Turkish Journal of Agriculture and Forestry

Volume 42 | Number 1 Article 6

1-1-2018

Seed fatty acid compositions and chemotaxonomy of wild safflower(Carthamus L., Asteraceae) species in Turkey

YUSUF ARSLAN

BURCU TARIKAHYA HACIOĞLU

TÜBİTAK

Turkish Journal of Agriculture and Forestry

http://journals.tubitak.gov.tr/agriculture/

Research Article

Turk J Agric For
(2018) 42: 45-54

doi:10.3906/tar-1708-68

Seed fatty acid compositions and chemotaxonomy of wild safflower (*Carthamus* L., Asteraceae) species in Turkey

Yusuf ARSLAN*, Burcu TARIKAHYA HACIOĞLU
General Directorate of Agricultural Research and Policies, Republic of Turkey Ministry of Food, Agriculture and Livestock, Ankara, Turkey

Received: 21.08.2017 • **Accepted/Published Online:** 02.01.2018 • **Final Version:** 12.02.2018

Abstract: Safflower is an important oilseed crop for the dry regions of the world. In the present study, we aimed to find out the seed oil content and composition of the wild relatives of safflower. For this purpose, 71 populations from 5 naturally growing *Carthamus* species were collected from Turkey: *Carthamus dentatus* (30 accessions), *Carthamus lanatus* (30 accessions), *Carthamus persicus* (2 accessions), *Carthamus glaucus* (6 accessions), and *Carthamus tenuis* (3 accessions). Our research covered all naturally growing safflower species in the flora of Turkey. The seeds belonging to 71 accessions were germinated in cabinets and transferred to vials in order to obtain seedlings and then sown in the field manually. After harvesting, the seed oil content (%) and fatty acid compositions were determined. The seed oil content ranged between 10.50% and 20.40% in all accessions. Thirteen fatty acids, mainly linoleic (58.8% to 82.6%), oleic (7.3% to 22.8%), and palmitic acids (4.8% to 8.8%) were determined. The fatty acid compositions were more or less similar in all investigated species; however, the seed oil contents were different. For the evaluation of fatty acid properties as a taxonomic character, cluster analysis was performed. The dendrogram based on the fatty acid ratios discriminated section *Carthamus* and section *Atractylis* of the genus *Carthamus*; however, the resulting tree was not compatible with the previously constructed phylogeny trees in detail.

Key words: *Carthamus*, chemotaxonomy, fatty acid composition, seed oil content

1. Introduction

Safflower belongs to the genus *Carthamus* (Asteraceae), which includes ca. 25 species worldwide. In the flora of Turkey, there are five wild relative species of this genus. The sectional distributions of Turkish species are as follows: *C. dentatus* (Forssk.) Vahl., *C. lanatus* L., *C. glaucus* M.Bieb., and *C. tenuis* (Boiss. & Blanche) Bornm. in the section *Atractylis* and *C. persicus* Desf. ex Willd. in the section *Carthamus* (Vilatersana et al., 2000, 2005; Bowles et al., 2010). The only cultivated species of the genus is *Carthamus tinctorius* (safflower), which is cultivated in over 20 countries (Esendal, 2001; Carapetian, 2005; Singh and Nimbkar, 2006; Peng et al., 2008; Bagmohammadi et al., 2012; Agrawal, 2013). Safflower is an edible and biodiesel oilseed crop (Işigigür et al., 1995; Patrascoiu et al., 2013) that is recommended to plant in dry regions of the world. The oil obtained from the seeds of safflower is healthy, due to its high unsaturated oil content, while the remaining meal is rich in proteins (Pavlov and Todorov, 1996; Corleto et al., 1997). Therefore, safflower is considered one of the best oilseed crops for human nutrition, because its oil contains very high levels of polyunsaturated (linoleic acid, 70% to 75%) or monounsaturated (oleic acid, 70% to 75%) fatty acids (Singh and Nimbkar, 2006). Safflower oil contains approximately 6% to 8% palmitic acid (C16:0), 2% to 3% stearic acid (C18:0), 16% to 20% oleic acid (C18:1), and 71% to 75% linoleic acid (C18:2) (Velasco and Fernandez-Martinez, 2001). The flowers are used medicinally for preventing and treating certain diseases (Li and Mundel, 1996; Hofbauer and Pelikan, 1997; Uher, 1997; Nimbkar, 2002; Jiang et al., 2005; Peng et al., 2008). Safflower seeds contain 27%–40% oil with mainly stearic, palmitic, oleic and linoleic acids (Weiss, 1983; Lata and Prakash, 1984; Knowles, 1989) and 15.6% to 21.5% protein (Ahmadzadeh et al., 2014). Cultivated safflowers may contain either very high levels of linoleic (87%–89%) or very high levels of oleic acid (>85%) (Futehally and Knowles, 1981; Dajue, 1993; Fernandez-Martinez et al., 1993).

The safflower cultivation area and production levels are rather low when compared with those of other oil seed crops because of its lower seed yield and lower tolerance to environmental stress factors (Li and Mundel, 1996). Scholars suggest that seed yield and tolerance may increase with breeding programs, if there is sufficient

* Correspondence: yarslantarm@gmail.com

genetic variation for the desired traits in the germplasm. Wild safflower species are important source of genes to enrich the cultivated safflower gene pool with desirable traits. Wild safflower species are highly crossable with *C. tinctorius* L. and it is possible to improve new varieties that are tolerant to biotic and abiotic stress factors (Peng et al., 2008; Sabzalian et al., 2009; Majidi et al., 2011; Mayerhofer et al., 2011). Although safflowers are fully self-pollinated plants, environmental factors may affect the self-pollination rate (Kadam and Patankar, 1942; Claassen, 1950; Boch, 1961; Eckert, 1962; Butler et al., 1966; Levin and Butler, 1966; Rubis et al., 1966; Levin et al., 1967; Knowles, 1969; Pandey and Kumari, 2008; Baloch et al., 2010). Previous investigations reveal that *Carthamus tinctorius* can artificially and naturally hybridize with several wild relatives (Kadam and Patankar, 1942; Claassen, 1950; Ashri and Knowles, 1960; Ashri and Efron, 1964; Ashri and Rudich, 1965; Imrie and Knowles, 1970; Khidir and Knowles, 1970, Estilai and Knowles, 1976; Estilai, 1977; Heaton and Klisiewicz, 1981; McPherson et al., 2004; Majidi et al., 2011).

