Morphological and Chemical Variation of *Origanum vulgare* L. from Lithuania

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Abstract

The germplasm of oregano has been gathered from indigenous populations and examined in the field collection. Infraspecific variability of oregano has been identified at phenotype level by morphometric and chemical methods. ANOVA indicated significant differences for majority of variables. On the basis of morphological characters, the accessions of oregano have been attributed to *O. vulgare* subsp. *vulgare* L. The essential oil isolated from aerial parts was analysed by GC and GC-MS. The accessions varied highly in essential oil content (0.25-1.51%). The results of essential oil analysis show rich composition and high variation of oil compounds. The populations of oregano found in Lithuania could be defined as sabinene - β-ocimene - β-caryophyllene - germacrene D chemotype. One can expect that the germplasm of *O. vulgare* is a potentially important source of genetic variation. Indigenous populations of oregano might be interesting for breeding of winter hardy varieties of this species.

INTRODUCTION

The genus *Origanum* L. includes annual, biennial or perennial herbs that occur mostly in warm and mountainous areas. *Origanum vulgare* L. is the most wide spread among all the species within the genus. It is distributed all over Europe, West and Central Asia up to Taiwan (Ietswaart, 1980). *O. vulgare* plays a primary role among culinary herbs in world trade (Oliver, 1997). Recent findings report the antibacterial, fungicidal and antioxidant properties of oregano (Bernáth, 1997; Paster et al., 1995). Most of the commercial oregano comes from wild populations in Turkey and Greece (Arnold et al., 1993; Skoula and Kamenopoulos, 1997). Despite its economic importance the genetic resources and variability, its potential for utilization have not yet been fully explored (Putievsky et al., 1997). This is due to its high heterogeneity and complex taxonomy. A number of studies have shown that variation within species may occur in its morphological and chemical features (Chalchat and Pasquier, 1998; D'Antuono et al., 2000; Kokkini et al., 1994, 1997a,1997b; Skoula et al., 1999). Moreover, the research on germplasm conservation is very sparse outside the Mediterranean region. On the other hand, there is a need to have a representation of the whole genetic diversity of the genus and not just of what might be of interest to the industrial sector.

Populations of *O. vulgare* in Lithuania are characterized by limited distribution and by low sources of raw material. The plant is treated as a threatened species, collection from the wild is regulated by legislation (Anonymous, 1999). The habitats of oregano are open, sunny slopes, and mesotrophic meadows. It prefers slightly acid and neutral soils.

The aim of this study was to characterize the populations of *O. vulgare* growing in Lithuania based on morphological, agronomic, and chemical data obtained using multivariate methods.

MATERIALS AND METHODS

The accessions of oregano (in total 14) were gathered from indigenous populations during the field trips in 1996-2001. All accessions were multiplied and transplanted in 2001 using mother plant roots. They were planted at 50 cm and 20 cm distance between rows and within rows, respectively. The collection holds two accessions of foreign origin (No. 270 from Holland and No. 298 from Poland) and the other two (No. 260, 397) of local long time cultivation.

The characterization of accessions has been carried out according to the morphometric analysis of phenotype and content as well as composition of essential oil. The examination of the morphological and yield characters was performed on reproductive shoots at the stage of full flowering. A total of 10 traits related to the agronomic and morphological characters were measured in 30 randomly selected individuals. The measures of length and weight of the leaves apply to the middle leaf of the shoot. The color of flowers was estimated according to the standard color chart of Royal Horticultural Society (1995). The botanical identification of the plants was based on the description of subspecies, given by Ietswaart (1980). Voucher specimens are kept in the herbarium of the Institute of Botany (BILAS).

