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Some morphological and chemical characteristics of oregano (*Origanum vulgare* L.) in Latvia

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Abstract. By European Cooperative Programme for Plant Genetic Resources (ECPGR), oregano (*Origanum vulgare* L.) is included on the list of priority species of medicinal and aromatic plants. In Latvia, it is important to cultivate oregano for keeping biodiversity and for meeting the needs of medicinal plant`s production. 44 accessions of oregano from the *ex situ* collection of genetic resources of medicinal and aromatic plants, attached to the Latvia University of Life Sciences and Technologies, were analysed during 2012–2014. Plants` morphological characteristics were described by the Draft Descriptor List of oregano, using the methodology of ECPGR. The essential oil was isolated using solvent-free microwave extraction method and analysed by gas chromatograph Hewlett Packard 6890 equipped with flame ionization detector FID and polar capillary column HP 20M. The results showed, that oregano accessions differ morphologically. Accessions are characterized with dense branching and the possibility to create big biomass. Local oregano is poor in content of essential oil, but 17 compounds were identified as the principal. As well as the correlation between the content of essential oil and colour of flowers in full flowering stage was observed – it is higher for accessions with dark flowers. Also, the influence of meteorological conditions per vegetation period (year) on chemical characteristics was significant ($p < 0.05$).

Key words: essential oil, Draft Descriptor List, accessions.

INTRODUCTION

Oregano (*Origanum* spp.) is one of the most used medicinal, spice- and ornamental plants in the world (Hammer & Spahillari, 2000). Oregano can be used in the development of a sustainable agrosystem and for the reconstruction of over-cropped land (Asdal et al., 2006). By European Cooperative Programme for Plant Genetic Resources (ECPGR), oregano (*Origanum vulgare* L.) is included on the list of priority species of medicinal and aromatic plants. In Europe, also in Latvia, wild populations of oregano are limited. For meeting the needs of oregano production as well as for preservation of local biodiversity, oregano should be grown in conventional and organic cultivation systems (Asdal et al., 2006).

Oregano cultivation needs to get as rich and qualitative biomass as possible. It is better to cultivate the genetic resources of medicinal plants collected from natural habitats as they are adapted to the local agroecological conditions and possible stress situations in a specific environment. That is why oregano genetic resources must be explored carefully with the aim to select the most productive plants. It is important to evaluate the quantitative and qualitative indices of cultivated accessions, their productivity, organoleptic and biochemical properties, winter hardiness, resistance to diseases and pests, biotic stress susceptibility and other parameters (Sivicka et al., 2015).

Oregano plants bloom from June to the end of vegetation, but during August produce seeds. Because of heterogeneity, it creates various, morphologically and chemically different forms, which are related to the place of their occurrence (Asdal et al., 2006; Nurzyńska-Wierdak, 2009). Oregano differs by colour of flowers and leaves, in a density of inflorescence as well as in plant height and other indices (Droushiotis & Dell, 2002).

The essential oil is a complex mixture of volatile compounds that are present in aromatic plants (Leyva-López et al., 2017). Oregano essential oil is characterized by changeable chemical composition, depending on the population or variation, cultivation conditions and developmental stage of plants in herb harvesting period (Osińska et al., 2000; Nurzyńska-Wierdak, 2009).

An International Standard, which specifies the quality requirement for dried oregano, claims that whole oregano must contain 1.8% of essential oil and ground oregano – about 1.5% of essential oil (Tucker & Rollins, 1989). Oregano essential oil has antimicrobial and antioxidant agents as well as antioxidant properties, effective in retarding lipid peroxidation and scavenging free radicals (Ortega-Ramirez et al., 2016). The main components are terpenes, but aldehydes, alcohols and esters are also presented as minor components (Leyva-López et al., 2017).

The essential oil composition is variable with obvious appearances of different chemotypes (Asdal et al., 2006; Lukas et al., 2011). The research of European oregano genetic resources showed, that by main compounds it is possible to define carvacrol/thymol, linalool and sesquiterpene oregano chemotypes (Lukas et al., 2011). The chemical composition of oregano essential oil is genetically controlled (D'Antuono et al., 2000; De Mastro et al., 2004; Węglarz et al., 2006; Lukas et al., 2011).

