Fatty acid composition in *Linum* species: Species delimitation and diversity

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Abstract. Flax (*Linum usitatissimum* L.) is one of the most important cultivated oil-producing plants that is highly evaluated for its fatty acid components. Studying and using wild relatives of cultivated crop plants as a spore of gene pool for breeding and hybridization is considered very important now. Therefore, the aim of present investigation was to provide data on saturated and unsaturated fatty acids of three Linum species, *Linum usitatissimum*, *L. austriacum* and *L. album*. We also studied the magnitude of oil composition variability within populations of these species and attempted to find out if oil composition data can be used in the *Linum* species delimitation. The saturated (C16:0, C18:0 and C20:0) and unsaturated (C18:1, C18:2, C18:3 and C20:1) fatty acids identified by GC represented inter- and intraspecies variations in the linseed and wild linum species. The linolenic ($C_{18:3}$), linoleic ($C_{18:2}$) and oleic acid as unsaturated fatty acids had an average of 52.7 %, 12.4 % and 20.6 % respectively in cultivated flax (*L. usitatissimum*), an average of 51.4 %, 18.8 and 20.3 % in *L. austriacum,* and 4.3 %, 60.4 %, 22.5 % in *L. album* respectively. Correlations between saturated and unsaturated fatty acids showed that with increase of linolenic acid in linseed oil, the oleic acid decreased. We found that unsaturated fatty acids (linoleic and linolenic) were higher in wild species as compared to those of the cultivated flaxseed, while precursor saturated fatty acids were lower in the studied wild species. Cluster analysis and a PCoA plot revealed that the *Linum* species differed in their oil profiles and the populations of each species were placed in a separate group. These data can be used in the *Linum* species delimitation.

Key words: *Linum*, polyunsaturated fatty acid, species delimitation

Introduction

Linum is the largest genus of *Linaceae* with more than 200 species. These species mainly grow in temperate and subtropical regions of the world (Rogers 1982; Muir & Westcott 2003). About 22 members of this species grow in Iran (Sharifnia & Assadi 2001). Interspecific hybridization between flax and congeneric species has been reported in *Linum* (Jhala & al. 2008).

Flax (*L. usitatissimum L.)* is one of the most important cultivated plants for its oil and fiber. It is used for different purposes like nutriceuticals, industry, biopharmaceuticals, animal feed, and human foods (Rowland & al. 1995; Lorgeril & al. 1999; Bhathena & al. 2002; Berglund 2002; Paschos & al. 2007; Zhao & al. 2007).

Among dietary plant oils, flaxseed oil has one of the highest contents of essential fatty acids, including precursor saturated fatty acids; palmitic (PAL, C16:0, 6 %) and stearic (STE, C18:0, 4.4 %), and unsaturated fatty acids, omega-3 (alpha-linolenic acid, ALA, 18:3, cisΔ9,1 2,15; 50.1 %), omega-6 (linoleic

acid, LIO, 18:2, cisΔ9,12; 15.3 %), and omega-9 (oleic acid, OLE, 18:1, cisΔ9; 24.2 %) (Muir & Westcott 2003). Decreasing linoleic / linolenic acid ratio is one of the indices considered in healthy diets (Wood & al. 2004).

In recent years, breeders have been engaged in developing new and improved varieties for environmental adaptation and more healthy oils (Rowland 1991; Muir & Westcott 2003; Cloutier & al. 2012).

Composition of fatty acids has been used in plant species delimitation and taxonomy (Shorland 1963, Wolff & al. 2001). Although there are published reports on fatty acid composition of cultivated linum *(Linum usitatissimum* L.) and pale flax (*L. bienne* Mill.), no study has been carried out into the other *Linum* species' fatty acid composition. The authors have studied the fatty acid components, including precursor saturated fatty acids and unsaturated fatty acids, for the first time in *L. austriacum* and *L. album,* so as to compare them with those of cultivated flax *L. usitatissimum.* We also tried to study the oil composition variability in the studied species and to reveal the use of oil composition data in the *Linum* species delimitation.

