The Slovak gene pool of German chamomile (*Matricaria recutita* L.) and comparison in its parameters

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ABSTRACT: Plant habit and production of secondary metabolites in chamomile plants depend on endogenous and exogenous factors that can be divided into two groups (Franz 1982): a) morpho-ontogenetic variability, b) genetic variability or genetic determinateness. The activity of these factors is reflected in biomass production, flower drug production, content and composition of essential oil and some other characteristics of chamomile stands. This study presents genetic and environmental variations of essential oil and its sesquiterpene composition – (-)-α-Bisabololoxide A, (-)-α-Bisabololoxide B, (-)-α-Bisabolol and Chamazulene – in a wild chamomile population growing in the East-Slovakian Lowland in comparison with bred varieties that are cultivated in Slovakia at this time. The highest contents of (-)-α-Bisabololoxide A (mean: 39.90%) and (-)-α-Bisabololoxide B (mean: 9.75%) are typical of chamomile plants whose flower anthodia were collected at various places in the East-Slovakian Lowland. These results show that it is a Bisabololoxide chemotype B of chamomile population (Lawrence 1986). Qualitative and quantitative characteristics of the chamomile essential oil of selected cultivars indicate (-)-α-Bisabolol (mean: 39.75%) and Chamazulene (mean: 16.75%) as dominant compounds. The chamomile varieties bred for a high content of the main compounds belong to the chemotype C group (Lawrence 1986). Parameters of the composition of essential oil from cultivated chamomile varieties, (-)-α-Bisabolol & Chamazulene, markedly exceeded the parameters measured in chamomile collected from the wild nature in the East-Slovakian Lowland.

Keywords: German chamomile; *Matricaria recutita* L.; East-Slovakian Lowland; diploid & tetraploid varieties; morpho-ontogenetic & genetic variability

A long German Chamomile, *Matricaria recutita* L., history and genesis of chamomile plant use stems from its origin. The native country of the chamomile species was Asia, Northern Africa, Southern and Eastern Europe. The ancient Egyptians considered Chamomile to be a sacred present from the Sun God because chamomile flowers were useful against a high fever and sunstroke. The notions of chamomile healing power came from antique scientific works of Hippocrates, Pliny, Dioscurides and Galen into old herbals and current phytomedicine. Finally, a very old folk saying in Slovakia says: "*An individual should always bow before the curative powers of the chamomile flower tea*." Nowadays, Chamomile is the most favoured and most frequently used medicinal plant over the world. Phytotherapeutically useful are primarily flower anthodia, and this drug (*Chamomillae Flos*) is included into the pharmacopoeias of 26 countries all over the world.

A substantial part of drug effects is determined by the essential oil content. Among the essential oil constituents (-)-α-Bisabolol and Chamazulene are the most active. (-)-α-Bisabolol has demonstrated its anti-inflammatory, anti-microbial, vulnerary activity and anti-ulcer activity. Chamazulene also has anti-inflammatory effects. Both substances have shown spasmolytic effects (Morgan 1996).

Evaluation of drug production considered essential oil quantities and chamazulene percentage content by the 70's. Progressive development and innovation of analytic methods along with new pharmacological methods of active components began to emphasize effects of other constituents.

An impulse for totally new evaluation of the drug production appeared if the identification of four chief chemical types of chamomile differing in the qualitative and quantitative composition of chemical compounds in the essential oil was carried out (Schilcher 1987). This fact was very important in these parts of the world where diploid chamomile population are characterised by a high content of Bisabololoxide constituents occurring in large quantities. With regard to this observation very intensive plant breeding programs were launched considering the flower anthodia yield, essential oil production and chamazulene and bisabolol content (active components with the most precious pharmacological characteristics).

The worldwide cultivation areas of chamomile lie in Argentina, Egypt, Germany, Hungary, Poland, Spain, Bulgaria, Belorussia, Russia, Czech Republic, Slovakia, Balkan peninsula, Ukraine and also in Bolivia and Brasilia (Luppold 1984; Seitz 1987).

About by 1955 the total production of Czechoslovakian chamomile came from the collection in the wild nature. In the second half of the 80's, areas under chamomile increased. This favourable fact was influenced by the solution of the complex problem related to large-scale production (Šalamon 1992). Besides some others, it was the breeding of new chamomile varieties that produce sufficiently high quantities of drug and essential oil with accent on the composition and stability of the chief constituents.

