Oil content and fatty acid composition of seeds of some Lithuanian wild crucifer species

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Abstract. Oil content and fatty acid composition were investigated in the following wild crucifer species during 2008: Nineteen samples of fanweed (*Thlaspi arvense* L.), eight samples of flixweed (*Descurainia sophia* (L.) Webb ex Prantl), 17 samples of wallflower mustard (*Erysimum cheiranthoides* L.), one sample of sea rocket (*Cakile baltica* Jord. ex Pobed.) and two samples of garlic mustard (*Alliaria petiolata* (M. Bieb.) Cavara et Grande). The greatest amount of oil was found in seeds of flixweed (32.2%) and wallflower mustard (30.6%). Seeds of garlic mustard contained the least amount of oil (15.8%). Fatty acids were dominated by unsaturated acids (oleic, linoleic and linolenic) in flixweed (71.4%), wallflower mustard (63.5%) and sea rocket (62.7%), by erucic acid in garlic mustard (49.3%) and fanweed (35.6%) seed oil. The seed oil of some investigated wild crucifer species could be suitable both for human consumption and industrial purposes.

Key words: *Brassicaceae*, unsaturated fatty acids, erucic acid, variation

INTRODUCTION

In recent years, there has been considerable interest in developing alternate crops for supplementing human needs for fuels, chemicals and feeds (Carr, 1985). The potential for wild germplasm to provide novel sources of economic traits in oil plant breeding programs has increased dramatically in the last 15–20 years with the development of biotechnology and its utilization as a breeding tool (Warvick, et al., 1999).

The *Brassicaceae*, which contain about 3,500 species and 350 genera, is one of the ten most economically important plant families (Koch, 2001). Crop *brassicae* display enormous diversity and are used as sources of oil, vegetables, mustard condiments, and fodder (Warvick et al., 1999). There are 16 *Brassica* collections in 11 countries in the EU (Astley et al., 2006). Wild crucifer species have already provided novel sources of important agronomic traits, such as male sterility and pest resistance for canola (Warvick et al., 1999).

In Lithuania there are 40 species and 87 genera of crucifer and most of them are wild flora. Such species are not sufficiently researched and assessed, because the main attention has been drawn to cultured oil plants. Local flora are still being formed by such evolutionary forces as natural selection, mutation, migration and hybridization, have a stable survival potential and greater possibility to create adaptive combinations of genes in ever-changing environmental conditions (Danusevičius, 2004).

The study of wild crucifers was started in 2008 at the Lithuanian University of Agriculture. The aim of this research was the search of Lithuanian flora plants for potential oil production and their selection in order to enrich the assortment of oil plants. The objective of the first stage of this work is to research interspecific and intraspecific biochemical diversity of seeds of the following species: (*Descurainia sophia* (L.) Webb ex Prantl; *Thlaspi arvense* L.; *Cakile baltica* Jord. ex Pobed. (*Cakile maritima* Scop.); *Erysimum cheiranthoides* L.; *Alliaria petiolata* (M. Bieb.) Cavara et Grande).

MATERIALS AND METHODS

Seeds of wild species were collected from all over the Lithuanian territory. The plant seeds were collected in the ripening period from June to September. Seeds of fanweed were the earliest to ripen and were collected from the middle of June till September. Seeds of flixweed, garlic mustard and sea rocket were collected in August. Of all the plants collected, seeds of wallflower mustard ripened and were collected last - in August and September.

Biochemical analyses were performed on the following numbers of samples: 19 samples of fanweed (*Thlaspi arvense*), eight samples of flixweed (*Descurainia sophia*), 17 samples of wallflower mustard (*Erysimum cheiranthoides*), one sample of sea rocket (*Cakile baltica*) and two samples of garlic mustard (*Alliaria petiolata*).