The essential oil was obtained from the air-dried inflorescences and leaves by hydro distillation for two hours. The essential oil content was expressed in percent of air-dried plant material. The oil was stored at 20°C until analysis. GC and GC-MS were used to analyse the essential oils of inflorescences of field accessions. The Carlo Erba-Fisons 8261 chromatograph with a flame ionization detector (FID) on a silica capillary column DB-5, 0.32 mm x 25 m was used. Detector temperature was 260°C, the initial column temperature was at 40°C and increased to 250°C at the rate of 4°C/min. Helium was used as a carrier gas at a flow rate of 1.6 ml/min.

Differences among accessions were tested by one-way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Morphological Variation

On the basis of morphological characters oregano growing in Lithuania could be attributed to *O. vulgare* L. ssp. *vulgare*, though the examined accessions were highly variable in almost all discriminative characters. Bracts ovate with acuminate tops, hirtellous, membranous, purple or greenish violet, highly heterogeneous in size - 2.5-5.5 mm long and 1-3 mm wide. Calyces were more or less homogenous in length (2.5-3.0 mm), with glandular trichomes, and violet tooth. Corollas were 2.5-6.0 mm long. The wide variation in color of corollas was observed, ranging from red-purple to purple. Although the bisexual flowers are common, in some plants female as well as male flowers have been found. They were smaller in size and more intensive in color of upper lip. Spikes ovoid, gathered in the paniculate type of inflorescence. Leaves ovate with acute tops, 10-50 mm long, 5-25 mm wide with inconspicuous sessile glands.

The one-way analysis of variance (ANOVA) revealed significant differences ($p<0.001$) among accessions within all of measured characters except the length of calyces (Table 1). The highest variability was detected for height of shoots ($F=38.34$), and length ($F=27.80$) and width ($F=15.64$) of leaves. The boxplots represented distribution of some characters among accessions (Fig. 1). The interquartile ranges between minimum and maximum value markers illustrate wide intervals of parameter variation.

The height of plants was the most variable parameter and the average height of accessions fluctuated from 18 to 59 cm. The lowest plants were those of accession No. 396 and the highest ones of accession No. 394. Length of inflorescences and weight of raw material are perhaps the most important parameters when the productivity of an accession is estimated. The lowest average weight was of accessions No. 340, 260 and 270 and the highest was of accession No. 280. The difference of the mean weight of raw material among other accessions was statistically insignificant. Many accessions, especially No. 271, 280, 279 and 395 were very heterogeneous in respect of this parameter. Accessions No. 281, 340, 397 were highly homogeneous.

Chemical Variation

The previous studies on *O. vulgare* from the Mediterranean indicated rich content of essential oil with carvacrol as a major constituent presenting in very high quantity (De Mastro, 1997; Kokkini et al., 1994). The essential oil content of the investigated accessions ranged from 0.25 to 1.51% in inflorescences and from 0.09 to 0.98% in leaves. The investigated accessions could be attributed to the low essential oil types of this species. The results confirmed the findings known for ssp. *vulgare* (D'Antuono, 2000; Kokkini, 1997a). The variation in the average amount of essential oil among wild populations and field accessions is shown in Fig. 2. The t-test comparison showed that the difference of values in oil yield obtained from wild populations and field accessions is not statistically significant, which confirms an assumption of genetic stability of this character (Table 2). Some of the accessions (No. 397, 395, 337) distinguished themselves by sufficiently high amount of essential oil. Low oil content was observed in both foreign accessions (No. 298 and 270).

The identified components of the essential oils of inflorescences are listed in Table 3. The oils were a complex mixture of about 60 compounds, 43 of which were identified representing more than 90% of the total essential oil. Quantitatively, the most important components (>5%) in essential oil were β-caryophyllene, germacrene D, sabinene, β-ocimene, and terpinen-4-ol. As some authors have reported, carvacrol, p-cymene, γ-terpinene, sabinene, cis-ocimene (Gounaris et al., 2002; Lawrence and Reynolds, 1984), or germacrene, terpinen-4-ol, β-bisabolene (Sezik et al., 1993) dominate in essential oil of ssp. *vulgare*. Two compounds from previous list (carvacrol and β-bisabolene) were not detected in our oil analyses.