This study presents the genetic and environmental variations of essential oil and its sesquiterpene composition – (-)-α-Bisabololoxide A, (-)-α-Bisabololoxide B, (-)-α-Bisabolol and Chamazulene – in the wild chamomile population growing in the East-Slovakian Lowland in comparison with the bred varieties that are cultivated in Slovakia.

MATERIAL AND METHODS

Plant materials

Plant material, chamomile anthodia (42 different samples), was collected from natural sites (together 30 localities) in the East-Slovakian Lowland during four years (1995, 1996, 1997 and 1998).

Chamomile flower anthodia of diploid varieties Bona and Novbona and tetraploid varieties Lutea and Goral were cultivated on a large-scale on agricultural farms in Nova Ľubovňa, Plavnica and Streda nad Bodrogom. All cultivated varieties complied with the variety trial requirements (for distinctness, homogeneity and stability). New varieties were approved in the period 1986–1992.

Flower chamomile drug (*Chamomilae Flos*) was dried in a sheltered room under standard laboratory temperature.

Methods of isolation and identification

Steam distillation

Chamomile essential oil was isolated by steam distillation (READ 1992). Hydro-distillation lasted for 2 hours into *n*-hexane, sample weights were 2 g of drug dry matter. A modified distillation apparatus by Coocking & Middleton was used (HUMPHREY 1992).

Gas chromatography (GC)

The compounds of essential oil were determined by means of Hewlett-Packard 5890 Series II system, with capillary column HP-5, FID detector, split-splitless system for injection and automatic injector HP 7673.

The operating conditions were: injection temperature 150°C, detector temperature 250°C, carrier gas nitrogen. Sample sizes 1.0 μl were used and manual type of injection.

The composition of chamomile essential oil was determined by capillary GC analysis: Hewlett-Packard 5890 Series II with FID and split-splitless system for injection. The column HP-5 (50 m long × 0.20 mm i. d.) was used. The following temperature program was used: 90ºC (0 min), then 10ºC/min to 150ºC (5 min), 5ºC/min to 180ºC (3 min), 7ºC/min to finally isothermal 280ºC for 25 min; nitrogen was used as carrier gas. Detector temperature 250°C, carrier gas nitrogen (flow velocity 274 mm/s), auxiliary gases were nitrogen (30 ml/min), hydrogen (30 ml/min), air (400 ml/min).

Peak areas and retention times were measured by electronic integration with a Hewlett-Packard 3396 Series II integrator.

Major components of essential oil were determined using standard compounds – (-)-α-Bisabolole, Chamazulene, trans-en-in-Dicycloether and (-)-α-Bisabololoxide A and B. Qualitative identification of selected components was carried out by comparing retention times of all detected components with retention times of standard compounds – (-)-α-Bisabolole, Chamazulene, trans-en-in-Dicycloether, (-)-α-Bisabololoxide A and B.

Results are presented in percentage. The percentage of single chromatographic peak areas was measured on the basis of the area of single peaks to the total peak area ratio. Results were statistically evaluated using *t*-test at the 0.05 level.

RESULTS AND DISCUSSION

Plant habit and production of secondary metabolites in the chamomile depend on the endogenous and exogenous factors that can be divided into two groups (FRANZ 1982):
a) morpho-ontogenetic variability,
b) genetic variability or genetic determinateness.

The activity of these factors is reflected in biomass production, flower drug production, content and composition of essential oil and some other characteristics of chamomile stands.

Morpho-ontogenetic variability of chamomile

The exogenous morphological characteristics of chamomile plants are not stable. Great variability and convertibility were confirmed in a representative sample of chamomile individuals in the following parameters: plant height, average flower anthodium size and weight, between the wild population and bred chamomile varieties (Table 1).

Comparison of the morphological characteristics in the diploid and tetraploid chamomile varieties and in wild chamomile confirmed evident differences. In regard to the mentioned morphological characteristics of chamomile the flower chamomile drug of different origin and varied visual properties is introduced into the world markets.

The range of the ecological amplitude of chamomile species gives to chamomile the ability to adapt itself to less suitable soil and climatic conditions (DOVIAK, ANDRAŠČÍK 1986). Morphological variability is very high in the chamomile species. The exogenous morphological characteristics of chamomile plants are not stable (Table 1). Therefore this plant represents a suitable material for the study of variability and morphological variability of the traits such as plant height, plant diversity and biomass production of roots, stems, leaves, flower anthodia and number of flower anthodia (ŠALAMON 1998). Study of this problem was realised

Table 1. Morphological characteristics of chamomile plants (*Matricaria recutita* L.)