Investigation of seed oil content and composition were carried out at the Institute of Cattle-breeding of the Lithuanian Veterinary Academy. The amount of oil was determined by means of petroleum ether extraction using the equipment Soxterm Multistat/SX PC by "Gerhardt". To determine fatty acids, the oils were extracted by the Folch method (Folch & Less, 1957) and methylated with a 2% solution of sodium methylate (NaOMe), according to Christopherson & Glass (1969). The mixture of fatty acid methyl esters was analyzed using a gas chromatographer GC-2010 SHIMADZU with a hydrogen flame detector, using an Alltech capillary column AT^{TM}-FAME (30 m, ID: 0.25 mm) . Change of column temperature was programmed from 150^{o}C (starting from 3 minutes) to 240°C., speed of temperature increase was 4°C min^{-1}. Fatty acids were identified by duration of retention compared to durations of fatty acid retention in mixtures of known composition. Amount of fatty acids (% of total amount of acids) was calculated with "GCsolution", a chromatographic data processing programme . In the course of the research 20 fatty acids were identified. Fatty acids comprising less than 1% of total fatty acids have not been reported individually in the article and their sum (Table 1) was named -'other fatty acids'.

Data were processed statistically with the statistical package *Statistica 5*. Differences were determined using analysis of variance (*ANOVA*). The relationships among samples were analysed with cluster analysis based on the standardised values of oil contents and fatty acid (linoleic, linolenic, oleic and erucic) composition. Distance among samples was Euclidean and distance between clusters – were unweighted pair-group means.

Meteorological conditions. A hydrothermal coefficient (HTC) for the assessment of the seeds ripening period was calculated using the Seleninov formula :

HTC = P/0.1 T,

- P is the total amount of precipitation (mm) over a period,
- T is the sum of positive temperatures over the same period.

An HTC < 0.3 represents extremely intense drought, 0.4–0.5 intense drought, 0.6–0.7 moderate drought, 0.8–0.9 slight drought, 1.0–1.5 for optimal precipitation and > 1.6 for excessive precipitation (Dirsė, 2001).

Over the research years the most favourable conditions for plant growing were in the central region of Lithuania (Fig. 1), the least favourable dry conditions were in the eastern region of Lithuania. In July dry climatic conditions (HTC 0.4–1.0) prevailed all over the country except southern region. Wet conditions were observed in the western region in August.

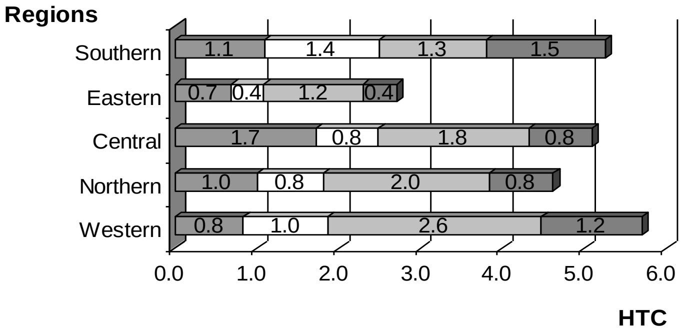

Fig. 1. June July August September Hydrotermical coefficient (HTC) of different Lithuania regions (2008).

RESULTS AND DISCUSSION

The greatest oil content of the studied species was found in the seeds of flixweed – 32.2% on average (Table 1) and that of separate samples reached 37.6%. Oil content of wallflower mustard was slightly less – it amounted to 30.1% and separate seed samples also reached 37.6%. Similar amounts of oil were determined in the seeds of sea rocket and fanweed – 25% and 25.5% respectively (20.10–33.47%). The least amount of oil (15.8%) was found in the seeds of garlic mustard. Oil variation in the studied seeds samples was not significant, variation coefficients were quite similar (Table 1) ranging from 12.7 to 14.6%. Of the two samples of garlic mustard studied, variation was slight – 5.1%.

According to the data presented by other authors, similar amounts of oil (30.3–32,2% (Dolya et a.,1973; Carr, 1993; Goffman et al.,1999)) were found in the seeds of flixweed; however, seed oilcontent of the genotypes studied by Peng et al. (1997) were as high as 44.2% (32.2–44.2). With reference to our research data, similar or slightly greater amounts of oil were found in the seeds of wallflower mustard and fanweed, while according to the data proposed by Isbell (2008), oil content in fanweed seeds amounted to 36%.

