ESL 2: Genetic resources of *Thymus vulgaris* L. and *T. vulgaris* x *T. Marschallianus* Willd. in the Czech Republic

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Abstract

Two varieties of *Thymus vulgaris* L. (´Krajový´ and ´Winter´) and three its hybrids with *T. Marschallianus* Will. (variety ´Mixta´ and two accessions of variety ´Lemona´) were evaluated according the Draft Descriptor List *Thymus vulgaris* L. and analysed for the essential oil content and composition in years 2014 and 2015. All evaluated accessions were found morphologically and/or chemically different. Varieties ´Krajový´, ´Winter´ and ´Mixta´ were assessed as a thymol type with the thymol (39.1 – 69.6 %), o-cymene (6.3 – 24.9 %) and γ-terpinene (2.75 – 13.8 %) as main oil components. The two accessions of ´Lemona´ variety were found significantly different each other: one of them (income No. 3239) was assessed as a terpineol type with the terpineol acetate (75.7 %) and α-terpineol (16.9 %) as main oil components and the other one (income No. 2757) as a geraniol type with the geranyl acetate (±42.4 %) and geraniol (±20.8 %) as main oil components. Only the accession with income No. 3239 was proved as a ´Lemona´ variety due its citral (±9.3 %) content, though even this content is too small compare to original ´Lemona´ where about 20 % of citral was declared.

Keywords: Thyme, descriptor list, essential oil, thymol, citral, terpineol acetate

Introduction

A wide breeding program for thyme was begun to work in the Czech Republic in the period 1951 – 1979. An old Czech origin variety of *Thymus vulgaris* L. ´Krajový´, which was made up as positive selection of wild populations and accepted as a variety in 1952, was a basic parental material. A new variety ´Aroma´ was made from ´Krajový´ by selection methods and then propagated vegetativelly to fixed high amount of essential oil (up to 2 %) and thymol content (about 60 %). ´Aroma´ was accepted as a variety in 1966 and then it became a new parental material for next breeding. It was (as well as the ´Krajový´ variety) fertile so the hybridization by plants from wild population of *Thymus marschallianus* Willd. was tested and focused on increasing of plant mass yield and possible mechanized harvesting. The two new varieties have been developed by this hybridization: ´Lemona´ (accepted in 1975) and ´Mixta´ (accepted in 1979). ´Lemona´ was characterized by untypical lemon aroma which comes from citral (about 20 % of essential oil) content. ´Mixta´ was chemically comparable to ´Aroma´ variety, but 3-4 days earlier, with high plant material yield and habitus suitable for mechanized harvesting. Both hybrid varieties are sterile, flowers have not stamens.

All thyme breeding program was carried out at Research and Breeding Institute of Vegetable growing in Olomouc but after 1994 when it was cancelled a lot of original plant material has got unfortunately lost or damaged. Later on, some materials were repatriated from other institutions, Czech as well as foreign, but it was not a case of ´Aroma´ variety.

Czech collection of genetic resources of medicinal and aromatic plants currently includes 3 accessions of *Thymus vulgaris* L. (varieties ´Krajový´, ´Winter´ and ´French Summer´) and 2 hybrid accessions *T. vulgaris* x *T. Marschallianus* Will. (´Lemona´ and ´Mixta´). Four of these materials were eval-

uated according the Draft Descriptor List *Thymus vulgaris* L. (ECP/GR, 2011) and analysed for the essential oil content and composition in years 2014 and 2015.

Materials and Methods

A 5 years old, well-developed overgrowths of five *Thymus vulgaris* and *T.vulgaris x T. Marschallianus accessions* (Tab. 1) were used for evaluation and comparison. The evaluation of morphological descriptors was made according the Draft Descriptor List *Thymus vulgaris* L. (ECP/GR, 2011). Dry thyme stems in full bloom were submitted to hydrodistillation with a Clevenger-type apparatus. The EO was co-distilled with 500 ml of distilled water for 4 h and collected and stored in glass vials in dark at 4 °C until the GC analysis. Hydrodistillation was performed one to four times for each sample and the mean values of the extraction yields are reported. GC-MS analyses were carried out on an Agilent 7890A (Agilent technologies) gas chromatograph equipped with an Agilent 5975C MS detector (Agilent technologies) and fitted with an HP-5MS capillary column (30 m, 0.25-mm ID, 0.25-µm film thickness, Hewlett-Packard). The injection port temperature was 200 °C and MS detector temperature was 150 °C. The column temperature ranged from 60 °C (15 min) to 180 °C at a rate of 3 °C min^{-1}. The samples were diluted 1:50 (hexane) and the injection volume of each sample was 1 µl. The samples were injected using a split automatic injector (split ratio 1:100) and with helium as a carrier gas at a flow rate of 1.0 $ml.min^{-1}$. The measurement of the peak areas was performed with an HP E.02.02 ChemStation.