Origin of chamomile	Plant height (m)	Average size of flower anthodia (mm)	Average weight of flower anthodia (g)
Wild population			
(East-Slovakian Lowland)	0.20–0.30	15	0.0020
Diploid varieties			
Bohemia	0.40–0.70	20	0.0030
Bona	0.15–0.30	15	0.0020
Novbona	0.20–0.40	20	0.0025
Tetraploid varieties			
Lutea	0.35–0.50	30	0.0040
Goral	0.40–0.60	35	0.0045

under major emphasis on the exogenous conditions of environment in which plants and their population were grown. Variation of the chamomile diploid type, grown in the autochthonous localities, is considered as the varieties and cultivars, not as the interspecific taxa.

Other studies show the results of comparison of morphological traits for the purpose of differentiation between both diploid and tetraploid plants grown under very different ecological conditions in Germany (Europe) and Ethiopia (Africa) (LETCHAMO 1990; LETCHAMO, WOMEL 1989). The results demonstrated that the length of germinal cells, diameters of bloom grains, diameters and weight of flower anthodia, plant height and course of flowering phases did not expressly show the differentiation between the two examined groups. In regard to expressive differentiation other characteristics were chosen – stoma number, number of chloroplasts in the stoma cells and number of bloom germinal utricles.

Identification of chamomile chemotypes in the East-Slovakian Lowland

German Chamomile, *Matricaria recutita* L., can be found in secondary plant communities in the East-Slovakian Lowland, such as trodden societies on dry and moist soils, weed societies, and dump societies. A large-scale monitoring of the chamomile gene pool was realised for the species identification of the wild population chemotypes in 1995–1998 in the East-Slovakian Lowland.

Percentage of essential oil content in dry chamomile flower heads and its qualitative and quantitative characteristics that were determined by the GC-analysis are presented in Table 2 and Fig. 1. Percentage contents of essential oil from chamomile flower anthodia ranged from 0.30 to 0.97 in all examined samples.

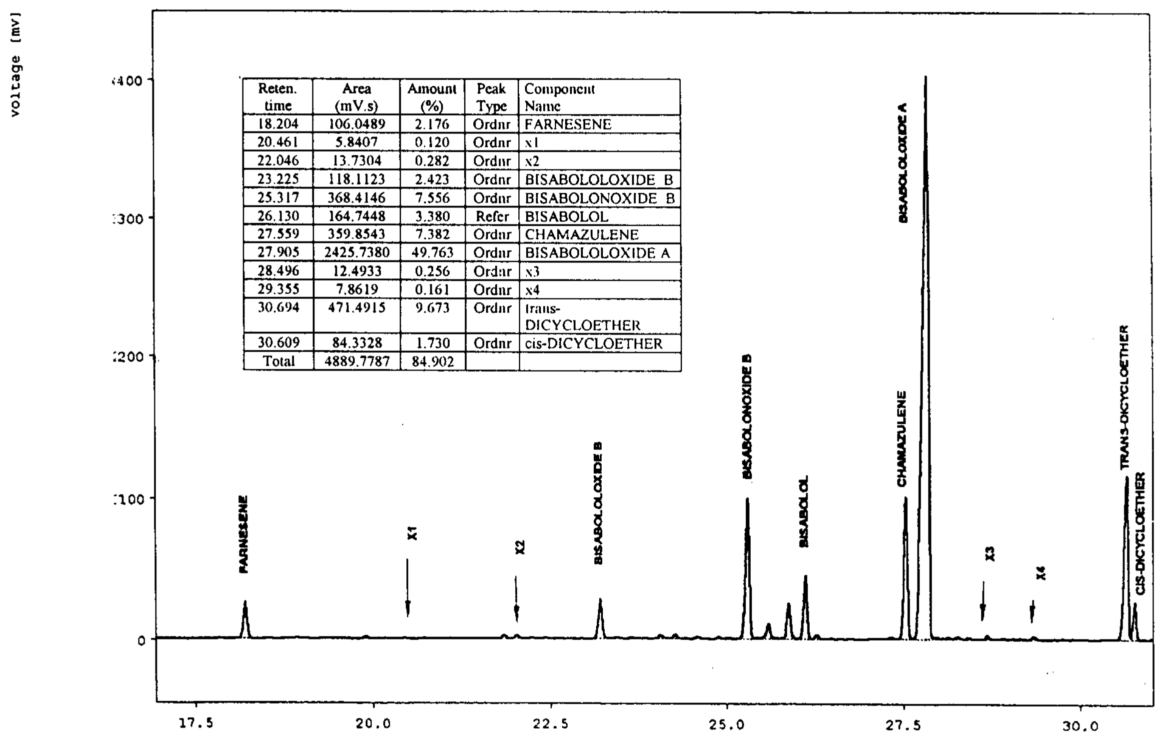

Fig. 1. The characteristic GC-analysis of essential oils from the East-Slovakian chamomile plant population