Table 1. Oil content and fatty acid composition in seeds of some Lithuanian wild crucifer species.

Content, %		*Thlaspi arvense*	*Erysimum cheiranthoides*	*Descurainia Sophia*	*Cakile baltica*	*Allaria petiolata*	*F*
Oil content in dry matter	m ± s	25.5	30.1	32.2	25.0	15.8	8.76*
	min–max	20.1–33.5	23.2–37.6	23.2–37.6		15.2–16.3	
Fatty acids: Linoleic	m ± s	20.9±0.6	27.2±1.1	18.3±1.3	19.6	18.7	165.5*
	min–max	19.7–21.9	24.7–29.0	16.5–20.9			
Linolenic	m ± s	12.5±0.9	29.1±1.4	42.1±1.0	23.9	6.6	1,434.8*
	min–max	11.7–13.5	26.2–32.0	41.0–44.2			
Oleic	m ± s	10.9±1.1	7.3±0.7	11.0±0.5	19.0	8.5	72.7*
	min–max	9.3–13.7	5.9–8.8	10.2–11.5			
Palmitic	m ± s	2.6±0.2	4.4±0.3	5.7±0.2	4.4	3.1	424.0*
	min–max	2.4–2.9	4.0–4.9	5.4–5.9			
Erucic	m ± s	35.6±1.6	18.9±1.0	7.5±0.4	18.2	49.3	961.9*
	min–max	32.2–38.2	16.5–20.8	7.0–8.2			
Stearic	m ± s	0.3±0.0	1.5±0.2	1.4±0.1	1.5	0.3	295.4*
	min–max	0.3–0.5	1.3–1.9	1.3–1.7			
Arachidic	m ± s	0.2±0.0	1.1±0.1	1.2±0.1	0.9	0.2	409.5*
	min–max	0.2–0.3	1.0–1.5	1.1–1.3			
Eicosenoic	m ± s	10.4±0.5	3.9±0.5	8.2±0.2	8.6	4.1	481.4*
	min–max	9.6–11.7	3.3–5.4	8.0–8.6	5.0		
Heneikoz	m ± s	1.8±0.1	1.6±0.2	0.7±0.1	0.6	0.6	104.4*
	min–max	1.4–2.1	1.3–1.9	0.6–0.9			
Arachidic	m ± s	0.4±0.1	1.1±0.2	1.0±0.0	0.8	0.8	87.7*
	min–max	0.3–0.6	0.4–1.2	1.0–1.1			
Nervonic	m ± s	3.0±0.2	1.5±0.2	0.6±0.1	0.7	6.3	429.7*
	min–max	2.6–3.5	1.3–1.8	0.5–0.7			
Other fatty acids	m ± s	1.47	2.4	2.2	1.9	1.7	

* $P < 0.005$

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Reference sources indicate that the oil content of sea rocket and garlic mustard seeds is greater, by up to 1.8 times (Miller et al., 1965; Dolya, et a.,1973; Ghars, 2008) and 1.4–1.7 times (Miller et al., 1965; Goffman et al.,1999), compared to that established by our research.

From the viewpoint of nutrition, the greatest amount of essential (Gruzdienė & Bagdonaitė, 2003) unsaturated acid (oleic, linolic, linoleic) was found in the oil of flixweed seeds – 71.4% on average (Table 1). A slightly less amount of acid was found in the oil of wallflower mustard (63.5%) and sea rocket (62.7%). The amount of unsaturated acid in fanweed was 58% on average. Among all the studied species, garlic mustard oil contained the lowest amount of unsaturated acid (33.8%). Oil from all the studied species was rich in linolic acid, this was especially characteristic of wallflower mustard oil. Evaluating the composition of fatty acids from the viewpoint of nutrition, linoleic acid, which is not synthesized in the human organism, is extremely important (Gruzdienė & Bagdonaitė, 2003). Flixweed oil was distinguishable with respect to the amount of linolenic acid; this index was far higher compared to the other studied species (from 6.4 to 1.4 times). In some samples of flixweed seeds oil the amount of linolenic acid topped 44%. Large amounts of this acid were also established in the wallflower mustard seeds (Table 1). Oil from fanweed and sea rocket seeds were the richest in oleic acid. The greatest amount of erucic acid, which is important from the technical point of view (Isbell, 1985), was found in oil from garlic mustard (49.31%) (Table 1). Fanweed oil amounted to 35.6% and was second highest with respect to the amount of erucic acid. The least amount of erucic acid (7.5%) was found in oil from flixweed seeds.