Oregano is covered with glandular trichomes (hairs) which serve as a reserve of essential oil. The trichomes are easily exposed with the help of a microscope. Thus, it is possible to prognose the content of essential oil. De Mastro et al. (2004) report that 1 cm^3 of oregano leaf blade contain from 207 to 734 oil hairs and the amount of essential oil in dry herb varies from 0.50 to 5.1%, depending on the population.

By Nurzyńska-Wierdak (2009), the content of oil systematically increased with the progressive development of the plant, reaching its maximum in the flowering period. The lack of significant differences between mean essential oil contents in oregano herb harvested in the initial phase of flowering and in full bloom was founded in this research. It means, that harvesting plants in full blooming stage make it possible to obtain high quality yield.

Previous researches with Latvian oregano showed the differences in biochemical compounds between accessions inside one natural population. It is important to understand the influence of the colour of petals on the content and composition of essential oil of oregano accession.

The aim of this research was to explore some morphological and chemical characteristics of oregano (*Origanum vulgare* L.) in Latvia.

MATERIALS AND METHODS

Plant Material and Growing Conditions. The samples for the experiment were selected from an *ex situ* collection of spice- and medicinal plants (latitude: N 56º39´47´´; longitude: E 23º45´13´´), attached to the Latvia University of Life Science and Technologies. There are 44 accessions of oregano genetic resources in this collection. The plants had been collected from nature in 2001–2006, using the modified method of Professor E. Muižarāja (Žukauska, 2008). The main point of this method is the initial visual division of an area into squares and zigzag passing through these squares as well as the random gathering of samples.

In spring 2012, 44 accessions were propagated by cloning and grown in the field conditions. After methodology of Draft Descriptor List *Origanum vulgare* L., plants` morphological characteristics were described in 2012–2014 (Žukauska & Sivicka, 2011). For research in essential oil, samples had been dried at +26 °C temperature in a special drying cabinet with ventilation.

The soil at the trial site was strongly altered by cultivation loam with organic matter content of 2.7 g kg^{-1}, soil reaction pH$_{KCl}$ 6.3, the phosphorus (P) content was 102 g kg^{-1} and potassium (K) level content was 207 g kg^{-1}. Plant care was provided for this collection.

Meteorological Conditions. According to data of the Latvian Environment, Geology and Meteorology Centre, the average air temperature in 2012 was +6.1 °C (0.2 °C above long-term average observations), +7.0 °C (1.1 °C above long-term average observations) in 2013 and +7.4 °C (1.5 °C above long-term average observations) in 2014. The quantity of rainfall was 832 mm (125% of normal) in 2012, 622 mm or 94% of normal in 2013 and in 2014–725 mm (107% of normal). In vegetation period (from May to the end of September), the average temperature was 14.3 °C and the total quantity of rainfall was about 373 mm in 2012, 11.4 °C and 330 mm in 2013 and 12.3 °C and 441.6 mm in 2014.

Isolation of essential oil. The research in oregano essential oil was made in the Department of Vegetable and Medicinal Plants of the Warsaw University of Life Sciences.

Isolation process description. Glass vessel (2 L) was filled with dry material (50 g). This method suggests adding as much water how much air-dried raw materials are. The risk of possible ignition of raw material is eliminated by this method. Prepared glass vessel with the moistened sample was placed in a microwave oven with a power 300 W for 30 minutes. The essential oil was extracted and collected in 2 mL glass vials for storage without light at 0–4 °C.

Chromatographic separation conditions. The analysis of distilled essential oils was performed using a gas chromatograph Hewlett Packard 6890 equipped with flame ionization detector FID and polar capillary column HP 20M. The separation conditions were: initial oven temperature 60 °C for 2 min, then the temperature increases of 4 °C per min. Injector chamber temperature was 220 °C for 5 min. As a carrier gas helium was used (1.1 mL per min). Injector chamber temperature was 210 °C, detector – 260 °C. Split 1:70. The essential oil was injected (0.1 µL) manually into the chromatography column. Reference mixtures components were identified based on the retention times of the patterns (RT) and by comparing their retention indices (RI) to a series of n-alkanes (C7-C30), analysed in the above described separation conditions, using the following formula:

$$\mathrm{RI} = 100\,\frac{\mathrm{tR`(x)} - \mathrm{tR`(n)}}{\mathrm{tR`\,(m)} - \mathrm{tR`(n)}} + 100\mathrm{n} \qquad (1)$$

where n – the number of carbon atoms in the smaller n-alkane, eluting before target compound; $\mathrm{tR`(x)}$ – retention time of target compound; $\mathrm{tR`(n)}$ – retention time of the reference n-alkane eluting immediately before targeting chemical compound; $\mathrm{tR`\,(m)}$ – retention time of the reference n-alkane eluting immediately after targeting chemical compound.

To obtain the percentage of the individual compounds in the essential oil normalization method was used without using the correction factor.

Data are presented as the mean, and diferences among means were determined by analysis of variance (ANOVA), using Microsoft Office Excel 16.0 version software, and comparisons were made with Fisher test ($p < 0.05$).

RESULTS AND DISCUSSION

Describing oregano accessions, the differences were found in characters as \`plant height\` and \`width of inflorescence\`. The plant height varied from 50.1 to 85.0 cm in 2012, from 45.0 to 75.2 cm in 2013, from 30 to 85.9 cm in 2014. In average, it was 68.14 cm in 2012, 59.7 cm in 2013, 74.12 cm in 2014 – more than the results in other research (Nurzyńska-Wierdak). It is recommended to cultivate oregano with a plant height up to 50 cm as productive accessions (Sivicka & Žukauska, 2011). The width of inflorescence varied from 8.4 to 15.8 cm in 2012, from 7.3 to 20.0 cm in 2013, from 9.5 to 20.4 cm in 2014. In average, it was 12.6 cm in 2012, 17.2 cm in 2013, 11.15 cm in 2014. It is recommended to cultivate oregano with width of inflorescence up to 10 cm in agrocenosis us productive accessions (Sivicka & Žukauska, 2011). Considering these two parameters, the data statistical analysis showed that the variability between accessions was significant ($p < 0.05$), but between samples of each accession - non-significant ($p > 0.05$). The variability between years was significant ($p < 0.05$).

Plant growth habit was prostate for 4 accessions (9%), semi-erect for 10 (23%) and erect for 30 (68%) of the accessions. It is recommended to grow plants with erect habitus in agrocenosis for mechanical harvest (Sivicka & Žukauska, 2011). Branching density was dense for 35 accessions (79%) of accession, for 9 (21%) it was sparse. It means, that Latvian oregano can produce high fresh biomass. The previous research showed, that plants created the average fresh biomass 12.72 g per plant in 2012, 127.50 g per plant in 2013 and 195.08 g per plant in 2014, but the average air-dry biomass per plant was 5.11 g in 2012, 55.90 g in 2013, 77.96 g in 2014 (Sivicka et al., 2015).

The density of the flowers was very sparse for 2 accessions (5%), sparse – for 5 accessions (11%), medium – for 10 accessions (23%), dense – for 15 accessions (34%) and very dense – for 12 accessions (27%). Colour of petals was pink for 34 accessions (77%), for others it varied from white to lilac. For understanding the influence of petals\` colour on the content and composition of oregano essential oil, accessions were grouped as dark-, semi-dark- and light-coloured petals for analysis (Table 1).

Results showed, that local oregano is poor in the essential oil. But the higher content of essential oil was observed for oregano with dark flowers. Also, the influence of meteorological conditions per vegetation period (year) on chemical characteristics was significant ($p < 0.05$). In scientific literature it was proved that during the vegetation period the influence of air temperature from +20 to +30 °C and of the quantity of rainfall of about 600 mm on oregano biomass is positive (Rzekanowski et al., 2008; Caliskan et al., 2010). The results suggestthat in 2012–2014 the meteorological conditions were not optimal for oregano cultivation and plant biomass creation. For local oregano, 17 compounds were identified as the principal (Table 2).

