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Variation in Protein, Oil and Fatty Acid Contents in Three Wild Species of Safflower (*Carthamus*) from West Azerbaijan, Iran

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Abstract: The present study was conducted to find out the variation in protein, oil and fatty acid contents in wild species in comparison to cultivated species. Whole plants and seeds were collected from three of these wild species as below: *C. oxyacantha* (2n=24) from three regions of Khoy, Miandoab and Nazloo of Urmia. *C. dentatus* (2n=20) from Sardasht area and *C. turkestanicus* (2n=64) from Shahindej, Piranshahr and Sero. For the determination of protein, oil and fatty acids, kjeldal, solvent extraction method and gas chromatography apparatus was used, respectively. After statistical analyses of seed protein content, the result points to meaningful variation between the species. The amounts of protein in these species was higher than in cultivated safflower. With respect to seed oil content, no significant differences were found between the three wild species and in addition, this parameter was lower as compared with cultivated safflower. A negative correlation was observed between the amounts of seed protein and oil. The extracted seed oil from these species identically consisted of four fatty acids namely palmitic, stearic, oleic and linoleic. This result indicates the qualitative similarity that exists between the species which is also similar to cultivated safflower. It is interesting to note that the seed oil of *C. Dentatus* having the highest ratio of oleic over linoleic acid is highly stable and is expected to have a longer shelf life than the seed oil of cultivated safflower. In addition, the nutritional value for human consumption of the seed oil in all three species was higher than the cultivated safflower oil. The combined amounts of mono- and polyunsaturated fatty acids in the three wild *Carthamus* species was over 90% which is beneficial to human health if consumed as edible oils.

Key words: *Carthamus*, fatty acids, oil, protein, wild safflower

INTRODUCTION

Although domesticated plants are considered to be the main source of food for human consumption, however, in some instances, wild plant species after going through rigorous steps of plant breeding have been successful in gradually joining the cultivated plants. The genus *Carthamus* is a member of the tribe Cynareae, sub-family Tubuliflorae and family Compositae and it contains about 25 species. These species are distributed from Spain and North Africa across the Middle East to Northern India and China^[1]. Eight of these species have been reported from Iran^[2]. Safflower (*Carthamus tinctorius* L.) is the only species from this genus that is commercially cultivated in over 20 countries^[3]. Safflower has a long history of cultivation as an oilseed crop, as a source of red dye, carthamin, which is extracted from its flowers and it is also important as a medicinal plant especially in China which is used for treatment, in the form of infusion, for circulatory system

related diseases^[4]. Five chromosome categories are found in the genus *Carthamus* with haploid chromosome number of 10, 11, 12, 22 and 32. The cultivated safflower and about 10 of the wild species including *C. oxyacantha* have 12 pairs of chromosomes and are closely related, permitting an easy transfer of useful genes from the wild to the domesticated species. The wild species, as successful weeds in the Middle East and North Africa, have accumulated genes for vigor and for disease and insect resistance. These wild species may well prove to be the most important sources of valuable germplasm. A number of these wild species, such as, *C. persicus* (syn. *C. flavescentis*), *C. lanatus*, *C. oxyacantha* and *C. palaestinus* were identified as good sources of resistance or tolerance to various diseases and pests^[5]. It has also been realized that the transfer of resistance to leaf spot from *C. lanatus* to the cultivated species using conventional methods will prove to be difficult as *C. lanatus* is distantly related and it is an allotetraploid (2n=44).

The reserve of oil and protein in oilseeds is of particular interest to man and *Carthamus* species are a suitable example. The oil functions as a reserve of high-energy food for use by the germinating seed. Almost all reports on the seed protein content of *Carthamus* is with regard to the cultivated species^[6] in which the range of protein content in different varieties and breeding lines were reported to be from 8.6 to 16.2%. The potential of cultivated safflower as a source of protein for animal and human consumption has been investigated^[7]. There exists a rather similar situation with the seed oil and fatty acid composition of *Carthamus* species for obvious reasons. However, extremely limited reports can be found on wild species, namely, *C. lanatus* and *C. baeticus*. A more closely related weedy species, *C. oxyacantha*, which is considered to be a progenitor of cultivated safflower, is a noxious annual weed in Iran, Pakistan and northwest India, adapted to habitats associated with people and crop cultivation^[4]. Seeds of this species having appreciable amounts of oil can be used for culinary purposes and as lighting fuel^[8]. Evaluation of the seed oil and the constituent fatty acids from this species can be found in limited reports^[9-11]. It is unfortunate that the economic value of the wild species of safflower have been neglected for a long time and that we have not been able to find a comprehensive and up to date review in support of present study. However, the need for such investigations have been emphasized^[12]. This project is in support of looking into the germplasm diversity and the possibilities of widening the genetic base of cultivated safflower.