The usage of chemical constituents as taxonomical markers is an ongoing question. There are many chemosystematic investigations on Asteraceae, mostly using flavonoids as taxonomic markers (Payne, 1976; Alvarenga et al., 2001; Emerenciano et al., 2001; Zidorn and Stuppner, 2001a, 2001b; Valant-Vetschera et al., 2001, Zidorn et al., 2002; Zidorn, 2006, 2008; Sareedenchai and Zidorn, 2010; Enke et al., 2012). Chemotaxonomical studies on fatty acid compositions of leaves (Mongrand et al., 2005) and on seed oil (Bagci et al., 2003) of various taxa are present as well.

In this investigation we aimed to find out the seed oil features of wild *Carthamus* species for improving the quality of cultivated safflower oil in future breeding programs. Another purpose was to discuss whether the seed oil compositions and amounts could be used as a taxonomical character by comparing with the molecular and morphological data in different *Carthamus* species from Turkey.

2. Materials and methods

2.1. Sampling and field experiments

Our research included all wild *Carthamus* species growing in Turkey. Seeds collected from 71 different locations in Turkey were the materials in our investigation: *C. dentatus* accessions were collected from 30 localities, *C. lanatus* from 30 localities, *C. glaucus* from 6 localities, *C. persicus* from 2 localities, and *C. tenuis* from 3 localities (Table 1). The abbreviations used for the wild safflower species were as follows: *C. dentatus* (CD), *C. lanatus* (CL), *C. glaucus* (CG), and *C. persicus* (CP). The species were identified by the second author. The identification key given in Tarıkahya Hacıoğlu et al. (2014) was used for this purpose. The herbarium material was deposited in IZ (Aegean Agricultural Research Institute Herbarium).

Field experiments were conducted in İkizce, Ankara (Central Research Institute for Field Crops research fields, 39°57′20.39″N, 32°48′53.00″E). All wild *Carthamus* species and the five cultivars were sown in March 2014, on a well-prepared seedbed, using an augmented test design. The plots were of eleven rows; each was 6 m in length, with 30 cm between plants within a row and 60 cm between rows. All plots were treated identically and weeds were cleared manually. The total precipitation was 444.2 mm during the growth season of 2013–2014, and the lowest and highest temperatures were –8.4 °C in February and 36.9 °C in August. The test field used for the research consisted of well-drained, deep or medium deep, slightly rocky or rockless, and loamy-clayey soils located on plains or near plain inclinations. The soil pH was 8.06, the salt ratio was 0.041%, the organic matter was 1.57%, and the lime ratio was 2.65%.

2.2. Extraction and gas chromatography

The oil content and composition were determined in the laboratories of the Central Research Institute for Field Crops of the Ministry of Food, Agriculture and Livestock (MoFAL). For extraction, derivatization and GC analysis, we followed the method given in Gölükcü et al. (2016) with some modifications. The seeds were thoroughly ground and then weighed (5 g) and the oil was extracted with petroleum ether in a Soxhlet extractor (Soxtherm 2000 automatic extractor) for oil measurements. Recovered crude oils were placed in a drying oven for petroleum ether evaporation at 35–40 °C for 3 h. After cooling in a desiccator, crude oils of all accessions were weighed on precision scales. The seed oil content was reported as mass percent (crude oil weight (g)/ground seeds weight × 100). For the determination of fatty acid compositions, oil (0.1 g) was dissolved in 10 mL of n-hexane and 0.5 mL of methanolic KOH solution (2 N) was added; the mixture was incubated for 30 min; thus fatty acids were esterified as methyl esters and placed in a Shimadzu AOC-20i automatic injector (split ratio 1:100) and then analyzed on a Shimadzu GC-2010 (Japan) GC system equipped with Teknokroma capillary column (100 m × 0.25 mm and 0.2 µm) and FID detector. Helium was used as carrier gas at a flow rate of 0.94 mL/min. Injector and detector temperature were 250 °C. Column temperature was programmed as follows: 140 °C for 5 min, increase 4 °C/min, and 240 °C for 20 min. FAMEs were identified by comparison of their retention times with those of reference standards (Restek FAME Mix 37).

2.3. Statistical analysis

Standard one-way analyses of variance were performed for each trait, using the JMP statistical software package

Table 1. Localities and altitudes of analyzed *Carthamus* accessions in Turkey.

Localities		Altitude (m)
C. dentatus (2n = 20)		
CD1	Afyon	998
CD2	Antalya, Serik	14
CD3	Antalya, Kemer	502
CD4	Muğla, Dalaman	122
CD5	İzmir, Kuşadası	253
CD6	İzmir, Karşıyaka	46
CD7	Manisa	46
CD8	Manisa, Salihli	86
CD9	Edirne	94
CD10	Bursa, Karacabey	24
CD11	Çanakkale, Biga	48
CD12	Çanakkale, Gelibolu	17
CD13	Afyon	1146
CD14	Antalya, Demre	10
CD15	Uşak	863
CD16	Kütahya, Altıntaş	1145
CD17	Kırşehir	1103
CD18	Kırıkkale	689
CD19	Sivas, Koyulhisar	674
CD20	Nevşehir, Ürgüp	1091
CD21	Kırıkkale	879
CD22	Karaman, Ermenek	1139
CD23	Afyon, Sandıklı	1013
CD24	Ankara, Çubuk	1170
CD25	Muğla, Fethiye	133
CD26	Antalya, Kalkan	107
CD27	Ankara, Kalecik	948
CD28	Ankara, Kalecik	948
CD29	İstanbul, Silivri	36
CD30	Nevşehir, Ürgüp	1091
C. glaucus (2n = 20)		
CG1	Antalya, Kemer	328
CG2	Karabük, Eskipazar	644
CG3	Sinop	6
CG4	İçel, Anamur	12
CG5	Adana	96
CG6	Sinop	6