The variation in fatty acid composition of the studied species was small (variation coefficient was 2.4–9.8%).

Seed oil content and oil composition depend on variety, geographical location, local climatic and ecologic conditions, and meteorological conditions at the time of oil synthesis (Özer et al., 2003; Sudaraic & Vrataric, 2002). Comparing biochemical data of fatty acids obtained by us with data from other literature, it was noticed, that flixweed, wallflower mustard and sea rocket synthesized a lesser amount of erucic acid and a greater amount of unsaturated acids respectively, especially the amount of linoleic acid. Garlic mustard and sea rocket accumulated a greater amount of erucic acid and respectively a lesser amount of unsaturated acids, especially linoleic acid. Such differences in oil content and fatty acids composition might be caused by genotype and the ecogeographical conditions of Lithuania.

The cluster analysis of fatty acid composition and content of 45 *Brassicaceae* samples was used to produce a dendrogram (Fig. 2). The dendrogram clearly showed five main groups of samples corresponding to the five species studied. A similar dendrogram was obtained when data of essential fatty acids (namely linoleic, linolenic, oleic and erucic) as well as oil content was used (not shown). This confirms the effect of essential fatty acid composition on discrimination and characterization of the studied species samples. For a linkage distance higher than 3.5, two sub-clusters, can be defined in the cluster of *Descurainia Sophia,* and three in the cluster of *Erysimum cheiranthoides.*

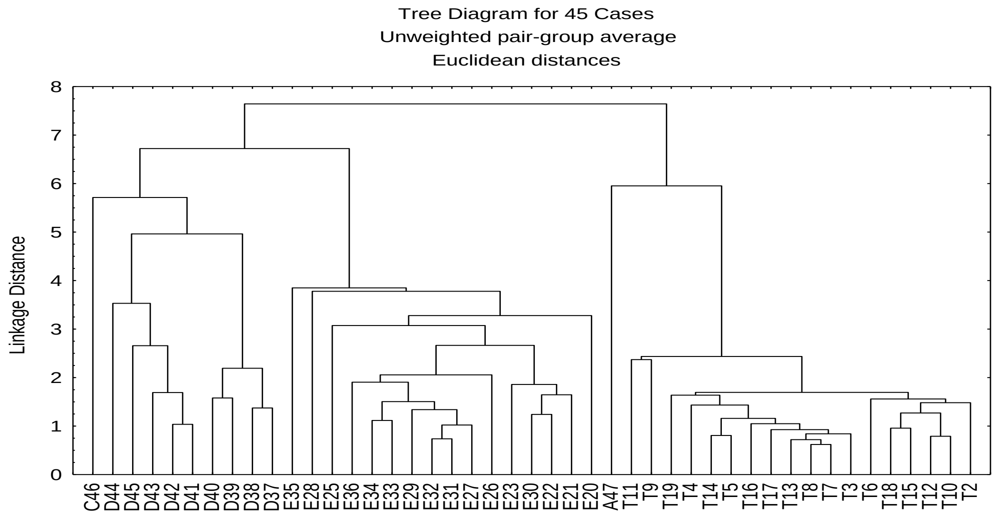

Fig. 2. Dendrogram of the *Brassicaceae* seed samples (from *Descurainia sophia* (D), *Cakile baltica* (C), *Erysimum cheiranthoides* (E), *Alliaria petiolata* (A) and *Thlaspi arvense* (T)) based on their oil content and fatty acid (linoleic, linolenic, oleic and erucic) composition.

