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Fatty Acid Composition of *Matthiola longipetala* ssp. *Bicornis* from Turkey

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ABSTRACT

The fatty acid composition and morphological characteristics of *Matthiola longipetala* ssp. *bicornis* was studied. Wild *Matthiola* plants and seeds were collected from its native areas in Turkey. The native plants were cultivated in two different sowing times (autumn sowing & late winter sowing) and some morphological characters and seed oil composition were measured. Plant height, number of siliqua, siliqua length, seed yield, thousand seeds weight and seed length values were found to be the highest in autumn sowings. The quantative analysis of seed oil of cultures and wild plants were compared and oil ratio were obtained as 21, 11 and 11% in autumn sowing, late winter sowing cultures and native plants respectively. γ-linolenic acid was found as main component at all *Matthiola* plants. The ratio of γ-linolenic acid was obtained 65.6, 63.0 and 62.6% in autumn sowing, late winter sowing and wild plants, respectively. The other main constituents of the all oils were obtained as oleic and linoleic acids. The ratios of oleic and linoleic acids were obtained 11.04% and 10.70% in autumn sowing, 13.18% and 11.53% in late winter sowing, 12.46% and 11.01% in wild plants, respectively.

Key Words: *Brassicaceae;* Fatty acid; Gamma-linolenic acid; *Matthiola*; Morphology

INTRODUCTION

The Brassicaceae (Cruciferae) family consists of 13-19 tribes, 350 genera and about 3500 species in the world (Onyilagho *et al.*, 2003). Turkey is one of the richest countries in the world in terms of the number of species of the Brassicaceae and with its 571 species it is second only to the United States, where there are 653 native species in 61 genera (Al-Shehbaz *et al.,* 2007). *Matthiola* is a member of Brassicaceae family and *Matthiola incana* is an important ornamental plant of the genus (Hisamatsu *et al.*, 1997). Several studies concerned with *M. incana* were focused on seed oil content, growth conditions and oil quality (Yaniv *et al.,* 1991; Yaniv *et al*., 1997; Heuer *et al.*, 2005; Dirmenci *et al.*, 2006; Hammami *et al.,* 2006; Kirimer *et al.*, 2006). Nine *Matthiola* L. species and three taxons were naturally found in Turkey. *M. longipetala* ssp. *bicornis* is a native plant for Turkey and distributed throughout North, Central, South and South-East Anatolia. Plant height is 50 cm, flowers are whitish or yellowish to purple and siliquae terete, up to 15 cm. Stigma has two long acute horns and one much shorter (Davis, 1982).

Current research in nutritional medicine indicates that the omega fatty acids are essential components of the human diet and the most important omega-6 fatty acid is gamma-linolenic acid (Sajilata *et al.*, 2008). It was shown that administration of γ-linolenic acids (GLA) from natural sources can correct both the biochemical abnormality and the clinical disorders (Levy *et al.*, 2001). Several plant species, such as evening primrose (*Oenothera biennis*), common borage (*Borago officinalis*), blackcurrant (*Ribes nigrum*) and various *Echium* species, also contain the GLA and stearidonic acid in their leaf lipids and as major components of their seed (Zhou *et al.*, 2006). Therefore, *O. biennis* was assented as one of the 25 additional foods for growth stimulation and GLA was sold in soft gel capsules with the amount of 500-1000 mg or in the other plant oils (Flider, 2005).

There are a few reports about *M. longipetala* and these studies were focused on the volatile oils and fenolic acids of this species. Furthermore Hammami *et al.* (2006) have worked about isolation and identification of three phenolic glycosides for the first time from the flowers of *M. longipetala*. Hammami *et al.* (2007) analyzed the chemical composition of the volatile parts of *M. longipetala* flowers. Most representative compounds found eugenol (19.93%), bicyclogermacrene (13.60%), heptacosane (7.85%) and tetradecanoic acid (5.57%).

In this study, morphological characteristics and seed oil compositions was quantified and evaluated in both wild and cultivated form *M. longipetala* ssp. *bicornis*. To the best of our knowledge, no information exists about morphological characteristics and seed oil composition of cultivated plants of *M. longipetala* ssp. *bicornis.*

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MATERIALS AND METHODS

Plant material: Wild *M. longipetala* ssp. *bicornis* plants and seeds were collected from native stands of Tahtakopru Dam and nearby area (latitude: 39° 57.0'; longitude: 29° 39.0') in Islahiye/Gaziantep–Turkey at an altitude of 500-550 m, in June 2005. Plants were identified according to Flora of Turkey and East Aegean Islands (Davis, 1982). Gaziantep Islahiye Tahtakopru Dam that collected the wild plant and seeds has a climate between Mediteranean and Territorial climate a Mediterranean climate (Table I).