Localities		Altitude (m)
C. lanatus (2n = 22)		
CL1	Antalya, Manavgat	45
CL2	Antalya, Manavgat	50
CL3	Antalya, Demre	451
CL4	İzmir, Kuşadası	9
CL5	Karabük, Eskipazar	644
CL6	Kastamonu, Taşköprü	386
CL7	Sinop	6
CL8	Amasya, Suluova	485
CL9	Bursa	422
CL10	Ankara, Akyurt	1060
CL11	Ankara, Kalecik	934
CL12	Ankara, Temelli	790
CL13	Kırıkkale	794
CL14	Kırıkkale	892
CL15	Kırıkkale	892
CL16	Yozgat, Sorgun	1090
CL17	Kayseri, İncesu	1109
CL18	Tekirdağ, Malkara	125
CL19	Ankara, Şereflikoçhisar	1599
CL20	Ankara, Sincan	856
CL21	Ankara, Gölbaşı	1065
CL22	Niğde	1155
CL23	Eskişehir	906
CL24	Nevşehir, Topaklı	1220
CL25	Ankara, Kalecik	715
CL26	Ankara, Anayurt	795
CL27	Ankara, Sincan	822
CL28	Ankara, Sincan	856
CL29	Ankara, Kalecik	1060
CL30	İstanbul, Silivri	36
C. persicus (2n = 24)		
CP1	Ankara, Gölbaşı	1074
CP2	Şanlıurfa	454
C. tenuis (2n = 20)		
CT1	Antalya, Kalkan	103
CT2	Antalya, Kalkan	144
CT3	İçel, Erdemli	22

(SAS Institute, 2002). Significant differences ($P < 0.05$) were detected between localities for all studied oil content and composition traits. Standard deviations (SDs) were calculated for each population for different studied characteristics. All analyses were carried out on the corrected values. Correlation among studied traits was calculated using the Pearson correlation procedure implemented in the JMP software. Principal component analysis (PCA) was performed with the palmitic, stearic, oleic, and linoleic acid values of each accession by using Microsoft Excel 2010/XLStat.Version2014.5.03. For chemotaxonomic evaluation of the species, the mean value of each fatty acid was calculated by using JMP 5.0.1a. Standardized trait mean values were used to perform the cluster analyses (CA) using NTSYS-pc (Rohlf, 2004). CA was conducted based on Euclidean distances and applying the unweighted pair group method with arithmetic mean (UPGMA).

3. Results

Four main fatty acids (stearic, palmitic, oleic, and linoleic acids) were identified in the wild relative species of safflower. All investigated safflower accessions were linoleic type. Docosahexaenoic acid (C22:6), pentadecanoic acid (C15:0), arachidic acid (C20:0), linolenic acid (C18:3), nervonic acid (C24:1), lignoceric acid (C24:0), behenic acid (C22:0), myristic acid (C14:0), and eicosenoic acid (C20:1) were less than 1% of the total fatty acid content.

In all five species, variation was low in oil content and linoleic acid among different accessions. In addition, the average oil contents for *C. lanatus*, *C. dentatus*, *C. persicus*, *C. glaucus*, and *C. tenuis* ranged between 10.5% and 20.4%. The linoleic, oleic, palmitic, and stearic acid amounts were 58.8%–82.6%, 7.3%–22.8%, 4.8%–8.8%, and 1.8%–4.9%, respectively (Table 2). In our research, five saturated (palmitic acid, stearic acid, arachidic acid, behenic acid, and lignoceric acid) and three unsaturated

Table 2. Oil content and composition for *Carthamus* species grown under field conditions.

		Oil content (%)	Palmitic acid (C16:0)	Stearic acid (C18:0)	Oleic acid (C18:1)	Linoleic acid (C18:2)
C. lanatus (30 accessions)	Min.	10.50	5.43	2.00	9.81	63.43
	Max.	20.00	8.79	4.88	22.85	80.07
	Mean	14.72	6.60	3.02	13.41	74.61
	SD	2.17	0.64	0.79	2.66	3.46
	CV	14.49	9.55	25.70	19.50	4.56
C. dentatus (30 accessions)	Min.	11.90	5.42	1.84	7.35	58.79
	Max.	20.30	8.02	4.20	17.56	82.57
	Mean	16.05	6.75	3.14	13.46	73.54
	SD	2.31	0.63	0.49	2.39	4.02
	CV	14.16	9.19	15.23	17.46	5.38
C. persicus (2 accessions)	Min.	13.60	4.85	2.43	13.51	67.95
	Max.	18.00	7.40	3.07	20.37	74.72
	Mean	15.20	6.32	2.80	15.88	71.97
	SD	2.43	1.32	0.33	3.89	3.56
	CV	13.07	17.03	9.64	20.02	4.04
C. glaucus (6 accessions)	Min.	17.30	6.19	2.62	7.44	69.70
	Max.	20.40	8.32	4.50	16.69	79.94
	Mean	18.88	7.03	3.49	12.23	75.02
	SD	1.28	0.84	0.62	3.12	3.41
	CV	6.20	10.88	16.10	23.32	4.15
C. tenuis (3 accessions)	Min.	13.40	7.30	2.90	11.89	71.44
	Max.	17.90	8.10	4.80	13.58	75.01
	Mean	15.50	7.76	3.57	12.69	73.52
	SD	2.26	0.41	1.07	0.85	1.86
	CV	11.93	4.35	24.39	5.46	2.06

(oleic acid, linoleic acid, and docosahexaenoic acid) fatty acids were identified. The mean percentages of saturated fatty acids in *C. lanatus*, *C. dentatus*, *C. persicus*, *C. glaucus*, and *C. tenuis* were 10.1%, 10.5%, 9.8%, 11.2%, and 12.0% and the percentages of unsaturated fatty acids were 89.0%, 87.8%, 88.9%, 88.0%, and 87.2% of the total fatty acids, respectively. In all five species, linoleic acid had negative correlations with oleic and stearic acids; stearic acid had a positive correlation with oleic acid (Figure 1).