Material and methods

Plant material

Twenty-seven plant specimens were collected from nine populations of three *Linum* species: 1 – cultivated flax *L. usitatissimum* L. (Oroomieh, Saveh and Shiraz regions), 2 – *L. austriacum* L. (two populations of West- and East-Azarbayejan provinces) and 3 – *L. album* L. (two populations of Fars province and one of Isfahan province).

Oil components

Linum seeds of the studied populations were used for oil extraction. Ten grams of linseeds were ground and separated on a 24-mesh Tyler sieve. Organic solvent was used for extraction after the Diederichsen & Raney (2006) procedure. The produced extracts were sterilized and samples were prepared so as to obtain fatty acid methyl esters (FAME). The methyl esters obtained were resuspended in 8 ml of Hexane for 5 hours. Then the extracted oil was resuspended in 1 cc methanol (2 M) and 3 ml Hexane. The obtained solution was injected into the gas chromatograph.

The fatty acid composition of the seed oil was analyzed by YL 6100 gas chromatography system with the following categories: oven temperature: 175 c, column: HP-INNOWAX (100 m * 0.25 mm * 0.2 um), carrier gas: hydrogen, 1 ml/min, Detector: FID. The identification of fatty acids in the samples was compared with the spectra of fatty acid patterns determined under the same conditions. Seventeen fatty acid components, including saturated fatty acids (12:0, 14:0, 16:0, 17:0, 18:0, 20:0 and 22:0) and unsaturated fatty acids (14:1c, 16:1c, 16:1t, 17:1c, 18:1c, 18:2, 18:2t, 18:3, 20:1c and 22:1c) were evaluated for the three *Linum* species.

Data analysis

The significant difference in the amount of saturated fatty acids (16:0, 18:0) and unsaturated fatty acids (18:1, 18:2, 18:3, 20:1) obtained in the studied *Linum* species and populations was analyzed by variance analysis test (ANOVA), following the least significant difference (LSD). Bilateral relationships between the fatty acids compositions were analyzed by the correlation analysis.

Grouping of the plant specimens on the basis of oil characteristics was studied by the Neighbor Joining (NJ) clustering method, maximum parsimony and NeighborNet method of networking, as well as by principal coordinate analysis (PCoA). These analyses were performed after 100 times bootstrapping/ permutations (Freeland & al. 2011; Huson & Bryant 2006).

Results

The mean value of 17 saturated and unsaturated fatty acids in nine populations of three *Linum* species are listed in Table 1. The linolenic ($C_{18:3}$), linoleic ($C_{18:2}$) and oleic acid as unsaturated fatty acid had an average of 52.7 %, 12.4 % and 20.6 %, respectively, in cultivated flax (*L. usitatissimum*). The mean value of stearic acid ($C_{18:0}$) as second abundant saturated fatty acid was 5.7 % (Table 1).

The fatty acids of lauric, myristoleic, palmitelaidic, linolelaidic, behenic, and erucic acids were not detected in the studied cultivated flax populations. Oroomieh population showed higher linoleic acid level, while Shiraz and Saveh samples were higher in linolenic acid and oleic acid, respec-

tively. ANOVA test showed significant differences (P<0.01) among the cultivated flax populations in these three important fatty acid components (data not shown). The mean values for important unsaturated fatty acids of linolenic, linoleic and oleic acids were 51.4 %, 18.8 and 20.3 %, respectively, in *L. austriacum* (Table 1). The mean value of stearic acid was 2.5 %. Except for two saturated fatty acids, lauric and myristoleic, the other fatty acids were detected in the studied samples of *L. austriacum* (Table 1). The amount of linoleic, linolenic and oleic acid did not differ significantly in the three studied populations (P>0.1). In *L. album,* the average values of linolenic, linoleic and oleic acids were 4.3 %, 60.4 % and 22.5 %, respectively. The average value of unsaturated fatty acid (stearic acid) was 3.8 %. Fatty acid components estimated in *L. album* showed variation among the studied populations. Fars population samples (L.al 3) had a higher amount of linoleic and linolenic acid (Table 1). The ANOVA test showed significant differences among all fatty acid compositions among the studied populations (P>0.01). Generally, omega-6 fatty acid (linoleic acid) was higher in wild species than in cultivated flax.