Cultivation trials: The field trials were carried out at the experimental area of University of KSU Faculty of Science Avsar Campus, during 2005-2006. This location, in southern Turkey, has typical Mediterranean-type climatic conditions (latitude: 39° 57.0'; longitude: 29° 39.0' altitude 600 m). Average annual rainfall is 600 mm. The soil was characterized by low water capacity, low humus content.

Seeds of wild *M. longipetala* ssp. *bicornis* were sown to field in 2.1 m length with 30 cm between rows individual plots consisted of 4 m^2 areas. The experimental field was designed to be sowing at the different sowing times as followed: autumn (second week of October 2005) and end of winter (end of February). In each experiment, treatments were arranged in a randomized complete block design with three replications. Nitrogen fertilizer at the rate of 2.5 kg ha^{-1} as ammonium nitrate was applied before sowing. Field was irrigated under dry conditions during vegetation period. Plants were harvested when the pods were ripe (yellowish-brown). Before harvest, plant heights (cm) were measured and after each harvest the number of branches, peduncle length (mm), number of siliqua, siliqua width (mm), siliqua length (cm), seed width (mm), seed length (cm), seed yield (g/plant), yield of fruit in siliqua (number/siliqua), thousand seed weight (g) and ratio of fatty acid were determined. These morphological characteristics were measured using ten plants for each plot at two different sowing times also these characters were obtained wild plants collecting native area. Emergence, flowering and harvest date of *M. longipetala* ssp. *bicornis* are given in Table II.

Lipid extraction and methylation: *Matthiola* seeds were dried overnight at 50ºC and ground into powder in a Moulinex coffee grinder. Sample powder (5 g) was introduced into a cellulose cartridge and extracted in a Soxhlet apparatus with 250 mL of diethyl ether for 7 h. The solvent was evaporated on a rotatory evaporator at 40ºC and the lipid fraction residues were weighed (Punín Crespo & Lage Yusty, 2006; Özmen *et al.*, 2007). Three accessions were evaluated for each species and average values of these plants were recorded.

Two N (normal) KOH with methanol (2 g KOH/100 mL MeOH) and 0.1 g *Matthiola* oil were added in a 50 mL flask and boiled in a water bath for 25 min. Two mL of Pentane was added on the mixture and transferred into a separator funnel. Then pentane was removed under nitrogen stream (Kalender, 2002).

Gas chromatography of methylated fatty acids: Gas chromatography (GC) analysis was conducted in Control Laboratory in Mersin/Turkey. Qualification of the oil was analyzed with a Hewlett-Packard GC-6890II series GC with flame ionization detector. SE-54 fused silica capillary column (600 m x 0.25 mm i.d.; 0.25 μm film thickness) and nitrogen was used as a carrier gas with flow rate of 1.0 mL/min. One μL of the oil was injected into the column. The GC oven temperature was kept at 100ºC for 5 min and programmed to keep 200ºC at a rate of 2ºC/min and then kept at 200ºC. The injector temperature was 200ºC and the amount of injection was 1 μL.

Oil analysis was also repeated by GC-mass spectrometer (MS) in the Plant Physiology Laboratory in Biology Dept. of Kahramanmaras Sutcu Imam University and similar results were obtained. Qualification of the oil was analyzed on an Agilent 5975C MS coupled with an Agilent GC-6890II series. The GC was equipped with HP-88 capillary column (100 m x 0.25 mm i.d., 0.20 μm film thickness) and helium was used as carrier gas with flow rate of 1.0 mL/min. The GC oven temperature was programmed as follows: 170 (1 min), 220ºC at an increase of 5ºC/min, hold 10 min at 220ºC and 230ºC at of 10ºC/min then kept at 230ºC at 15 min. The injector temperature was 250ºC. The MS was operated in EI mode at 70 eV. Split ratio was 20:1. Mass range 35-400 m/z; scan speed (amu/s): 1000. The components of the oil were identified by comparing their retention indices and mass spectra with those of pure authentic samples and wiley7n.1, Famdbwax.L, Famedb23.L, libraries reference compounds.