According to the biplot diagram, 86.1% of the total variation was explained (Figure 1). The first four eigenvalues were 2.28, 1.16, 0.44, and 0.11. Among all accessions, CL4 consisted the highest stearic and palmitic acid content; CL2, CD14, and CP2 consisted the highest oleic acid content; and CD8, CL18, and CG1 consisted the highest linoleic acid content (Figure 2). The fatty acid contents of the rest of the accessions were close to mean values. For taxonomic evaluation, mean values for all determined seed fatty acids were used. The dendrogram (Figure 3) created by UPGMA grouped *C. lanatus* and *C. dentatus* as the closest relatives. These two combined with *C. glaucus*, *C. tenuis*, *C. persicus*, and *C. tinctorius*.

4. Discussion

The cultivated safflower seed oil content are 11%–47% (Weiss, 1983; Knowles, 1989; De Haro et al., 1991; Dajue et al., 1993; Fernandez-Martinez et al., 1993; Koutroubas et al., 2009; Rudra Naik et al., 2010; Yeilaghi et al., 2012; Agrawal et al., 2013; Fernández-Cuesta et al., 2014). According to our results, oil content in wild species of safflower was lower (10.5%–20.4%) than that of cultivated safflowers. The thick seed crust in the wild species could cause the low oil content values. Our research is not the first on the seed oils of the wild relatives of safflower: Knowles and Ashri (1958) reported the seed oil content in *C. lanatus* and *C. baeticus* as 16%–20% and 16%–21%, respectively; Carapetian and Zarei (2005) reported the seed oil content in *C. oxyacantha*, *C. dentatus*, and *C. turkestanicus* as 22.4%, 22.2%, and 24.23%, respectively. Sabzalian et al. (2008) analyzed the seed oil and fatty acid compositions of six safflower genotypes and 19 accessions of two wild species of safflower. The oil content was 29.2%–34.0%, 20.0%–30.8%, and 15.3%–20.8% in *C. tinctorius*, *C. oxyacantha*, and *C. lanatus*, respectively.

Carapetian and Zarei (2005) analyzed three wild safflower species: *C. oxyacantha*, *C. dentatus*, and *C. turkestanicus*. The oleic and linoleic acid ratios were as

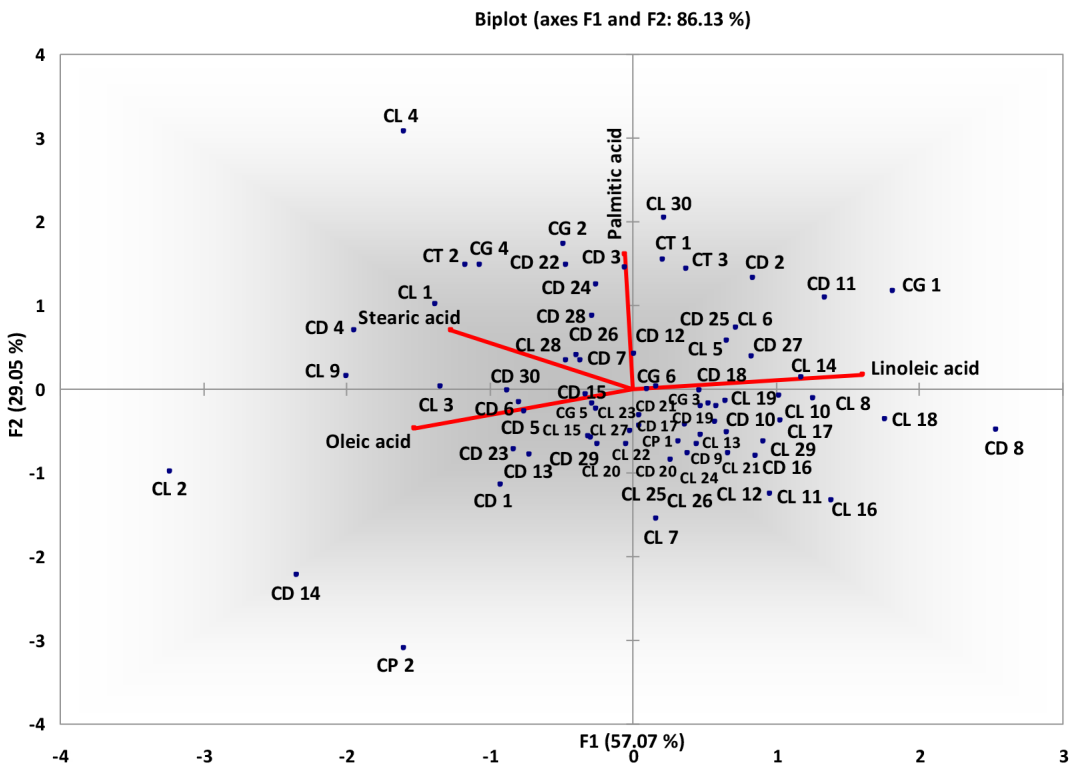

Figure 1. Biplot diagram of the main fatty acid components of the *Carthamus* accessions.