The ANOVA test showed significant differences among the studied *Linum* species for all studied fatty acids, except for margaric, oleic and linolelaidic acid (P<0.05, Table 2). Bilateral relationships between the fatty acids composition data were analyzed by correlation analysis. In cultivated flax samples, the amount of palmitic acid ($C_{16:0}$) was negatively correlated with the amount of palmitoleic acid (r = -0.786, (P<0.05). However, it was positively correlated with the amount of stearic acid (r = 0.959, P<0.01). Similarly, some significant positive correlations were recorded between ginkgolic ($C_{17:1}$), oleic and linoleic acid (r = 0.693, r = 0.831, P<0.05 and P< 0.01, respectively). Positive significant correlations (P<0.05) were revealed between linoleic ($C_{18:2}$), oleic ($C_{18:1}$, r = 0.721) and linolenic acid ($C_{18:3}$, r = 0.757), too. However, a negative correlation was shown between ginkgolic and linolenic acids (r = -0.725, P<0.05). Similar analyses of the populations of *L. austriacum* almost agreed with the above-mentioned results for cultivated flax, but differed in some points. For example, we found significant positive correlation between the fatty acids like oleic acid ($C_{18:1}$) and linoleic acid ($C_{18:2}$) content (r = 0.696, p<0.05), between linolenic ($C_{18:3}$) and oleic acid content ($C_{18:1}$) (r = 0.949, p<0.01), and between linolenic ($C_{18:3}$) and stearic acid content (r = 0.723, p<0.05). We also found significant negative correlations between oleic ($C_{18:1}$) and strearic acid (r = -0.891, p<0.01), and linolenic (r = -0.949, p<0.01) and arachidic acid ($C_{20:0}$). The results also showed a negative correlation between linoleic ($C_{18:2}$) and linolenic acid ($C_{18:3}$) content (r = -0.821, p<0.01).

In *L. album* populations, some significant positive correlations were recorded between stearic ($C_{18:0}$) and oleic acid ($C_{18:1}$) content (r = 0.739, P<0.01). A significant negative correlation was detected between oleic ($C_{18:1}$) and linoleic acid ($C_{18:2}$) content (r = -0.961, P<0.01), oleic with linolenic acid ($C_{18:3}$) content (r = -0.992, P<0.01), and between stearic and linoleic and linolenic acid contents (r = -0.882, r = -0.726, P<0.01).

The UPGMA clustering of *Linum* species and populations based on oil profile is presented in Fig. 1. It showed that the *Linum* species differed in their oil profiles and populations of each species were placed in a separate cluster/group. Two main clusters were formed. The first major cluster was formed by *L. usitatissimum* and *L. austriacum* populations. While the *L. album* samples formed the second major cluster. Of the three studied populations of *L. usitatissimum*, the samples collected from Shiraz population (plant numbers 10–12 in Fig. 1) were more distanced from the other two cultivated populations (Fig. 1). Difference between the populations oil profiles was also observed in *L. usitatissimum* and *L. album*, as plant samples of different populations were somewhat distanced from each other in both species.

A MDS plot based on oil components in three *Linum* species (Fig. 2) supported the clustering result. The MDS plot separated all three *Linum* species into three separate groups and placed the samples of *L. usitatissimum* and *L. austriacum* closer to each other. Similarly, *L. album* samples were placed far from these two species. The results were also in agreement with the ANOVA result that revealed a significant difference in oil components of the three studied species as well as between the populations in each species. The MDS plot also revealed a higher degree of intraspecies diversity in *L. album,* followed by *L. usitatissimum* with regard to oil profile.