Results

All evaluated accessions were found morphologically and/or chemically different. Varieties ´Krajový´, ´Winter´ and ´Mixta´were based on CG analysis of essential oil assessed as a thymol type with the thymol (39.1 – 69.6 %), o-cymene (6.3 – 24.9 %) and γ-terpinene (2.75 – 13.8 %) as main oil components. The two accessions of ´Lemona´ variety were found significantly different each other: one of them (income No. 3239) was assessed as a terpineol type with the terpineol acetate (75.7 %) and α-terpineol (16.9 %) as main oil components and the other one (income No. 2757) as a geraniol type with the geranyl acetate (±42.4 %) and geraniol (±20.8 %) as main oil components. Only the accession with income No. 3239 was proved as a ´Lemona´ variety due its citral (±9.3 %) content, though even this content is too small compare to original ´Lemona´ where about 20 % of citral was declared.

The essential oil profile of varieties ´Krajový´ and ´Winter´ (probably ´Deutscher Winter´) respond to result of Mewes et al. (2008) and both of these varieties as well as variety ´Mixta´ corresponds to the chemotypical requirement of the Pharmacopoea Bohemica MMIX (based on Pharmacopoeia Europaea) for Thymi herba where minimally 1.2 % of essential oil content and 40.0 % of thymol and carvacrol in the essential oil is required.

Morphological differences between the varieties with identical chemotype is possible to find for example in Plant height (´Krajový´ 292 mm (average of both evaluated years), ´Winter´ 204 mm, ´Mixta´ 309 mm), Flower length (´Krajový´ 7.3 mm, ´Winter´ 4.5 mm, ´Mixta´ 4.8 mm) and Male sterility (´Krajový´ absent, ´Winter´ and ´Mixta´ present).

Tab. 1 Plant material and the description of its origin

ECN / Income No.	Taxon	Origin, the year of inclusion in collection	Variety	Male sterility
A8900004 / 3420	T. vulgaris	2003, Seva-Flora Valtice, CZE	Krajový	no
A8900011 / 2194	T. vulgaris	1987, Royal Sluis, NDL	Winter	yes
A8900003 / 3238	T. vulgaris x T. Marschallianus	2001, UJEP Brno, Kraví hora, CZE	Mixta	yes
A8900002 / 2757	T. vulgaris x T. Marschallianus	1980, VŠÚZ Olomouc, CZE	Lemona	yes
A8900002 / 3239	T. vulgaris x T. Marschallianus	2001, UJEP Brno, Kraví hora, CZE	Lemona	yes

Tab. 2 Essential oil content and quality – the content of major compounds

Income No.		3420		2194		3238		2757	3239	
Year of analysis		2014	2015	2014	2015	2014	2015	2015	2014	2015
Number of analysis		1	4	1	3	1	2	4	1	2
Essential oil content (%)	Av.	0.84	1.80	0.80	1.74	0.80	2.70	3.41	1.36	2.33
	SD	-	0.12	-	0.36	-	0.43	0.48	-	0.11
o-Cymene (%)	Av.	**24.86**	**11.71**	**11.39**	**14.16**	6.26	**10.96**	0.23	-	0.15
	SD	-	0.82	-	1.42	-	0.68	0.05	-	0.02
γ-Terpinene (%)	Av.	4.61	**13.70**	5.85	**13.84**	2.75	7.58	0.77	-	0.20
	SD	-	0.43	-	0.43	-	0.42	0.20	-	0.09
α-Terpineol (%)	Av.	tr.	-	tr.	-	tr.	tr.	**16.93**	tr.	tr.
	SD	-	-	-	-	-	-	0.90	-	-
Geraniol (%)	Av.	-	-	-	-	-	-	-	**18.27**	**22.07**
	SD	-	-	-	-	-	-	-	-	1.60
Citral (%)	Av.	-	-	-	-	0.18	-	-	7.92	9.96
	SD	-	-	-	-	-	-	-	-	0.67
Thymol (%)	Av.	**52.13**	**61.92**	**58.70**	**55.95**	**69.60**	**39.13**	-	0.27	0.57
	SD	-	0.68	-	1.10	-	1.15	-	-	0.10
Terpineol acetate (%)	Av.	-	-	-	-	-	-	**75.74**	-	-
	SD	-	-	-	-	-	-	0.92	-	-
Geranyl acetate (%)	Av.	-	-	-	-	-	-	-	**43.42**	**41.95**
	SD	-	-	-	-	-	-	-	-	0.56

tr. = traces; the compounds exceeding 10 % are bold marked

References

ECP/GR, 2011: Draft Descriptor List Thymus vulgaris L. (available from http://www.ecpgr.cgiar.org/fileadmin/templates/ecpgr.org/upload/NW_and_WG_UPLOADS/MAP_Descriptors/Thymus_vulgaris_DRAFT_DESCRIPTOR_LIST_FINAL.pdf)

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