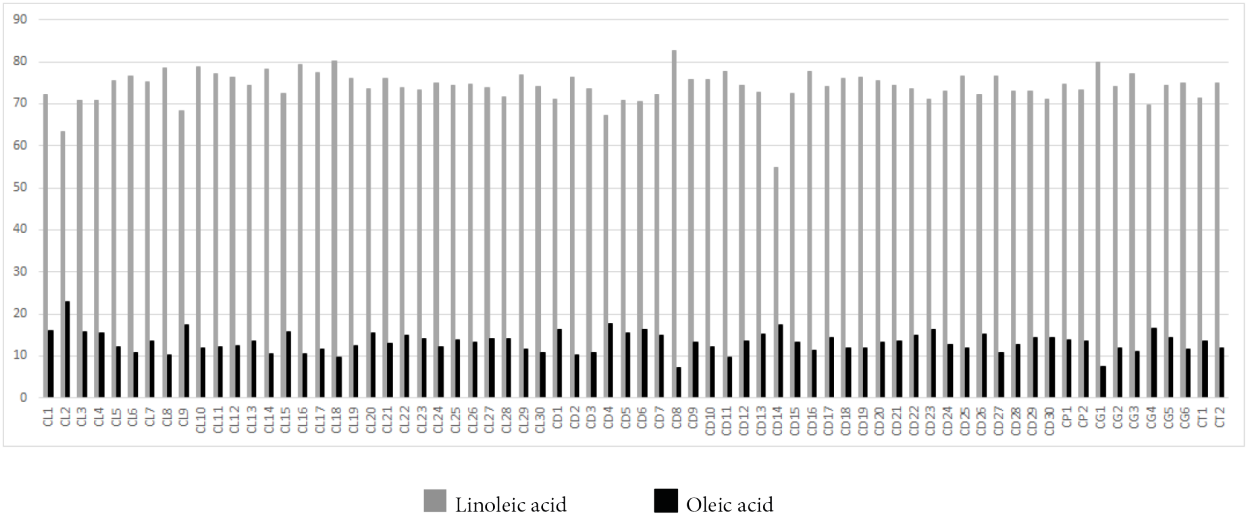

Figure 2. Bar diagram of the main two fatty acid (linoleic and oleic acids) components of the *Carthamus* accessions.

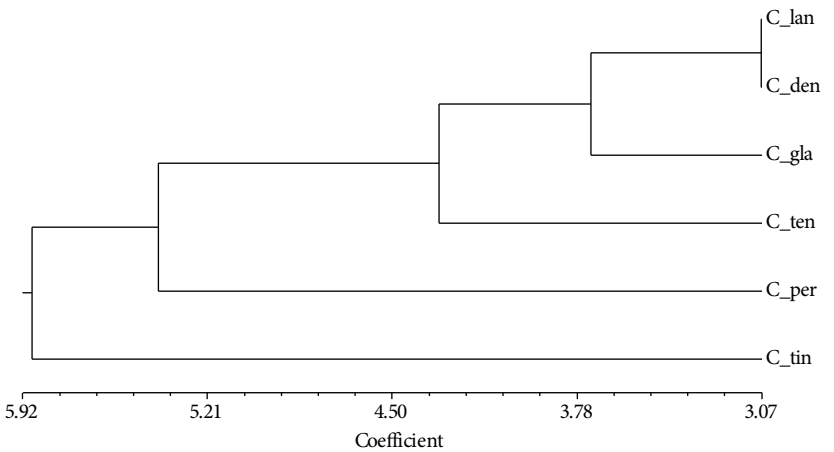

Figure 3. CA dendrogram created with UPGMA with mean values of fatty acids of wild *Carthamus* species.

follows: *C. oxyacantha* 15.7% and 78.4%; *C. dentatus* 21.1% and 72.4%; *C. turkestanicus* 11.2% and 83.4%. Murthy and Anjani (2007) investigated the seed oil compositions of seven *Carthamus* species (*C. oxyacantha*, *C. tinctorius*, *C. palaestinus*, *C. glaucus*, *C. creticus*, *C. lanatus*, and *C. turkesthanicus*) collected from Pakistan. In their investigation the fatty acid ratios of oleic acid and linoleic acid were 11.3%–23.4% and 61.4%–82.1%, respectively. The oleic acid in seed oil of *C. tinctorius*, *C. oxyacantha*, and *C. lanatus* genotypes was 12.2%–15.4%, 14.1%–19.3%, and 16.7%–19.8% and linoleic acid ranged between 71.1%–76.1%, 63.9%–75.4%, and 62.5%–71.1%, respectively (Sabzalian et al., 2008). According to Hamdan and Fernández-Martínez (2008), as reported from Knowles (1989), Knowles and Hill (1964), and Fernández-Martínez et al. (1993), the amount of linoleic acid generally accounts for 70%–80%; however, high oleic varieties (the oleic acid amount ranging from 35% to 91%) have also been described. *Carthamus lanatus* and *C. dentatus* were investigated in the mentioned previous works and revealed results similar to our findings. Our research is the first to investigate all wild relatives of safflower naturally growing in Turkey.

The results of our study and previous works show that wild safflower species do not contain high oleic acid. All wild *Carthamus* accessions investigated in our study were linoleic type; therefore, CD8, CG1, and CL18 accessions

could be potential gene sources for linoleic type safflower breeding purposes in order to gain some other desired properties. CD8, CG1, and CL18 accessions belonging to three different species (*C. dentatus*, *C. lanatus*, *C. glaucus*) all grow at low altitudes (86–328 m).

Nazari et al. (2017) planted 27 genotypes belonging to *C. tinctorius*, *C. palaestinus*, *C. oxyacanthus*, *C. lanatus*, and *C. glaucus* under normal and drought-stress conditions for 2 years. According to the results of this research, similar and stable responses for fatty acid composition were gathered in all investigated species. Therefore, the similarity in fatty acid profiles and almost the same pattern of changes under drought stress showed that the wild species, especially the crossable ones, are good candidates to be used in breeding of cultivated safflower.