Table 1. **Fatty acid components in the studied *Linum* species and populations.**

Specimen		Lauric acid	Myristic acid	Myristoleic acid	Palmitic acid	Palmitelaidic acid	palmitoleic acid	Margaric acid	Ginkgolic acid	Stearic acid	Oleic acid	Linolelaidic acid	Linoleic acid	Linolenic acid	Arachidic acid	Paullinic acid	behenic acid	Erucic acid
L.us 1	Mean	.0000	.1000	.0000	6.4667	.0000	.2000	.1000	.1000	5.2333	21.633	.0000	13.966	51.466	.1000	.2000	.0000	.0000
	SD	.0000	.0000	.00000	.05774	.00000	.00000	.00000	.00000	.05774	.05774	.00000	.05774	.05774	.00000	.00000	.0000	.0000
L.us 2	Mean	.0000	.1000	.0000	7.0000	.0000	.1000	.1000	.0667	6.2333	23.433	.0000	12.533	49.966	.0000	.1333	.0000	.0000
	SD	.0000	.0000	.00000	.10000	.00000	.00000	.00000	.05774	.05774	.05774	.00000	.05774	.11547	.00000	.05774	.0000	.0000
L.us 3	Mean	.0000	.1000	.0000	6.7000	.0000	.1000	.1000	.0000	5.6667	16.8333	.0000	10.9667	56.7000	.2000	2.3333	.0000	.0000
	SD	.00000	.00000	.00000	.10000	.00000	.00000	.00000	.00000	.05774	.05774	.00000	.05774	.00000	.00000	.05774	.00000	.00000
L. usitatissimum	Mean	.0000	.1000	.0000	6.7222	.0000	.1333	.1000	.0556	5.7111	20.6333	.0000	12.4889	52.7111	.1000	.8889	.0000	.0000
	SD	.00000	.00000	.00000	.24381	.00000	.05000	.00000	.05270	.43716	2.95508	.00000	1.30043	3.06204	.08660	1.08449	.00000	.00000
L.au 1	Mean	.0000	.1000	.0000	4.8000	.1000	.1000	.0667	.0000	2.9333	19.2000	.0333	18.8000	52.1000	1.0000	.2667	.1000	.0333
	SD	.00000	.00000	.00000	.20000	.00000	.00000	.05774	.00000	.05774	.60000	.05774	.10000	.70000	.20000	.05774	.00000	.05774
L.au 2	Mean	.0000	.1000	.0000	5.3333	.1000	.1000	.1000	.0000	2.3000	21.1000	.0333	19.0333	50.8333	.5333	.3333	.1000	.0000
	SD	.00000	.00000	.00000	.05774	.00000	.00000	.00000	.00000	.00000	.30000	.05774	.15275	.28868	.05774	.05774	.00000	.00000
L.au 3	Mean	.0000	.1000	.0000	5.3000	.1000	.1000	.1000	.0000	2.3667	20.6333	.0000	18.8000	51.4000	.6000	.3000	.1000	.0000
	SD	.00000	.00000	.00000	.00000	.00000	.00000	.00000	.00000	.05774	.15275	.00000	.00000	.10000	.00000	.00000	.00000	.00000
L. austriacum	Mean	.0000	.1000	.0000	5.1444	.1000	.1000	.0889	.0000	2.5333	20.3111	.0222	18.8778	51.4444	.7111	.3000	.1000	.0111
	SD	.00000	.00000	.00000	.27889	.00000	.00000	.03333	.00000	.30414	.92391	.04410	.14814	.66916	.24210	.05000	.00000	.03333
L.al 1	Mean	.2333	1.0000	.1333	8.5000	.1000	.2333	.1000	.1000	4.4000	26.3667	.0000	54.2000	3.6000	.3000	.0000	.1000	.0000
	SD	.05774	.10000	.05774	.30000	.00000	.05774	.00000	.00000	.10000	.45092	.00000	.20000	.00000	.00000	.00000	.00000	.00000
l.al 2	Mean	.0000	.1000	.0000	6.1333	.1000	.1000	.1000	.0000	3.3667	22.0000	.1667	63.0000	4.4333	.3000	.0000	.1667	.1000
	SD	.00000	.00000	.00000	.05774	.00000	.00000	.00000	.00000	.05774	.30000	.15275	.50000	.05774	.00000	.00000	.05774	.10000
L.al 3	Mean	.0000	.3000	.0000	6.3333	.0000	.2000	.1000	.1000	3.7333	19.3667	.0000	64.2000	4.9333	.4000	.0000	.2000	.0000
	SD	.00000	.20000	.00000	.45092	.00000	.00000	.00000	.00000	.05774	.05774	.00000	.60000	.05774	.00000	.00000	.00000	.00000
L. album	Mean	.0778	.4667	.0444	6.9889	.0667	.1778	.1000	.0667	3.8333	22.5778	.0556	60.4667	4.3222	.3333	.0000	.1556	.0333
	SD	.12019	.42426	.07265	1.16881	.05000	.06667	.00000	.05000	.45826	3.07399	.11304	4.74579	.58476	.05000	.00000	.05270	.07071
Total	Mean	.0259	.2222	.0148	6.2852	.0556	.1370	.0963	.0407	4.0259	21.1741	.0259	30.6111	36.1593	.3815	.3963	.0852	.0148
	SD	.07642	.29396	.04560	1.07263	.05064	.05649	.01925	.05007	1.38525	2.62643	.07121	21.84812	23.01513	.29488	.71008	.07181	.04560