Statistical analysis: Statistical evaluation of morphological analyses was performed using the SPSS 13.0 software and Independent-Samples T Test was accomplished for all data.

RESULTS AND DISCUSSION

Morphological characteristics: Vegetation times of autumn and end of winter sowings of *M. longipetala* ssp. *bicornis* were 240 and 139 days, respectively (Table II). Morphological characteristics of wild and cultivated *M. longipetala* ssp. *bicornis* indicated differences between wild plant, autumn and late winter sowings indicated significant ($P<0.05$) differences in plant height, number of siliqua, siliqua length, siliqua yield, seed length, peduncle length, seed width, number of seed in siluqua and thousand seed weight (Table III). Cultivation trials significantly influenced the morphological characteristics of *M. longipetala* ssp. *bicornis*. As general two cultivation experiments (autumn & late winter sowings) gave high morphological value. Irrigation, fertilization and peck up the ground to weed was increased the product yield. The highest values in morphological characteristics were obtained in autumn sowings, the longer growing period lead to the formation of more branches, flowers and seeds.

Plant heights of autumn sowing (22.0-40.0 cm) were higher than in late winter sowing (15-25 cm) and wild plants

Table I: Mean of air temperature and precipitation in Islahiye/Gaziantep during experimental years (2004-2005)

Months	Temperature (ºC)						Annual Rainfall (mm)		Humidity (%)	
	Min.		Max.		Mean					
	2004	2005	2004	2005	2004	2005	2004	2005	2004	2005
January	1.9	2.9	7.3	11.4	4.3	6.4	311.0	82.2	73.2	62.9
February	1.5	2.9	9.0	11.3	4.8	6.4	149.5	99.0	68.9	65.7
March	7.0	6.4	19.0	16.8	12.5	11.4	6.5	188.4	50.0	61.7
April	9.6	11.3	22.3	22.3	15.9	16.7	30.6	55.6	46.5	60.0
May	14.0	15.5	25.9	28.5	19.7	22.0	57.0	18.8	57.6	53.9
June	19.5	19.8	32.3	32.2	25.9	25.7	-	8.0	59.8	55.6
July	22.7	24.1	36.8	35.5	29.7	29.3	6.5	-	59.1	60.6
August	22.8	24.2	34.8	36.2	28.1	29.7	0.5	-	65.6	60.9
September	19.1	20.2	34.6	32.9	26.7	26.3	-	5.0	53.4	55.4
October	15.2	12.7	29.0	26.0	21.4	18.5	8.1	61.6	58.4	56.7
November	7.6	6.8	16.8	16.6	11.3	11.1	298.7	88.4	69.8	64.9
December	1.2	5.3	11.2	14.2	5.3	9.2	82.4	88.9	64.0	67.9

Table II: Emergence, flowering and harvest date of *M. longipetala* ssp. *bicornis*

Sowing date/locations	Emergence	Flowering	Harvest
Autumn sowing Kahramanmaras	14.10.2005	02.03.2006	08.06.2006
Late winter Kahramanmaras	02.03.2006	05.05.2006	12.07.2006

Table III: Minimum-Maximum and Average Values of Some Morphological Characteristics of *Matthiola longipetala* ssp. *bicornis*