The greatest differences were found among samples of *Descurainia sophia* (Fig. 2). Two sub-clusters had statistically significant differences (P < 0.05) in oil quantity (1st - 36.45% and 2nd - 27.9%) and in amounts of acids: arachidic (1.12% and 1.25%), behenic (0.36% and 0.41%), docosanoic (0.19% and 0.06%) and docosatetraenoic (no docosanoic acid was found in the oil of the 1st sub-cluster or the 2nd sub-cluster – 0.21%).

Seed oil content and oil composition depend on variety, geographic location, local climatic and ecologic conditions, and temperature at the time of oil synthesis. Samples of the obtained clusters could be attributed to different regions of Lithuania: 1st sub-cluster to the northern and central regions, 2nd cluster to the south-eastern, eastern and western regions. But the meteorological conditions of these regions also differed (Fig. 1). A moderately strong linear interdependence between amount of oil in seeds and the HTC (r = 0.78 (P < 0.05)) of July (when ripening of flixweed seeds and synthesis of oil occurred) was established. Similar correlations were also determined between the HTC of July and amount of arachidic, behenic, docosanoic, docosatetraenoic acid as well as between the amount of oil and these acids. Thus, it is difficult to state that two chemotypes of flixweed were found in different regions of Lithuania and that differences in amount of oil and fatty acids depended solely on ecological, especially meteorological, conditions of a site of cultivation.

With reference to the composition of fatty acids, three sub-clusters of wallflower mustard are distinguishable (Fig. 2). The 1st cluster comprises 85% of wallflower mustard samples, the other two include one sample each. Samples of the 3rd cluster differed from other fatty acids the most in terms of composition. Statistically significant differences from the 1st cluster were observed in the amounts of palmitic, palmitoleic and erucic acid. In the oil of the 2nd cluster there was recorded a sample of myristic acid (0.08%), which was not detected in any of the other samples. No interdependence between the seed ripening period, HTC and oil or fatty acid content was established.

According to the research data, variation of fanweed oil composition among different samples was considerably less compared to wallflower mustard and flixweed (Fig. 2). No interdependence between the seed ripening period, HTC and other investigated parameters was established. It was observed, however, that the amount of oil in samples was the least (20–21.95%) when HTC over the period of seed ripening was $\leq$ 0.6 (drought) and $\geq$ 2.6 (considerable excessive precipitation). In this case, both lack of moisture and its excess were limiting factors for oil synthesis. Moreover, a statistically significant correlation between the amount of linolenic acid in oil and HTC (r = 0.303) over the period of seed ripening was found. Also negative correlations between erucic (r = -0.34), tricosanoic (r = -0.52) and docosanoic (r = -0.30) acids and HTC were established. A negative, moderately strong, linear correlation between erucic acid, the main acid of fanweed oil, and oleic (r = -0.86, $P < 0.05$), linoleic (r = -0.73), eicosenoic (r = -0.71), tricosanoic (r = 0.85) acids was found.

CONCLUSIONS

The greatest amount of oil was found in the seeds of *Descurainia sophia* (32.2%) and *Erysimum cheiranthoides* (30.6%), the least amount was found in seeds of *Alliaria petiolata* (15.8%). In the seed oil of *Descurainia sophia*, *Erysimum cheiranthoides*, and *Cakile baltica*, unsaturated (oleic, linoleic, linolenic) oils dominated. The greatest amount of erucic acid was determined in the seed oil from *Alliaria petiolata* and *Thlaspi arvense*. Comparing biochemical data of fatty acids obtained by us with data from other literature from countries other than Lithuania, *Descurainia sophia*, *Erysimum cheiranthoide* and *Cakile baltica* synthesized less erucic acid and more unsaturated acids, especially linoleic acid. *Alliaria petiolata*, *Thlaspi arvense* accumulated more erucic acid and less unsaturated acids, especially linoleic acid.

The results obtained show intraspecific fluctuation amplitude of oil content and fatty acid composition of the investigated species. Investigations under conditions of a single environment and similar agro-technology would show morphological, biochemical and phenological differences among samples of the species and stability of the characteristics studied.

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