Abbreviations: **L.us** – *L. usitatissimum*: **1** – Oroomieh, **2** – Saveh, **3** – Shiraz populations; **L.au** – L. *austriacum*: **1** – West azarbayejan1, **2** –West azarbayejan2, **3** – East Azarbayejan populations); **L.al** – *L. album*: **1** – Fars1, **2** – Fars 2, **3** – Isfahan populations.
Sd – standard deviation.

Table 2. ANOVA test based on fatty acid compositions in the studied *Linum* species.

			Sum of Squares	df	Mean Square	F	Sig.
Lauric acid	Between Groups	(Combined)	0.036	2	0.018	3.769	.038
	Within Groups		0.116	24	0.005		
	Total		0.152	26			
Myristic acid	Between Groups	(Combined)	0.807	2	0.403	6.722	.005
	Within Groups		1.440	24	0.060		
	Total		2.247	26			
Myristoleic acid	Between Groups	(Combined)	0.012	2	0.006	3.368	.051
	Within Groups		0.042	24	0.002		
	Total		0.054	26			
Palmitic acid	Between Groups	(Combined)	17.887	2	8.944	17.848	.000
	Within Groups		12.027	24	0.501		
	Total		29.914	26			
Palmitelaidic acid	Between Groups	(Combined)	0.047	2	0.023	28.000	.000
	Within Groups		0.020	24	0.001		
	Total		0.067	26			
Palmitoleic acid	Between Groups	(Combined)	0.027	2	0.014	5.920	.008
	Within Groups		0.056	24	0.002		
	Total		0.083	26			
Margaric acid	Between Groups	(Combined)	0.001	2	0.000	1.000	.383
	Within Groups		0.009	24	0.000		
	Total		0.010	26			
Ginkgolic acid	Between Groups	(Combined)	0.023	2	0.011	6.526	0.005
	Within Groups		0.042	24	0.002		
	Total		0.065	26			
Stearic acid	Between Groups	(Combined)	45.943	2	22.971	139.613	0.000
	Within Groups		3.949	24	0.165		
	Total		49.892	26			
Oleic acid	Between Groups	(Combined)	27.067	2	13.534	2.133	0.140
	Within Groups		152.284	24	6.345		
	Total		179.352	26			
Linolelaidic acid	Between Groups	(Combined)	0.014	2	0.007	1.434	0.258
	Within Groups		0.118	24	0.005		
	Total		0.132	26			
Linoleic acid	Between Groups	(Combined)	12216.962	2	6108.481	756.139	0.000
	Within Groups		193.884	24	8.079		
	Total		12410.847	26			
Linolenic acid	Between Groups	(Combined)	13690.779	2	6845.389	2020.117	0.000
	Within Groups		81.327	24	3.389		
	Total		13772.105	26			
Arachidic acid	Between Groups	(Combined)	1.712	2	0.856	37.425	0.000
	Within Groups		0.549	24	0.023		
	Total		2.261	26			
Paullinic acid	Between Groups	(Combined)	3.681	2	1.840	4.684	0.019
	Within Groups		9.429	24	0.393		
	Total		13.110	26			
Behenic acid	Between Groups	(Combined)	0.112	2	0.056	60.400	0.000
	Within Groups		0.022	24	0.001		
	Total		0.134	26			
Erucic acid	Between Groups	(Combined)	0.005	2	0.003	1.273	0.298
	Within Groups		0.049	24	0.002		
	Total		0.054	26			

