids (U/T) in the accessions varied from 0.88 to 0.91 (Table 2).

Within the species, there was also variation of the fatty acids content in seed oil of the accessions. Palmitic acid (C16:0) content ranged from 5.48 to 7.59% in *C. tinctorius*, 6.09 to 8.33% in *C. oxyacantha* and 7.44 to 8.78% in *C. lanatus*, however, the corresponding ranges for stearic acid (C18:0) in these species were 1.72 to 2.89, 2.50 to 4.87 and 3.14 to 4.79%. Oleic acid (C18:1) content varied from 12.40 to 15.43, 14.11 to 19.28 and 16.17 to 19.77% in the cultivated safflower and the wild species of *C. oxyacantha* and *C. lanatus*, respectively. The variations for linoleic acid (C18:2) content in the three species were from 71.05 to 76.12, 63.90 to 75.43 and 62.47 to 71.08%, respectively (Table 3). Linolenic acid (C18:3) content in seed oil of all accessions was very low and ranged from 0.21 to 0.56, 0.13 to 0.33 and 0.12 to 0.26% in the species of *C. tinctorius*, *C. oxyacantha* and *C. lanatus*, respectively (Table 3).

The fatty acid composition of the cultivated safflower in our study was in agreement with those reported by others [15, 16]. The fatty acid profile of seed oil in the cultivated and two wild species of *Carthamus* were almost the same, suggesting the suitability of the seed oil of wild species for human consumption and industrial purposes. The seed oil of all three species was very rich in the unsaturated fatty acid of linoleic which makes the oil nutritionally valuable and a preventative of cardiovascular disorders such as coronary heart diseases, atherosclerosis and high blood pressure [21]; however, the seed oil of two wild species had a slightly higher oleic acid content than the cultivated safflower.

The high similarity in fatty acid composition of the seed oil from cultivated and wild accessions of safflower also indicates that hybridization between these species for genetic improvement of agronomic traits in cultivated safflower may have no adverse effects on its oil quality. *C. oxyacantha* has the same chromosome number as the cultivated safflower and hybridization between these two species has been successful in reciprocal crosses [10].

Our findings indicate that although wild safflower species had a lower average oil content than the cultivated type, some accessions of *C. oxyacantha* had a seed oil content comparable to cultivated genotypes. Additionally, the fatty acid profiles of oil in all three species were alike and possibly suitable for human consumption and industrial purposes. Furthermore, *C. oxyacantha* is easily crossed with cultivated safflower and can be used as a new source of biotic and abiotic stress resistance genes in breeding programs of safflower.

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