Characteristics	Islahiye (Wild plant)			Kahramanmaras (Autumn sowing)			Kahramanmaras (Late winter sowing)				
	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Sig.	F Value
Plant height (cm)	18.00	32.00	25.00 ^{b}	22.00	40.00	36.00 ^{a}	15.00	25.00	19.70 ^{b}	***	18.00
Number of branches	1.00	4.00	2.60	2.00	4.00	3.00	2.00	8.00	4.00	NS	2.50
Peduncle length (mm)	1.20	2.00	1.60 ^{b}	1.20	3.00	2.00 ^{a}	1.20	2.90	1.90 ^{a}	*	3.70
Number of siliqua	8.00	32.00	20.80 ^{b}	42.00	287.00	136.50 ^{a}	10.00	50.00	27.00 ^{b}	***	11.00
Siliqua width (mm)	0.90	1.70	1.20	1.00	1.40	1.10	0.90	1.40	1.10	NS	2.20
Siliqua length (cm)	4.00	7.00	5.00 ^{b}	4.10	7.20	6.30 ^{a}	3.50	7.60	4.90 ^{b}	***	17.50
Seed width (mm)	0.90	1.70	1.10 ^{a}	0.70	1.30	0.90 ^{b}	0.70	1.20	1.00 ^{ab}	*	4.30
Seed length (cm)	1.50	2.00	1.70 ^{b}	1.50	2.00	1.90 ^{a}	1.50	2.30	1.80 ^{b}	**	8.00
Number of seed in siliqua (number/capsule)	20.00	40.00	26.00 ^{b}	14.00	40.00	32.00 ^{a}	10.00	36.00	25.00 ^{b}	*	4.00
Seed yield (g/plant)	0.01	0.30	0.10 ^{b}	0.65	3.00	1.60 ^{a}	0.10	0.70	0.30 ^{b}	***	12.70
Thousand seed weight (g)	0.29	0.52	0.37 ^{b}	0.39	0.63	0.44 ^{a}	0.31	0.55	0.38 ^{b}	*	2.06

*P<0.05, ** P<0.01, *** P<0.001, NS: Non Significant

Table IV: The oil amount value of *Matthiola longipetala* ssp. *bicornis* (%)

Sowing date/locations	Minimum	Maximum	Mean	F value	Significance
Autumn sowing Kahramanmaras	18.00	25.00	21.00 ^{a}	40.70	**
Late winter Kahramanmaras	10.00	13.00	11.00 ^{b}		
Wild plants Islahiye	9.00	13.00	11.00 ^{b}		

**P<0.01

(18-32 cm) but all the data were found to be lower than Davis (1982)'s report (50 cm). The number of siliqua in autumn sowing (136.5 siliqua/plant) was higher than late winter sowing and wild plant (27.0 & 20.8 siliqua/plant, respectively). Yaniv *et al.* (1997) were found that the siliqua number of *M. incana* between 18-87 siliqua/plant. Ecological variations such as altitude, location, light and temperature could affect the agronomical observations and more branches and taller plant height per plant directly influence the number of fruits/seeds per plant (Kizil, 2006).

Mean thousand seed weight of *Matthiola* plant in autumn sowing (0.44 g) were higher than late winter sowing (0.38 g) and wild plant (0.37 g). Max values for these two characteristics were obtained autumn sowing. Yaniv *et al.* (1997) were found that the thousand seed weight of *M. incana* between 1.2-2.4 g. An increase in seed length and width also contributed to increase in thousand seed weight of the plant. Max seed width was observed from wild (1.10 cm) and max seed length were obtained autumn sowing (1.90 cm). Especially siliqua number, siliqua length and thousand seeds weight were found the key factors that affect the crop yield. These characteristics were superior in the autumn sowing than late winter plants and natural plants, so we could expect that the yield of seed oil would be high in autumn sowings. Decreases in several properties such as plant height, thousand seed weight, seed yield, days to flower were reported as a result of delayed sowing (Ozturk, 2000; Karaguzel *et al.*, 2005; Firoz *et al.*, 2009).

Fatty acid composition: The oil amounts of cultivated and wild *M. longipetala* ssp. *bicornis* were given in Table IV.

Table V: Composition of fatty acids of the *Matthiola longipetala* ssp. *bicornis*

Fatty acid (%)		Sowing Date/locations		
	Carbon Number	Autumn sowing Kahramanmaras	Late winter Kahramanmaras	Wild plants Islahiye
Miristic acid	14:0	---	0.12	0.16
Pentadecanoic acid	15:0	---	---	0.03
Palmitic acid	16:0	5.24	4.73	5.47
Palmitoleic acid	16:1	---	0.33	0.33
Heptadecanoic acid	17:0	---	---	0.05
Cis-10 Heptadecenoic acid	17:1	---	---	0.04
Stearic acid	18:0	2.88	3.18	3.21
Oleic acid	18:1	11.04	13.18	12.46
Elaidic acid	18:1 trans9	2.27	2.23	2.45
Linoleic acid	18:2	10.70	11.53	11.01
Linolenic acid	18:3	0.31	0.35	0.34
Gamma-linolenic acid	18:3	65.64	63.02	62.61
Arachidic acid	20:0	0.24	0.27	0.30
11c-Eikosenoic acid	20:1	0.22	0.27	0.25
11c-4c-Eikosadienoic acid	20:2	0.20	---	0.09
Arachidonic acid	20:4	0.21	0.14	0.17
Behenic acid	22:0	---	---	0.08
Lignoseric acid	24:0	---	---	0.07
Sum		98.95	99.35	99.12