Table 2. Essential oil content and its composition of dry chamomile flowers from natural sites in the East-Slovakian Lowland

Localities in the East-Slovakian Lowland	% of essential oil	Basic composition of essential oil (%)			
		Fa	Bo	Ch	BoA
Michalovce ('95,'96,'97,'98)	0.55–0.91	1.2 – 6.1	2.6–6.8	6.3–7.5	28.9–40.4
Vojany ('95, '96, '97, '98)	0.40–0.71	5.3–10.5	3.4–6.1	5.0–9.1	39.8–48.4
Vysoká nad Uhom ('95, '96, '97)	0.75–0.96	8.1–13.2	4.4–6.8	4.4–10.1	38.5–48.9
Vybuchanec ('95, '96)	0.52–0.97	6.0–6.1	6.6–6.7	5.4–5.5	43.1–43.6
Trebišov ('96, '97)	0.50–0.60	11.7–13.6	2.9–3.0	10.1–10.2	36.1–42.5
Veľké Raškovce ('97, '98)	0.65–0.70	4.2–18.0	3.2–3.5	6.9–10.1	33.1–37.8
Krišovská Liesková ('96, '98)	0.53–0.90	4.0–12.2	2.5–6.6	3.8–4.0	36.7–42.2
Bajany ('95, '98)	0.60 –0.74	1.5–3.2	3.8–5.1	4.7–7.4	32.8–52.9
Nižný Hrabovec ('95)	0.61	3.8	4.9	10.8	34.6
Vranov nad Topľou ('95)	0.82	4.1	5.2	7.6	48.3
Moravany ('95)	0.93	4.6	9.6	9.9	37.9
Sírnik ('95)	0.86	4.0	6.2	10.6	40.0
Malé Raškovce ('96)	0.63	9.9	4.8	10.2	36.2
Rakovec nad Ondavou ('96)	0.92	8.0	5.0	11.0	42.3
Čierne Pole ('96)	0.72	8.3	4.5	9.8	43.8
Tibava ('96)	0.85	12.6	5.1	9.0	36.8
Stretava ('96)	0.60	2.9	7.7	10.8	45.6
Beša ('96)	0.62	7.4	6.0	8.3	45.3
Veľké Kapušany ('96)	0.55	7.9	6.9	6.9	48.7
Zálužice ('97)	0.75	15.5	5.2	4.4	33.9
Pavlovce nad Uhom ('97)	0.60	15.2	6.8	6.3	41.6
Pozdišovce ('97)	0.30	17.2	6.8	5.8	26.6
Bracovce ('97)	0.74	16.1	4.7	6.9	36.2
Malčice ('97)	0.82	15.9	2.4	8.9	30.2
Trhovište ('97)	0.90	15.7	5.1	6.2	38.6
Vojčice ('98)	0.97	2.10	5.1	7.6	42.8
Novosad ('98)	0.70	1.5	4.0	12.0	39.9
Hraň ('98)	0.60	2.9	5.5	9.5	35.8
Zemplínska Branč ('98)	0.50	0.4	3.2	7.7	49.2

Fa – Farnesene, Bo – (-)-α-Bisabolol, Ch – Chamazulene, BoA – (-)-α-Bisabololoxide A

Table 1 presents the essential oil composition of dry chamomile flowers from individual natural sites and years of their collection in the East-Slovakian Lowland.

The highest contents of (-)-α-Bisabololoxide A (mean: 39.9%) and (-)-α-Bisabololoxide B (mean: 9.75%) are typical of chamomile plants whose flower anthodia were collected at various places in the East-Slovakian Low-

Table 3. Quantity and composition of the essential oil isolated from the flower anthodia of chamomile varieties cultivated in Slovakia