The use of *O. vulgare* as medicinal plant is believed to be due to biological properties of p-cymene and carvacrol. Bernáth (2002) has noted that there are intraspecific taxa of oregano having no "oregano" character that is based on the presence of carvacrol. Investigated populations could be attributed to the kind of plants that did not contain carvacrol. All our accessions showed large proportions of mono- and sesquiterpenes hydrocarbons, while there were only a few oxygenated components in the essential oil. The monoterpenes hydrocarbons were represented by sabinene (5.99-10.09%), β-ocimene (2.22-15.32%), γ-terpinene (2.36-4.13%) and in much lower concentrations of p-cymene (0.52-4.60%), α-thujene (0.40-1.60%), α and β-pinene (0.45-0.74% and 0.72-0.90%, respectively), myrcene (1.23-2.38%), and limonene (0.82-1.10%). The sesquiterpenes fraction was represented mainly by β-caryophyllene (12.64-20.17%), germacrene D (6.63-8.62%). The main oxygenated monoterpenes were 1,8-cineole (1.24-5.58%) and terpinen-4-ol (2.42-9.20%), while α-cadinol (2.50-3.45%) was the major oxygenated sesquiterpene. The relative quantity of constituents (totalling 19) did not exceed 1% of the essential oil.

The results obtained showed differences among the accessions of oregano in respect to morphological traits and chemical constituents of essential oils, indicating the existence of infraspecific variation and chemical polymorphism. The populations of *O. vulgare* ssp. *vulgare* could be defined as sabinene - β-ocimene - β-caryophyllene - germacrene D chemotype. The oregano found in Lithuania is considered to be a poor source of essential oils though of good resistance to the cold and could be used for breeding of winter hardy varieties of this species.

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Tables

Table 1. Summary statistics of the *Origanum vulgare* characters and the level of differentiation of accessions on each character according to ANOVA Fisher's statistics (F).

Characters	Mean	SE	Range of means	F
Long of calyx (mm)	2.69	0.22	2.5-3.0	0.92
Long of corolla (mm)	3.34	1.21	2.5-6.0	8.11
Long of bract (mm)	4.14	0.84	2.5-5.5	6.35
Width of bract (mm)	1.86	0.61	1.0-3.0	3.35
Height of shoot (cm)	38.63	0.38	18.0-59.0	38.34
Number of internodes	12.04	0.11	7.0-18.0	10.68
Length of leaf (mm)	26.7	0.04	10-50	27.80
Width of leaf (mm)	13.8	0.02	5-25	15.64
Length of inflorescence (cm)	19.1	0.38	2.0-40.0	14.28
Weight of raw material (g) (part of shoot 30 cm from the top)	7.56	0.27	0.54-32.6	15.93

Table 2. The t-test comparison of essential oil content in *Origanum vulgare* wild populations and field accessions.

Part of plant	Wild populations		Field accessions		t	df	P
	Mean	SE	Mean	SE			
Inflorescences	0.82	0.107	0.75	0.095	0.43	12	0.675
Leaves	0.52	0.118	0.36	0.005	1.195	12	0.255

SE - standard error; t - Student's statistic; df - degree of freed; P - significant level

Table 3. Percentage composition of the essential oils (GC area %) of *Origanum vulgare* from Lithuania.