The CA dendrogram (Figure 3) was created by UPGMA for the infrageneric taxonomic treatment of *Carthamus* species with mean fatty acid ratios. According to the dendrogram, *C. lanatus* and *C. dentatus* grouped as the closest relatives, which had the biggest achenes according to Tarıkahya Hacıoğlu et al. (2012). These two species combined with *C. glaucus*, *C. tenuis*, *C. persicus*, and *C. tinctorius* in the dendrogram. The recent phylogeny inference based on the nuclear polymorphism of the internal transcribed spacer (ITS) region was performed by Mihoub et al. (2017), but only *C. lanatus* and *C. tinctorius* were included in the phylogram. Our previous work consisted of all five *Carthamus* species that naturally grow in Turkey (Tarıkahya Hacıoğlu et al., 2014). According to the phylogeny tree constructed with ITS data (Tarıkahya Hacıoğlu et al., 2014), *Carthamus tinctorius* was the closest relative of *C. persicus* as in our recent results. The only conflict with the phylogeny tree was the position of *C. tenuis*, which formed a cluster with *C. dentatus* in the ITS phylogeny tree. *C. tenuis* grouped with *C. glaucus*, *C. dentatus*, and *C. lanatus* in the dendrogram based on fatty acid compositions. Our dendrogram grouped the species of the genus *Carthamus* under the section *Atractylis* and the species of the section *Carthamus* linked to this group as former classifications of *Carthamus* species (Vilatersana et al., 2000, 2005; Bowles et al., 2010).

Due to the low oil content of wild safflower accessions, crossing with wild relatives for an increase in oil content in breeding programs is an unlikely scenario. However, oil content and fatty acid composition are not the only breeding criteria for seed oil plants; drought tolerance, insect resistance, and many other reasons could be the purpose of the breeding. Wild relatives of the safflower in Turkey are all linoleic type. Three accessions, namely CD8, CG1, and CL18, could be potential gene sources for linoleic type safflower breeding purposes. For the taxonomic treatment, the fatty acid composition of investigated *Carthamus* species gave a clue for sectional classification, while it was not compatible with the ITS phylogeny tree in detail.

Acknowledgments

The General Directorate of Agricultural Research and Policies, Republic of Turkey, MoFAL financed our research. We are grateful to *İlhan* Subaşı and Hasan Keyvanoğlu for their valuable contributions to the research.

References

Agrawal R, Tsujimoto H, Tandon R, Rao SR, Raina SN (2013). Species-genomic relationships among the tribasic diploid and polyploid *Carthamus* taxa based on physical mapping of active and inactive 18S–5.8 S–26S and 5S ribosomal RNA gene families, and the two tandemly repeated DNA sequences. Gene 521: 136-144.

Ahmadzadeh S, Kadivar M, Saeidi G (2014). Investigation of oil properties and seed composition in some safflower lines and cultivars. J Food Biochem 38: 527-532.

Alvarenga SAV, Ferreira MJP, Emerenciano VP, Cabrol-Bass D (2001). Chemosystematic studies of natural compounds isolated from Asteraceae: characterization of tribes by principal component analysis. Chemometr Intell Lab 56: 27-37.

Ashri A, Efron Y (1964). Inheritance studies with fertile interspecific hybrids of three *Carthamus* L. species. Crop Sci 4: 510-514.

Ashri A, Knowles PF (1960). Cytogenetics of safflower (*Carthamus* L.) species and their hybrids. Agron J 52: 11-17.

Ashri A, Rudich J (1965). Unequal reciprocal natural hybridization rates between two *Carthamus* L. species. Crop Sci 5: 190-191.

Bagci E, Bruehl L, Aitzetmuller K, Altan Y (2003). A chemotaxonomic approach to the fatty acid and tocochromanol content of *Cannabis sativa* L. (Cannabaceae). Turk J Bot 27: 141-147.

Bagmohammadi H, Pahlevani M, Ahmadikhah A, Razavi SE (2012). Genetic variation of safflower (*Carthamus tinctorius* L.) and related species revealed by ISSR analysis. Plant Breeding and Seed Science 66: 139-150.

Baloch FS, Kurt C, Arıoğlu H, Özkan H (2010). Assaying of diversity among soybean (*Glycin max* (L.) Merr.) and peanut (*Arachis hypogaea* L.) genotypes at DNA level. Turk J Agric For 34: 285-301.

Boch R (1961). Honey bee activity on safflower (*Carthamus tinctorius* L.). Can J Plant Sci 41: 559-562.

Bowles VG, Mayerhofer R, Davis C, Good AG, Hall JC (2010). A phylogenetic investigation of *Carthamus* combining sequence and microsatellite data. Plant Syst Evol 287: 85-97.

Butler GD Jr, Werner EG, Leven MD (1966). Native bees associated with safflower in Southern Central Arizona. J Kansas Entomol Soc 39: 434-436.

Carapetian J, Zarei G (2005). Variation in protein, oil and fatty acid contents in three wild species of safflower (*Carthamus* L.) from West Azerbaijan, Iran. International Journal of Botany 1: 133-137.

Claassen CE (1950). Natural and controlled crossing in safflower (*Carthamus tinctorius* L.). Argon J 42: 381-384.

Corleto A, Alba E, Polignano GB, Vonghio G (1997). Safflower: a multipurpose species with nexploited potential and world adaptability. Proceedings of the IVth International Safflower Conference, Bari, Italy: pp 23-31.

Dajue L (1993). Progress of safflower (*Carthamus tinctorius* L.) research and production in China. In: Third International Safflower Conference, Beijing, China: pp 35-46.

Dajue L, Mingde Z, Rao VR (1993). Characterization and Evaluation of Safflower Germplasm. Beijing, China: Geological Publishing House, 260 pp.

De Haro A, Del Rio M, López JC, García MA, Palomares MJ, Fernández-Martínez J (1991). Evaluation of a world collection of safflower (*Carthamus tinctorius* L.) for oil quality and other seed characters. Sesame and Safflower Newsletter 6: 94-99.

Eckert JE (1962). The relation of honey bees to safflower. Am Bee J 102: 349-350.

Emerenciano VP, Militão JSLT, Campos CC, Romoff P, Kaplan MAC, Zambon M, Brant AJC (2001). Flavonoids as chemotaxonomic markers for Asteraceae. Biochem Syst Ecol 29: 947-957.