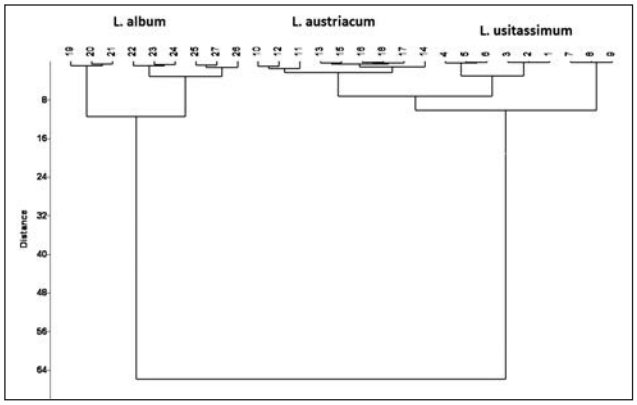

Fig. 1. UPGMA dendrogram of the *Linum* species and populations based on the oil profile.

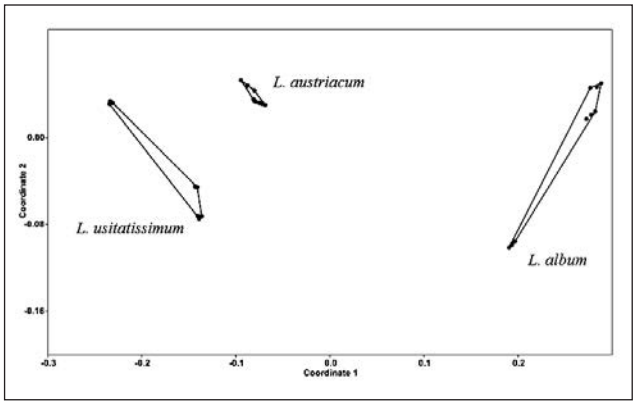

Fig. 2. MDS plot of the *Linum* species and populations based on the oil profile.