Table VI: The variance analysis result of important fatty acids of *Matthiola longipetala* ssp. *bicornis*

Fatty acid ratio and composition	Sowing Date/locations					
	Autumn sowing Kahramanmaras	Late winter Kahramanmaras	Wild plants Islahiye	F Value	Error	Sig.
Palmitic Acid	5.24 [a]	4.73 [a]	5.47 [a]	4.87	0.17	NS
Oleic Acid	11.04 [a]	13.18 [c]	12.46 [b]	79.49	4.71	**
Linoleic Acid	10.70 [a]	11.53 [a]	11.01 [a]	2.48	2.48	NS
Linolenic Acid	0.31 [a]	0.35 [a]	0.34 [a]	2.16	0.01	NS
Gamma Linolenic Acid	65.64 [a]	63.02 [a]	62.61 [a]	51.97	0.31	**

The sowing time had a significant influence on seed oil ratio. The oil ratio of *M. longipetala* ssp. *bicornis* seed was 21% for autumn sowings, 11% for late winter sowing and wild plants ($P<0.05$). The oil ratios of *M. incana* were found 21-29% and 11.09% by Yaniv *et al*. (1997) and Heuer *et al.* (2005), respectively.

As regards fatty acids, they were greater in wild plants. Palmitic, stearic, oleic, elaidic, linoleic, linolenic, γ-linolenic, arachidic, 11c- eicosenoic, arachidonic acids were found in all samples: however, pentadecanoic, heptadecanoic, cis-10-heptadecanoic, behenic, lignoceric acids were detected in wild plants only. No miristic and palmitoleic acids were found in autumn sowing whereas 11-c, 14-c-eicosadienoic acid was absent in late winter sowing (Table V).

The main components of *M. longipetala* ssp. *bicornis* were found to be palmitic, oleic, linoleic and γ-linolenic acids therefore, statistical analyses were done on these four components (Table VI). γ-linolenic acid was found as the main component in *M. longipetala* subs. *bicornis* oils at each three experiments and GLA ratio of plants in autumn sowing (65.64%) was highest than late winter sowing (63.02%) and wild plants (62.62%). GLA ratio was found 68% and 43% in *M. incana* (Yaniv *et al.,* 1997) and *M. tricuspidata* (Heuer *et al.,* 2002), respectively. Yaniv *et al.* (1997) reported that the coolest temperatures prevailing the maturation period (May-June) and inducing increase in the level of GLA, maturation should occur under a much cooler temperature. Oleic acid ratio (13.18%) was found different in cultivated and wild plants ($P<0.01$). Oleic and linoleic acid ratios of *M. incana* were found 13% and 11%, respectively (Yaniv *et al.*, 1997) and these values were similar with our results. However, oleic (26.57%) and linoleic (12.44%) acid ratios of *M. tricuspidata* (Heuer *et al.*, 2002) were higher than *M. longipetala* ssp. *bicornis.* Palmitic acid ratio of *M. longipetala* ssp. *bicornis* in this study was found to be lower than *M. incana* (9%) and *M. tricuspidata* (9.5%) (Yaniv *et al*., 1997; Heuer *et al.*, 2002).

Harvesting and sowing time are critical operation especially in plants with indeterminate inflorescence and high seed shattering during ripeness. Levy *et al.* (1993) reported that *Oenothera* plants as a GLA source, the degree of desuturation of the fatty acid composition of the plant is inversely related to temperature prevailing during seed maturation. As confirmed by Yaniv *et al.* (1997), cold temperature increases the GLA content in *M. incana*. Various factors that affect effective substance amount in plant were indicated (Ceylan, 1994). Oil ratio and chemical components could change according to plant parts, maturing periods, echological factors and genetic structure (Unger, 1980; Nagao & Yamazaki 1984; Ceylan, 1994).

CONCLUSION

Matthiola longipetala ssp. *bicornis* has the very high

GLA content and this plant must be investigated for biological activity and medicinal uses. *M. longipetala* ssp. *bicornis*, grown in fall had better results. However, more studies must be done about the culturing time to get more yields.

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