Table 1. The influence of year and petals` colour on the average content of oregano essential oil, mL 100 g 10^{-1} dry matter

Year	Dark petals	Semi-dark petals	Light petals
2012	0.11 ± 0.01	0.10 ± 0.01	0.13 ± 0.02
2013	0.10 ± 0.02	0.10 ± 0.02	0.11 ± 0.01
2014	0.27 ± 0.01	0.15 ± 0.01	0.17 ± 0.01

Table 2. The influence of year and petals` colour on principal compounds of oregano essential oil, %

	Dark petals			Semi-dark petals			Light petals		
	2012	2013	2014	2012	2013	2014	2012	2013	2014
Limonene	0.37	0.44	0.49	0.24	-	-	1.10	1.20	1.22
β-phellandrene	2.35	3.31	3.33	2.13	-	1.18	5.78	7.12	6.11
1.8-cineole	-	-	-	0.61	-	-	3.35	1.98	3.61
γ-terpinene	0.29	-	-	0.32	-	-	2.66	2.23	0.85
ρ-cymene	3.52	3.08	3.96	2.33	-	1.41	5.39	8.70	4.93
Linalool	4.47	3.28	3.26	6.47	0.93	1.16	8.87	5.38	5.65
α-terpineol	2.09	2.44	3.09	2.26	1.30	1.62	2.16	2.35	2.42
Borneol	0.40	0.37	0.28	0.96	0.26	0.24	1.78	0.96	0.37
γ -cadinene	0.54	0.54	0.91	0.80	0.97	0.77	1.22	1.07	1.42
β-caryophyllene	5.22	5.52	13.08	4.85	12.28	1.59	7.76	6.94	8.41
Germacrene D	4.98	4.51	0.87	6.85	9.92	-	5.63	6.00	5.96
Caryophyllene oxide	23.58	25.69	15.53	16.73	27.19	30.30	11.23	12.30	9.92
Spathulenol	5.22	5.55	7.49	5.32	5.47	6.97	3.09	3.59	3.13
Farnesyl acetate	1.85	1.97	0.87	1.80	2.22	1.54	1.23	1.56	1.20

For accessions, the most important compounds were caryophyllene oxide, β-caryophyllene, spathulenol, germacrene D and linalool. Oregano samples were not rich in thymol and carvacrol. From our results, sesquiterpene chemotype can be defined for oregano accessions.

By Nurzyńska-Wierdak (2009), oregano from Poland had the greatest content of sabinene and germacrene D, but the content of sabinene and β-pinene as well as germacrene-D-4-ol, thymol, and carvacrol depended on the climatic conditions and were the most changeable in the study years. Particularly it is concluded also in this research (Sivicka et al., 2017).

By De Mastro et al. (2004), about 44 components with the predominance of carvacrol, thymol, γ-terpinene, linalyl acetate, germacrene D and cis-ocymene were detected in oregano from Italy. Carvacrol ranged between 0.18 and 66.74%, thymol from

0.20 to 43.68% as well as linalyl acetate between 51.27 and 60.93%, thus outlining a new oregano chemotype.

By Lukas et al. (2011), *Origanum vulgare* L. wild populations from Italy, Israel, Turkey, Greece and Croatia can be regarded as rich in thymol or carvacrol. Also, oregano mainly comes from wild populations in Turkey and Greece (Asdal et al., 2006). Some populations can be classified into thymol-'pure', carvacrol-'pure' and thymol/carvacrol mixed. Some populations showed the content of thymol or carvacrol till 20%. In Ukraine it was found, that for oregano propagation by cuttings increases the content of essential oil significantly in comparison with plants propagated by division (Boiko & Konik, 2010). As oregano accessions from Latvia also were propagated by division, this factor could be negative for essential oil synthesis.

By Falco et al. (2013), oregano growth conditions influence the composition of essential oil significantly. In particular, the oil from plants grown in single rows was rich in sabinene, but plants grown in double rows – in ocimenes.

CONCLUSIONS

Despite of non-optimal meteorological conditions, oregano accessions can produce high biomass in Latvia.

Oregano accessions from Latvia are poor in essential oil, but its` content is higher for plants with dark coloured flowers. Seventeen (17) compounds were identified as the principal, the caryophyllene oxide had the greatest content. Sesquiterpene chemotype can be defined for local oregano.

In the future, it is important to research the influence of propagation methods and agrotechnics on content and composition of oregano essential oil more carefully.

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