MATERIALS AND METHODS

Whole plants and seeds from three wild species of *Carthamus* were collected from various locations of West Azerbaijan province as follows: *C. oxyacantha* (2n=24) from three areas of Khoy, Miandoab and the Nazloo region of Urmia, *C. dentatus* (2n=20), being extremely scarce, from one location of Sardasht, *C. turkestanicus* (2n=64) from three areas of Shahindej, Piranshahr and Sero (Fig. 1). This study was planned and initiated in 2003 and seeds of all the species were collected separately from each mature wild growing plant in September. Five plants from each location were used for the measurement of protein and oil.

Seed samples were powdered with an electric grinder for the measurement of total protein by the kjeldal method using 0.5 g of oven dried sample. For oil extraction, one gram of seed was crushed using a mortar and pestle, followed by the addition of 5-10 mL of diethyl ether. The oil extraction with the solvent was repeated

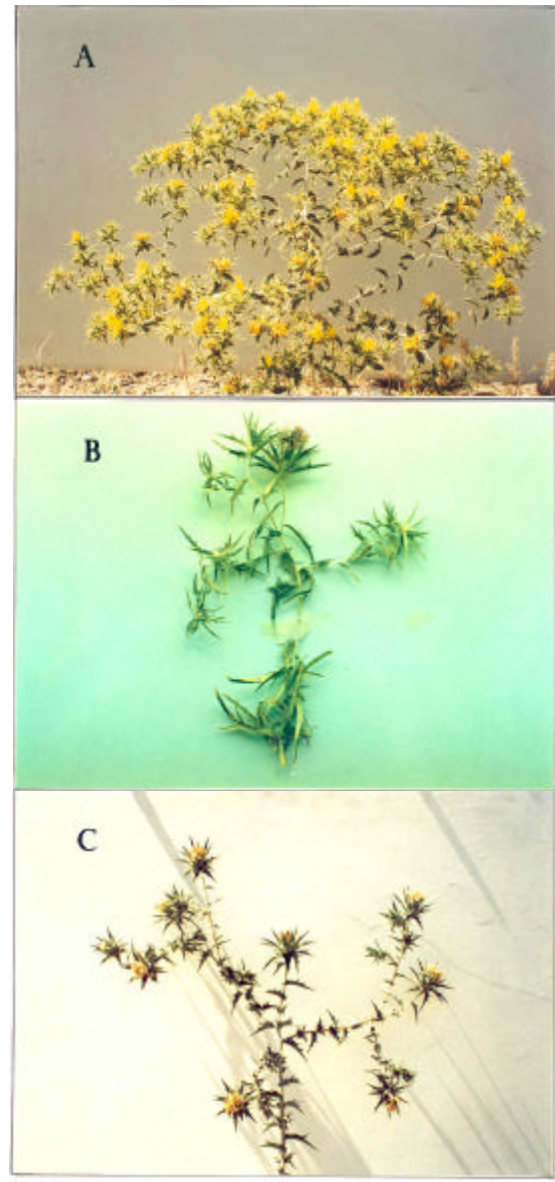


Fig. 1: Representative plants from the three species of wild safflower. (A) *C. oxyacantha*, (B) *C. dentatus*, (C) *C. turkestanicus*

three time and the oil was recovered by solvent evaporation at 60-70°C in an oven. Fatty acids were determined by gas chromatography after the preparation of their methyl esters. Esterification was accomplished by adding 1 mL n-heptan and 0.05 mL of 2 N KOH in methanol to 0.1 mL of the oil sample. The esters were separated using a Varian Aerograph model 2800 gas chromatograph equipped with a flame ionization detector. Nitrogen was used as the carrier gas. The 180 cm stainless steel column with 3 mm internal diameter was packed with