Component	R_t	Range	M	SD
α-thujene	5.17	0.40-1.60	1.24	0.00
α-pinene	5.32	0.45-0.74	0.65	0.18
sabinene	**6.31**	**5.99-10.09**	**7.45**	**2.23**
1-octen-3-ol	6.55	0.33-0.54	0.41	0.01
β-pinene	6.69	0.72-0.90	0.78	0.01
myrcene	6.90	1.23-2.38	1.94	0.63
3-octanone	7.20	0.25-0.41	0.34	0.05
p-cymene	7.45	0.52-4.60	3.35	0.55
linonene	7.60	0.82-1.10	0.94	0.23
1,8-cineole	7.82	1.24-5.58	4.32	1.40
(Z)-β-ocimene	**7.96**	**2.22-10.60**	**8.63**	**1.70**
(E)-β-ocimene	**8.45**	**7.57-15.32**	**12.11**	**2.56**
γ-terpinene	8.69	2.36-4.13	3.02	0.54
cis-sabinene hydrate	8.90	0.86-1.2	1.02	0.03
terpinolene	9.37	0.55-0.67	0.62	0.02
trans-sabinene hydrate	9.89	3.26-5.43	4.65	0.24
linalool	10.15	0.9-1.21	1.11	0.02
cis-p-menth-2-en-1-ol	10.40	0.22-0.45	0.32	0.01
allo-ocimene	10.52	0.25-0.38	0.33	0.01
trans-p-menth-2-en-1-ol	10.96	0.02-0.16	0.09	0.00
terpinen-4-ol	12.21	2.42-9.20	6.42	1.05
α-terpeniol	12.52	0.49-1.64	1.22	0.21
δ-elemene	16.37	0.05-0.18	0.11	0.01
α-copaene	17.43	0.08-0.11	0.10	0.03
β-bourbonene	17.69	0.10-0.77	0.56	0.02
β-elemene	17.94	0.12-0.19	0.14	0.01
β-caryophyllene	**18.98**	**12.64-20.17**	**17.50**	**2.31**
aromadendrene	19.31	0.02-0.07	0.04	0.00
β-gurjunene	19.46	0.05-0.22	0.15	0.01
α-humulene	19.61	0.77-1.01	0.86	0.23
allo-aromadendrene	19.77	0.33-1.17	0.93	0.25
germacrene D	**20.47**	**6.63-8.62**	**7.14**	**0.56**
α-amorphene	20.60	0.05-0.12	0.10	0.00
bicyclogermacrene	20.76	1.04-6.00	4.11	0.86
(E,E)-α-franesene	21.07	1.53-6.24	4.25	1.21
δ-cardinene	21.42	0.55-0.76	0.65	0.02
germacrene D-4-ol	22.21	1.13-1.56	1.25	0.51
spathulenol	22.93	2.26-2.50	2.32	0.23
caryophyllene oxide	23.0002	1.11-3.47	2.11	0.86
humulene epoxide II	23.50	0.02-0.13	0.08	0.01
epi-α-muurolol	24.66	0.77-0.86	0.81	0.12
6,10,14-trimethyl-2-pentadecanone	28.60	0.03-0.08	0.05	0.01
α-cadinol	30.20	2.50-3.45	2.91	0.74
Total, %		89.3-95.7	93.4	1.52