Discussion

In the present study, the fatty acid components of *L. austriacum* and *L. album* species were evaluated so as to compare them with those of cultivated flaxseed. The main fatty acids in flaxseed are precursor saturated fatty acids, palmitic ($C_{16:0}$) and stearic ($C_{18:0}$), as well as unsaturated fatty acids, oleic ($C_{18:1}$), linoleic ($C_{18:2}$) and α-linolenic ($C_{18:3}$). The amounts of saturated and unsaturated fatty acids calculated in this study significantly varied among cultivated flaxseed. There are some reports of variation in amounts of fatty acids in flaxseed cultivars (Bayrak & al. 2010; El-Beltagi & al. 2011; Gallardo & al. 2014; Pali & Mehta 2014). Different factors may affect flax oil, mainly such environmental factors as temperate climate during plant development, seed moisture at harvest (Casa & al. 1999; Adugna & Labuschagne 2003; Gallardo & al. 2014) and genetic variations in desaturase genes (Thambugala & al. 2013; Thambugala & Cloutier 2014). In the present study, three flaxseed populations are cultivated in different climates with different temperatures: Orumieh 5–17 °C, Shiraz 5–30 °C and Saveh 12–24 °C. Besides environmental factors, genetic study of these populations also showed variation among and between the populations (Noormohammadi and co-workers, pers. com.) which may affect the oil components.

The mean values of unsaturated fatty acids are in agreement with some researchers' reports (Diederichsen & Fu 2008, Bayrak & al. 2010, Pali & Mehta 2014), while Thambugala & Cloutier (2014) reported higher amounts of linolenic acid (up to 72 %) in flaxseed cultivars. Correlations between saturated and unsaturated fatty acids showed that with increase of linolenic acid in linseed oil, oleic acid decreased. Bhatty (1995) and Bayrak & al. 2010 also reported negative correlation between saturated and unsaturated fatty acids. Fatty acid components in the studied wild *Linum* species showed significant variation among the populations. Variation in percentage of fatty acids among populations in *L. austriacum* and *L. album* may be influenced by differences in their habitats and genetic structures. Sheidai & al. (2014 a, b) reported high genetic variation among *L. austriacum* and *L. album* populations in Iran.

We found that unsaturated fatty acids (linoleic and linolenic) were higher in wild species as compared to cultivated flaxseed, while precursor saturated fatty acids in wild species decreased. The latter also caused production of long-chain saturated fatty acids (20:0, 22:0) in wild species. It is interesting to know that lenoleic acid percentage in *L. album* was much higher than in other *Linum* species, while linolenic acid showed much lower values than in other species. This decrease may stem from Δ15 desaturase enzyme in *L. album*. This desaturase is a key enzyme for conversion of linoleic to linolenic acid (Damude & Kinney 2008). Further study is necessary to find the reasons for the low amount of linolenic acid. However, fatty acid components in the wild *Linum* species are competitive with *Linum* cultivars in terms of unsaturated fatty acids. Cluster analysis based on oil components also supported variations among the studied wild *Linum* species and cultivated flaxseed.

Our study revealed significant difference in oil composition among the *Linum* species. Similar studies performed into fatty acid (FA) compositions in *Pinus* spp., *Larix* (Larch), *Picea* (Spruce), and *Pseudotsuga* (Douglas Fir) spp. revealed a similar com-

position, including in particular the same delta5-olefinic acids. However, they display a considerably lower variability in *Larix* and *Picea* spp. than in *Pinus* spp. (Wolff & al. 2001). We have also observed that intraspecies oil composition variability is much lower than the interspecies dissimilarities, therefore, this observation supports the use of seed FA compositions as chemotaxonomic markers, as they practically do not depend on edaphic or climatic conditions (Wolff & al. 2001).

In conclusion, these important plant polyunsaturated fatty acids are essential components of cell membranes and precursors of signaling molecules (Ohlrogge & Browse 1995). They are also considered essential in mammalian tissues which cannot synthesize these fatty acids. Our results showed that the high amounts of important fatty acids provided by the wild species represent a good resource for oil consumption.

Interspecific hybridization and cytogenetic studies of the cultivated flax and wild species demonstrated that cultivated flax has the ability to hybridize and form viable F1 plants with some *Linum* species (Jhala & al. 2008). This is the first report on oil components in the wild *Linum* populations and it may pose a primary challenge for plant breeders to introduce new, desired traits from the wild species and develop novel, high-value opportunities. The present findings indicated that the oil profile can be used in taxonomic delimitation of *Linum* species and also suggested that oil data can be used in population studies.

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