20% DEGS (Diethylene Glycol Succinate) on chromosorb W, 80/100 mesh. The injector and detector temperatures were both set at 245°C and the column temperature was isothermal at 190°C. The identification of fatty acid methyl esters were carried out by comparing their relative retention time with those of standards. The relative area under each of the consecutive curves of palmitic, stearic, oleic and linoleic acids were calculated using an electronic CDS-101 digital integrator. The obtained data were subjected to analysis of variance according to a Completely Randomized Design. Means were separated with Duncan's Multiple Range Test and the correlation coefficients and regression was calculated.

RESULTS AND DISCUSSION

The average amounts of seed protein in the three species of *C. oxyacantha*, *C. dentatus* and *C. turkestanicus* were 18.32, 16.85 and 15.15%, respectively and the analysis of variance gave a significant F value as compared with the differences observed within species. However, the Duncan's Multiple Range Test detected the significant difference at 1% level only between *C. oxyacantha* and *C. turkestanicus* with respect to seed protein. On the other hand, no significant differences could be found between locations for seed protein. This result points to the existence of genetic variation for this character between the species. In a survey of 15 varieties and 9 breeding lines of cultivated safflower the range of seed protein content was reported to be 8.6-16.2% with an average of 11.32%^[6]. It can be concluded that the wild species of *Carthamus* investigated generally have a higher percentage of seed protein as compared with the cultivated species which may be due to the efforts made in the direction of increasing seed oil content for commercial use in the cultivated safflower which is inversely related with protein.

With respect to seed oil content, the average amounts in the three species of *C. oxyacantha*, *C. dentatus* and *C. turkestanicus* were 22.40, 22.20 and 24.23%, respectively and the analysis of variance did not give a significant F-value indicating the lack of statistically significant differences between the three species as compared with the observed variation within the species. In general there are very few reports on the seed oil content of wild species of safflower. In an older report, the seed oil content of *C. lanatus* and *C. baeticus* was measured to be within the range of 16-20 and 16-21%, respectively^[13]. On the other hand, in one single investigation, the seed oil content of *C. oxyacantha* has been reported to be 34.8% which is much higher than our

results^[11]. *C. oxyacantha*, being a common weed and one of the closest relatives of cultivated safflower is generally reported to have 28% seed oil^[4,8]. The general profile for seed oil content in wild species of safflower is much lower than the cultivated safflower that has a range of 30-42%^[8,12,14] indicating the results of breeding efforts that has been directed towards increasing oil content in the cultivated safflower. Results of this study indicates that the three wild species under investigation have higher amounts of protein and lower amounts of seed oil as compared with the cultivated safflower. Although the negative correlation observed between these two parameters is not significant ($r=-0.424$) but it is probable that breeding for higher oil content in the cultivated safflower has been responsible for lowering of seed protein.

Fatty acid analysis of the seed oil from the three wild species identically consisted of two saturated (palmitic, C16:0 and stearic, C18:0) and two unsaturated (oleic, C18:1 and linoleic, C18:2) fatty acids which is essentially similar to the cultivated safflower. The average percentages of these fatty acids are presented in Table 1. Although the differences between species are significant for stearic, oleic and linoleic, but their overall amounts correspond with each other and they conform by the amounts found in the standard high linoleic cultivated safflower^[15,16]. In another investigation, lower amounts of linoleic acid and higher amounts of the remaining three fatty acids have been reported^[11,17].

The oil stability index (C18:1/C18:2) which indicates the shelf life of extracted oil was the highest in *C. dentatus* (Table 1) and this index in *C. oxyacantha* was similar with the average value found in cultivated safflower^[6]. The overall ratio of linoleic, the only essential fatty acid in human nutrition, over saturated fatty acids known as the Nutritional Quality Index, in the seed oil of the three wild species studied is 13.6 (Table 1) which is higher than the reported average of 8.9 in cultivated safflower^[6] indicating their high nutritional value for human consumption.