Figures

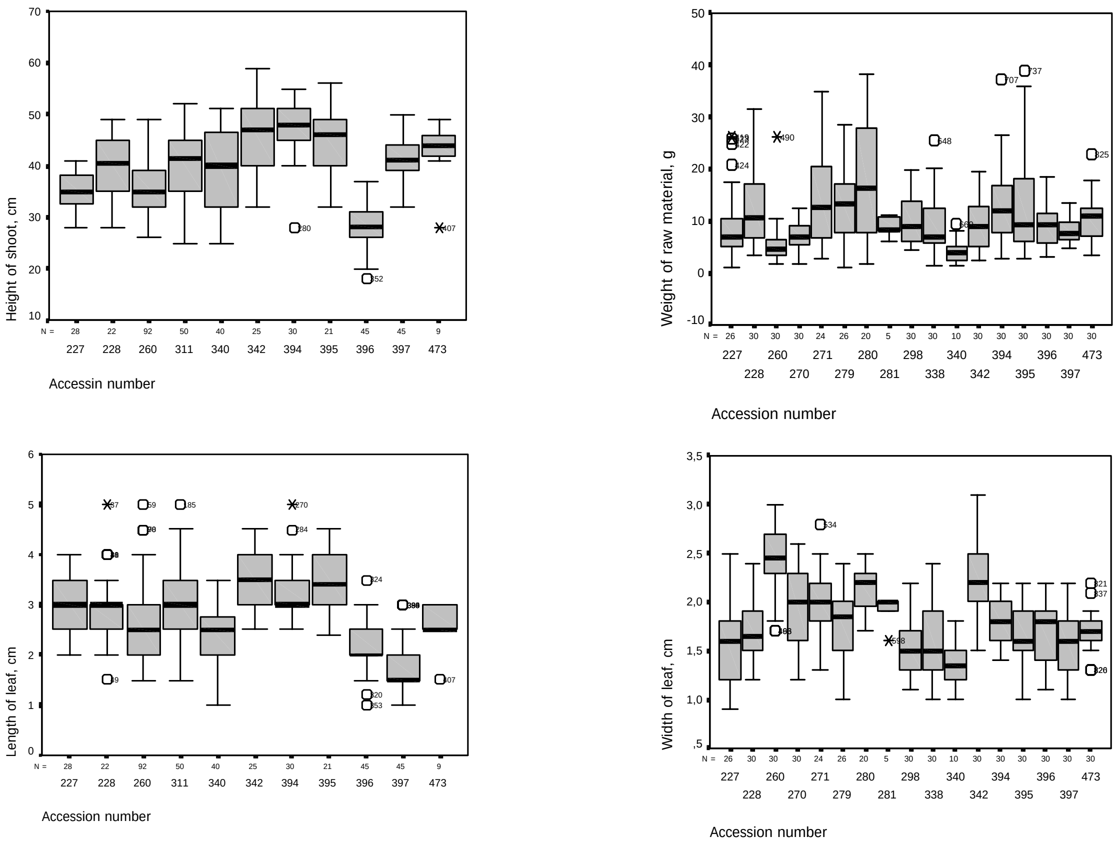

Fig. 1. Boxplots of *Origanum vulgare* height of shoots, weight of raw material, leaf length and width.

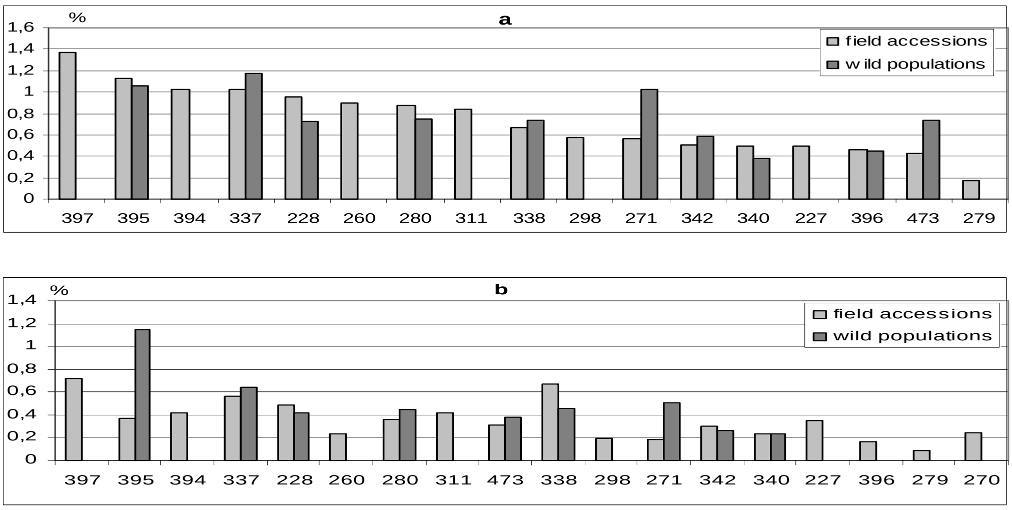

Fig. 2. The average amount of essential oil in inflorescences (a) and leaves (b) of *Origanum vulgare* wild populations and field accessions in 1998-2001.