Content of essential oil (%)	Bona variety 0.60 ± 0.02		Novbona variety 0.82 ± 0.04		Lutea variety 0.95 ± 0.02		Goral variety 1.10 ± 0.05	
	Fa	BoB	BnA	Bo	Ch	BoA	Dc	
	% in essential oil						-cis	-trans
Bona variety	4 ± 2.0	3 ± 0.5	0.5 ± 0.2	42 ± 2.0	20 ± 1.0	2 ± 1.0	13 ± 1.0	0.1 ± 0.05
Novbona variety	9 ± 3.0	2 ± 0.5	0.7 ± 0.2	39 ± 5.0	12 ± 3.0	1 ± 0.5	18 ± 1.0	0.1 ± 0.05
Lutea variety	5 ± 3.0	2 ± 0.5	0.6 ± 0.1	48 ± 2.0	16 ± 1.5	0.5 ± 0.5	12 ± 1.5	0.8 ± 0.1
Goral variety	9 ± 3.0	9 ± 2.0	0.5 ± 0.2	30 ± 3.0	19 ± 2.0	16.6 ±4.0	7 ± 2.0	1.3 ± 0.3

Fa – trans-α-Farnesene, BoB – (-)-α-Bisabololoxide B, BnA – (-)-α-Bisabolonoxide A, Bo – (-)-α-Bisabolol, Ch – Chamazulene, BoA – (-)-α-Bisabololoxide A, Dc – En-in-dicycloethers land. In regard to the sesquiterpenes, the chamomile anthodia contain (-)-α-Bisabolol (mean: 5.09%) and Chamazulene (mean: 7.65%). These results show the Bisabololoxide chemotype B of chamomile population (LAWRENCE 1986).

Satisfactory herb composition with a maximum of effective substances is the main condition for further herb cultivation and processing by the Slovak pharmaceutical industry as well as for export abroad. According to the study of chamomile pharmacodynamic properties, the sesquiterpenes: (-)-α-Bisabolol and Chamazulene are considered to be the most valuable constituents. Chamomile, which was collected from natural habitats, has the low (-)-α-Bisabolol and Chamazulene content. The highest amount of less important compounds, such as (-)-α-Bisabololoxide A and (-)-α-Bisabololoxide B, is typical of the chamomile population in the East-Slovakian Lowland. The content of Farnesene is very variable and dependent on the quantity of green flower parts (VALIGURSKY, ŠALAMON 1998). Chamomile flowers from natural plant societies are harvested manually by Gypsy people. This volume of dry chamomile raw material is higher than 10,000 kg each year. Unfortunately, the harvested raw material is of poor quality with the high content of Bisabololoxides, which is unfavourable for a world standard. Gradually, the chamomile varieties Bona, Novbona, Goral and Lutea were bred in Slovakia in the years 1972–1992.

Genetic variability of chamomile

Genetic approach to the chamazulene content (prochamazulene) in the chamomile essential oil was applied by the 60s (TÉTÉNYI 1960), the mechanism of inheritance in bisabolane type constituents was described as late as in the 90s (HORN et al. 1988; FRANZ et al. 1985). Considerable efforts concerning quantitative genetic aspects and their interactions in exogenous conditions were published in 1990 (MASSOUD, FRANZ 1990a,b). For successful breeding purposes of this medicinal plant it follows that there are plants with adequate genetic variability of the essential oil yield in the stands of Chamazulene – Bisabolol type chamomile. Therefore it is more effective to use plant selection for the high chamomile quality genotype. This breeding method is preferred opposite to cross breeding.

Yield of essential oil isolated from the Slovak bred varieties ranges from 0.60 to 1.10% (Table 3). Measured values represent standard parameters in comparison with foreign cultivars. Qualitative and quantitative characteristics of the chamomile essential oil of selected cultivars are shown in Table 3. In regard to the mentioned results, dominant compounds of essential oil are (-)-α-Bisabolol and Chamazulene. Essential oil was distilled from the average sample of dry flower drug. The chamomile varieties bred for a high content of main compounds belong to the chemotype C group (LAWRENCE 1986).

Differences were confirmed not only in the morphological characteristics but also in the content of the essential oil and its composition. Differences were found out both in wild-collected and large-scale cultivated chamomile populations. Presented parameters of qualitative and quantitative characteristics (Tables 2 and 3) have a direct influence on the therapeutic quality of this medicinal plant. Composition parameters of essential oil from cultivated chamomile varieties markedly exceeded parameters measured in the chamomile collected from the wild nature, and also diploid variety Bohemia, which was the only appreciated chamomile variety in Czechoslovakia (ŠALAMON, HONČARIV 1994).

At present good breeding methods, cultivation (direct sowing by seed-sowing machines, good agro-technical practices), harvesting (mechanical harvesters) and processing (special sorting machines, hot-air driers, stalk removers) produce in Slovakia the chamomile drug with high quality of essential oils.