Enke N, Gemeinholzer B, Zidorn C (2012). Molecular and phytochemical systematics of the subtribe Hypochaeridinae (Asteraceae, Cichorieae). Org Divers Evol 12: 1-16.

Esendal E (2001). Global adaptability and future potential of safflower, in: Proceedings of 5th International Safflower Conference. Williston, ND, USA: pp. 9-12.

Estilai A (1977). Interspecific hybrids between *Carthamus tinctorius* and *C. alexandrinus*. Crop Sci 17: 800-802.

Estilai A, Knowles PF (1976). Cytogenetic studies of *Carthamus divaricatus* with eleven pairs of chromosomes and its relationship to other *Carthamus* species (Compositae). Am J Bot 63: 771-782.

Fernández Cuesta Á, Velasco L, Ruiz Méndez MV (2014). Novel safflower oil with high γ-tocopherol content has a high oxidative stability. Euro J Lipid Sci Tech 116: 832-836.

Fernandez-Martinez J, Rio M, Haro A (1993). Survey of safflower (*Carthamus tinctorius* L.) germplasm for variants in fatty acid composition and other seed characters. Euphytica 69: 115-122.

Futehally S, Knowles PF (1981). Inheritance of very high levels of linoleic acid in an introduction of safflower (*Carthamus tinctorius* L.) from Portugal, in: First International Safflower Conference, Davis, CA, USA: pp 56-61.

Gölükcü M, Toker R, Tokgöz H, Çınar O (2016). The effect of harvesting time on seed oil content and fatty acid composition of some lemon and mandarin cultivars grown in Turkey. J Agr Sci 22: 566-575.

Hamdan YA, Fernández-Martínez LV (2008). Inheritance of very high oleic acid content and its relationship with several morphological and physiological traits. In: Proceedings of the 7th International Safflower Conference, Wagga Wagga, New South Wales, Australia.

Heaton TC, Klisiewicz JM (1981). A disease-resistant safflower alloploid from *C. tinctorius* L. × *C. lanatus* L. Can J Plant Sci 61: 219-224.

Hofbauer J, Pelikan J (1997). Production of safflower in Czech Republic, in: Proceedings of the IVth International Safflower Conference. Bari, Italy: pp. 43.

Imrie BC, Knowles PF (1970). Inheritance studies in interspecific hybrids between *Carthamus flavescens* and *C. tinctorius*. Crop Sci 10: 349-352.

Işigigür A, Karaosmanoglu F, Aksoy HA (1995). Characteristics of safflower seed oils of Turkish origin. J Am Oil Chem Soc 72: 1223-1225.

Jiang TF, Lv ZH, Wang YH (2005). Separation and determination of chalcones from *Carthamus tinctorius* L. and its medicinal preparation by capillary zone electrophoresis. J Sep Sci 28: 1244-1247.

Kadam BS, Patankar VK (1942). Natural cross-pollination in safflower. Indian J Genet Pl Br 2: 69-70.

Khidir MO, Knowles PF (1970). Cytogenetic studies of *Carthamus* species (Compositae) with 32 pairs of chromosomes. II. Intersectional hybridization. Can J Genet Cytol 12: 90-99.

Knowles PF (1969). Centers of plant diversity and conservation of crop germplasm, Safflower. Econ Bot 23: 324-329.

Knowles PF (1989). Safflower. In: Downey RK, Robbelen G, Ashri A, editors. Oil Crops of the World. New York, NY, USA: McGraw-Hill, pp. 363-374.

Knowles PF, Ashri A (1958). Wild Safflower in California. Improvement of cultivated safflower through plant-breeding program to obtain desirable characteristics of wild species. Calif Agric 12: 4-5.

Knowles PF, Hill AB (1964). Inheritance of fatty acid content in the seed oil of a safflower introduction from Iran. Crop Sci 4: 406.

Koutroubas SD, Papakosta DK, Doitsinis A (2009). Phenotypic variation in physiological determinants of yield in spring sown safflower under Mediterranean conditions. Field Crop Res 112: 199-204.

Lata TS, Prakash V (1984). Studies on the proteins from safflower seed (*Carthamus tinctorius* L.). J Agr Food Chem 32: 1412-1416.

Levin MD, Butler GD, Rubis DD (1967). Pollination of safflower by insects other than honeybees. J Econ Entomol 60: 1481-1482.

Levin M, Butler GD (1966). Bees associated with Safflower in South Central Arizona. J Econ Entomol 59: 654-657.

Li D, Mundel HH (1996). Safflower. *Carthamus tinctorius* L. In: Promoting the conservation and use of underutilized and neglected crops. 7. Institute of Plant Genetics and Crop Plant Research, Gatersleben/International Plant Genetic Resources Institute, Rome.

Majidi MM, Tavakoli V, Mirlohi A, Sabzalian MR (2011). Wild safflower species (*Carthamus oxyacantha* Bieb.): a possible source of drought tolerance for arid environments. Aust J Crop Sci 5: 1055-1063.

Mayerhofer M, Mayerhofer R, Topinka D, Christianson J, Good AG (2011). Introgression potential between safflower (*Carthamus tinctorius*) and wild relatives of the genus *Carthamus*. Bmc Plant Biol 11: 47.

McPherson MA, Good AG, Keith C, Topinka A, Hall LM (2004). Theoretical hybridization potential of transgenic safflower (*Carthamus tinctorius* L.) with weedy relatives in the New World. Can J Plant Sci 84: 923-934.

Mihoub I, Robert T, Ghashghaie J, Vilatersana R, Lamy F, Benmrid R, Lamothe-Sibold, M, Aid F (2017). Phylogenetic position of two endemic *Carthamus* species in Algeria and their potential as sources of genes for water use efficiency improvement of safflower. J Syst Evol 55: 34-43.

Mongrand S, Badoc A, Patouille B, Lacomblez C, Chavent M, Bessoule JJ (2005). Chemotaxonomy of the Rubiaceae family based on leaf fatty acid composition. Phytochemistry 66: 549-559.