Statistically significant differences for oleic and linoleic fatty acids were also detected between locations for *C. oxyacantha* and *C. turkestanicus* indicating the effect of the environment on the amounts of these two fatty acids. The main outcome from the calculations of correlation coefficients between the amounts of the four fatty acids is the consistent observation of a highly significant and negative correlation between the oleic and linoleic fatty acid in the seed oil of all three wild species (Table 2). This observation is consistent with the seed oil of the cultivated safflower^[14]. The scatter diagram of Fig. 2 amplifies this observation and indicates that this

Table 1: Average amount of fatty acids in the seed oil of *Carthamus* species from various locations in West Azerbaijan

	Palmitic (%) (C _{16:0})	Stearic (%) (C _{18:0})	Oleic (%) (C _{18:1})	Linoleic (%) (C _{18:2})	Stability index*	Nutritional quality**
<i>C. oxyacantha</i>	4.78±0.44	1.13±0.22	15.70±2.55	78.39±2.60	0.20	13.26
<i>C. dentatus</i>	4.90±0.29	1.20±0.31	21.10±1.68	72.80±1.39	0.29	11.93
<i>C. turkestanicus</i>	4.52±0.89	0.86±0.19	11.20±1.41	83.43±1.70	0.13	15.51

* oil stability index is the amount of C_{18:1} divided by C_{18:2} ** nutritional quality is the amount of C_{18:2} divided by (C_{16:0}+C_{18:0})

Table 2: Correlation coefficients between fatty acid percentage in *Carthamus* species from various locations in West Azerbaijan

Species	Fatty acids	Stearic	Oleic	Linoleic
<i>C. oxyacantha</i>	Palmitic	0.653**	-0.032 ^{NS}	-0.179 ^{NS}
	Stearic		0.238 ^{NS}	-0.424 ^{NS}
	Oleic			-0.974***
<i>C. dentatus</i>	Palmitic	0.807 ^{NS}	-0.805 ^{NS}	0.587 ^{NS}
	Stearic		-0.424 ^{NS}	0.123 ^{NS}
	Oleic			-0.949***
<i>C. turkestanicus</i>	Palmitic	0.126 ^{NS}	-0.083 ^{NS}	-0.475 ^{NS}
	Stearic		-0.464 ^{NS}	-0.121 ^{NS}
	Oleic			-0.832***

** and ***: significant at 1 and 0.1%, respectively, ^{NS}: non-significant

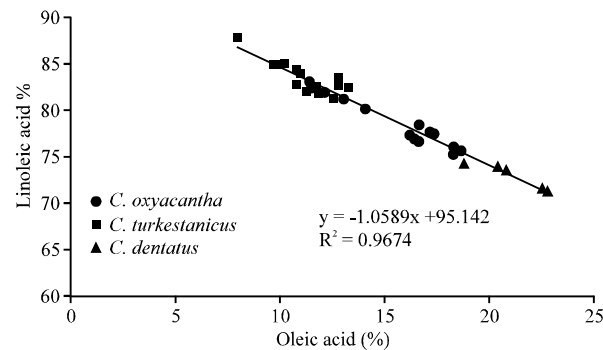


Fig. 2: Scatter diagram of the 35 pairwise observations between oleic and linoleic fatty acids in the seed oil of three wild species, namely, *Carthamus oxyacantha*, *C. dentatus* and *C. turkestanicus*

highly significant negative correlation ($r=-0.984^{***}$) is in line between the species. Although a considerable variation in the percentages of the oleic (8.0-22.8%) and linoleic (71.2-87.8%) fatty acids exists in the seed oil of the three species, but the combined percentages of oleic+linoleic (mono- and polyunsaturated fatty acids) has a much narrower range of from 92.9 to 96.2%. This is an indication of the high amount and the relative constancy of the overall percentage of unsaturated fatty acids in the seeds of the wild species of safflower under investigation which is beneficial to human health.

Keeping in view the composition of these oils, it is seen that the seed oil obtained from all three wild species can provide good quality edible oils. However, the only difficulty with these wild plants is the collection of their seeds.

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