CONCLUSIONS

German Chamomile, *Matricaria recutita* L., is the most favoured and most frequently used medicinal plant. Phytotherapeutically useful are primarily flower anthodia, and this drug (*Chamomillae Flos*) is included in the pharmacopoeias of 26 countries all over the world. Comparison of the morphological traits in the diploid and tetraploid chamomile varieties and wild chamomile confirmed the evident differences. In regard to the mentioned morphological characteristics of the chamomile the flower chamomile drug of diffe-rent origin and varied visual properties was introduced into the world markets. In the Slovak Republic wild chamomile flowers are usually collected by Gypsies and sold to pharmaceutical processing. The qualitative and qualitative characteristics of essential oil and its composition for these wild chamomile populations in the East-Slovakian Lowland were determined. The results show that there is a Bisababoloxide chemotype of chamomile with a lower content of essential oil. Gradually, the chamomile varieties Bona, Novbona, Goral and Lutea were bred in Slovakia in the years 1972–1992.

Composition parameters of essential oil from cultivated chamomile varieties, (-)-α-Bisabolol & Chamazulene, markedly exceeded parameters measured in the chamomile collected from the wild nature, and also diploid variety Bohemia, which was the only appreciated chamomile variety in formerly Czechoslovakia. At present good breeding methods, cultivation (direct sowing by seed-sowing machines, good agro-technical practices), harvesting (mechanical harvesters) and processing (special sorting machines, hot-air driers, stalk removers) produce in Slovakia the chamomile drug with high quality of chamomile essential oils.

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Genofond rumančeka kamilkového (*Matricaria recutita* L.) na Slovensku a porovnanie jeho parametrov

ABSTRAKT: Rumanček kamilkový (*Matricaria recutita* L.) patrí k jednej z najobľúbenejších a najžiadanejších liečivých rastlín. Sušený kvet rumančeka kamilkového (*Chamomillae Flos*) je starým liečebným prostriedkom, známym už v starovekom Egypte, Grécku a Ríme. Egypťania považovali túto rastlinu za posvätnú. Pre svoje zmierňujúce účinky pri horúčkach a úpaloch ju posudzovali ako dar od Boha Slnka. Tvorba sekundárnych metabolitov pri rumančeku kamilkovom závisí od endogénnych a exogénnych faktorov, ktoré sa podľa FRANZA (1982) dajú rozdeliť na: a) morfologicko-ontogenetickú variabilitu, b) genetickú variabilitu, resp. genetickú determinovanosť. Pôsobenie všetkých týchto faktorov sa odráža vo veľkosti tvorby biomasy, produkcie kvetnej drogy, obsahu, zloženia silice a ďalších charakteristík porastov rumančeka kamilkového. Uvedené výsledky kvantitatívno-kvalitatívnych charakteristík silice rumančeka kamilkového rôzneho pôvodu prezentujú významné rozdiely v obsahu jednotlivých kľúčových komponentov a v morfologických znakoch. Najvyššie zastúpenie (-)-α-bisabololoxidu A (priemer: 39,90 %) a (-)-α-bisabololoxidu B (priemer: 9,75 %) je typické pre rastliny, zbierané z rôznych lokalít Východoslovenskej nížiny. Výsledky potvrdili, že ide o bisabololoxidový chemotyp B (LAWRENCE 1986). Kvalitatívno-kvantitatívne parametre silice pestovaných odrôd sú charakteristické vysokým obsahom (-)-α-bisabololu (priemer: 39,75 %) a chamazulénu (priemer: 16,75 %). Populácie týchto rastlín patria ku chemotypu C (LAWRENCE 1986). Rôzne zastúpenie týchto zložiek ovplyvňuje rozdielnu farmakodynamickú hodnotu suchej rumančekovej drogy. Farmaceutický a kozmetický priemysel by mal uprednostňovať pre výrobu liečivých prípravkov surovinu tejto liečivej rastliny šľachteného bisabololového chemotypu. Táto sa veľkoplošne pestuje len na niektorých špecializovaných poľnohospodárskych podnikoch. Veľmi často sa však používa rumančeková kvetná droga zbieraná rómskym obyvateľstvom na východnom Slovensku, ktorá je cenovo oveľa lacnejšia. Účinnosť hromadne vyrábaných fytoterapeutických a kozmetických prípravkov sa takto znižuje.

Kľúčové slová: rumanček kamilkový; *Matricaria recutita* L.; Východoslovenská nížina; diploidné a tetraploidné odrody; morfologicko-ontogenetická a genetická variabilita

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