Murthy IYLN, Anjani K (2007). Fatty acid composition in *Carthamus* species. In 7th International Safflower Conference, Wagga Wagga, New South Wales, Australia: Directorate of Oilseeds Research, Rajendranagar, Hyderabad-500 (Vol. 30).

Nazari M, Mirlohi A, Majidi MM (2017). Effects of drought stress on oil characteristics of *Carthamus* species. J Am Oil Chem Soc 94: 247-256.

Nimbkar N (2002). Safflower rediscovered. Times Agricultural Journal 2: 32-36.

Pandey AK, Kumari A (2008). Pollination ecology of safflower (*Carthamus tinctorius* L.). In 7th International Safflower Conference. Wagga Wagga, Australia.

Patrascoiu M, Rathbauer J, Negrea M, Zeller R (2013). Perspectives of safflower oil as biodiesel source for South Eastern Europe (comparative study: safflower, soybean and rapeseed). Fuel 111: 114-119.

Pavlov DC, Tadorov NA (1996). Safflower (*Carthamus tinctorius* L.). In: Smartt J, Nwokolo E, editors. Food and Feed from Legumes and Oilseeds. Boston, MA, USA: Springer USA, pp. 245-257.

Payne WW (1976). Biochemistry and Species Problems in *Ambrosia* (Asteraceae-Ambrosieae). Plant Syst Evol 125: 169-178.

Peng S, Feng N, Guo M, Chen Y, Guo Q (2008). Genetic variation of *Carthamus tinctorius* L. and related species revealed by SRAP analysis. Biochem Syst Ecol 36: 531-538.

Rohlf JF (2004). NTSYS-pc: 2.11 Numerical Taxonomy and Multivariate Analysis System. New York, NY, USA: Exeter Software.

Rubis DD, Levin MD, McGregor SE (1966). Effects of honey bee activity and cages on attributes of thin hull and normal safflower lines. Crop Sci 6: 11-14.

Rudra Naik V, Bentur MG, Salimath PM, Parameshwarappa KG (2010). Introgression of non-spiny and high oil content in adapted generations of safflower (*Carthamus tinctorius* L.). Karnataka Journal of Agricultural Sciences 22: 39-43.

Sabzalian MR, Mirlohi A, Saeidi G, Rabbani MT (2009). Genetic variation among populations of wild safflower, *Carthamus oxyacanthus* analyzed by agro-morphological traits and ISSR markers. Genet Resour Crop Ev 56: 1057-1064.

Sabzalian MR, Saeidi G, Mirlohi A (2008). Oil content and fatty acid composition in seeds of three safflower species. J Am Oil Chem Soc 85: 717-721.

Sareedenchai V, Zidorn C (2010). Flavonoids as chemosystematic markers in the tribe Cichorieae of the Asteraceae. Biochem Syst Ecol 38: 935-957.

Singh V, Nimbkar N (2006). Safflower (*Carthamus tinctorius* L.). Genetic Resources, Chromosome Engineering, and Crop Improvement. New York, NY, USA: CRC, pp. 167-194.

Tarıkahya Hacıoğlu B, Arslan Y, Subaşı İ, Katar D, Bülbül AS, Çeter T (2012). Achene morphology of Turkish *Carthamus* species. Aust J Crop Sci 6: 1260-1264.

Tarıkahya Hacıoğlu B, Karacaoğlu Ç, Özüdoğru B (2014). The speciation history and systematics of *Carthamus* (Asteraceae) with special emphasis on Turkish species by integrating phylogenetic and ecological niche modelling data. Plant Syst Evol 6: 1349-1359.

Uher J (1997). Safflower in European horticulture. Proceedings of the IVth International Safflower Conference, Bari, Italy.

Valant-Vetschera KM, Wollenweber E (2001). Exudate flavonoid aglycones in the alpine species of *Achillea* sect. *Ptarmica*: chemosystematics of *A. moschata* and related species (Compositae, Anthemideae). Biochem Syst Ecol 29: 149-159.

Velasco L, Fernandez-Martinez L (2001). Breeding for oil quality in safflower. In Bergman JW, Mundel HH, editors. Proceedings of the 5th International Safflower Conference, Williston, ND, Sidney, MT, USA: pp. 133-137.

Vilatersana R, Garnatje T, Susana A, Garcia-Jacas N (2005). Taxonomic problems in *Carthamus* (Asteraceae): RAPD markers and sectional classification. Bot J Linn Soc 147: 375-383.

Vilatersana R, Susana A, Garcia-Jacas N, Garnetje T (2000). Karyology, generic delineation and dysploidy in the genera *Carduncellus*, *Carthamus* and *Phonus* (Asteraceae). Bot J Linn Soc 134: 425-438.

Weiss EA (1983). Oilseed Crops. Tropical Agriculture Series. London, UK: Longmans.

Yeilaghi H, Arzani A, Ghaderian M, Fotovat R, Feizi M, Pourdad SS (2012). Effect of salinity on seed oil content and fatty acid composition of safflower (*Carthamus tinctorius* L.) genotypes. Food Chem 130: 618-625.

Zidorn C (2006). Sesquiterpenoids as chemosystematic markers in the subtribe Hypochaeridinae (Lactuceae, Asteraceae). Biochem Syst Ecol 34: 144-159.

Zidorn C (2008). Sesquiterpene lactones and their precursors as chemosystematic markers in the tribe Cichorieae of the Asteraceae. Phytochemistry 69: 2270-2296.

Zidorn C, Gottschlich G, Stuppner H (2002). Chemosystematic investigations on phenolics from flowerheads of Central European taxa of *Hieracium* sensu lato (Asteraceae). Plant Syst Evol 231: 39-58.

Zidorn C, Stuppner H (2001a). Chemosystematics of taxa from the *Leontodon* section *Oporinia*. Biochem Syst Ecol 29: 827-837.

Zidorn C, Stuppner H (2001b). Evaluation of chemosystematic characters in the genus *Leontodon* (Asteraceae). Taxon 50: 115-133.