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RESEARCH ARTICLE

Content and composition of essential oil and content of rosmarinic acid in lemon balm and balm genotypes (*Melissa officinalis*)

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Abstract Lemon balm (*Melissa officinalis* L.) is used since ancient times because of its sedative, spasmolytic and antiviral effects. Its therapeutic impact is due to the content of essential oil and rosmarinic acid. A set of 68 *M. officinalis* genotypes was evaluated for content and composition of essential oil and the content of rosmarinic acid. For all genotypes the level of ploidy was determined. The 68 genotypes were clone plants grown and evaluated for two years at Quedlinburg. For analysis of secondary metabolites distillation, gas chromatography and high performance liquid chromatography was used. The content of essential oil varied in this study in ranges from 0.03 to 0.33% for the second cut 2010 and 0.01–0.35% for the second cut 2011. The rosmarinic acid content ranged in the year 2010 from 3.67 to 7.55% and in the year 2011 from 4.92 to 8.07%. Via statistical analyses two chemotypes of essential oil were found: chemotype citral and chemotype β-caryophyllene oxide. Ploidy was determined for all genotypes and two cytotypes were found: diploid 2n = 2x = 32 (62 of 68 genotypes) and triploid 2n = 3x = 48 (6 of 68 genotypes).

Keywords Content and composition of essential oil · Genetic resources · Chemotypes

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Introduction

The perennial crop plant lemon balm (*Melissa officinalis* L.), family Lamiaceae (syn. Labiatae) originating from Mediterranean Region or Western Asia (Hanelt and IPK 2001), is grown worldwide in temperate and subtropical regions. Beside essential oil (Aziz and El-Ashry 2009) a wide spectrum of other active constituents was found in lemon balm like phenolic acid derivatives, flavonoids, and triterpenes (Bomme et al. 2013).

Lemon balm is used for phytopharmaceuticals (*Melissa herba* and *Melissa folium*), as an aromatic plant and in traditional folk medicine. The essential oil, which contains mainly citronellal, (*E*)-citral, (*Z*)-citral and rosmarinic acid, a caffeic acid ester with 3,4-dihydroxyphenyllactic acid are the active pharmaceutical ingredients in lemon balm (Schilcher 2016), which has proven sedative, antibacterial and antivirus activity (Toth et al. 2003). There is also strong evidence that lemon balm has a positive effect on patients, who suffer from Alzheimer's disease (Moradkhani et al. 2010).

Lemon balm collections have been compiled for practical aspects therefore most accessions have lemon like scent (Adzet et al. 1992a, b; Bahtiyarca Bagdat and Cosge 2006; Basker and Putievsky 1978; Bomme et al. 2008; Mrlianova et al. 2002; Seidler-Łożykowska et al. 2013). In a set of 28 accessions Kittler et al. (2018) describe beside diploid populations with lemon like scent also 12 tetraploid accessions. Of these tetraploid accessions ten had a soap-like scent and belong to a chemotype which had germacrene D as the main component of the essential oil.

This study presents evaluation data for content and composition of essential oil, content of rosmarinic acid and level of ploidy. The collection includes beside accessions with typical lemon like scent also genotypes with off odours. These genotypes are named balm whereas genotypes with lemon like scent are called lemon balm in this study. This evaluation depicts genotypes, which can be useful for breeding new varieties with high content of rosmarinic acid and essential oil.

Materials and methods

Material and cultivation

The collection of the Bavarian State Institute for Agriculture at Freising, Germany (LfL) consisted of 68 genotypes of *M. officinalis*, preserved by vegetative maintenance (electronical supplement Table 1). These genotypes originated from botanical gardens, private collections, breeding material or varieties. Wild collected material was not included. The field trials were conducted in Quedlinburg in 68 plots of 20 clone plants and two repeats planted in a scheme of 50 cm × 45 cm. The trial was planted on 10/04/2009. The inner 6 plants of the plot were harvested as a representative probe. The second cut of the plants was harvested on 19/09/2010 and 21/09/2011, respectively, dried at Quedlinburg and subsequently analysed. Between the plots no ontogenetic differences could be found which were more extensive than within the plots, so all accessions of one trial were harvested on the same day, due to the mostly homogeneous developmental stage of the plants. The plants were cut 10 cm above the ground and were dried on a bench drying system (Lü-Ku GmbH, Germany, 30 °C, 72 h). Leaves and stems were separated by hand. To ensure a representative sample at least 100 g air-dried leaf material per sample were used. The experimental

Table 1 Summary of 6 essential oil components and rosmarinic acid of lemon balm (*Melissa officinalis*)

	Substance	Kovats retention index RI	CAS	Substance group	Odour	Co-el
1	Citronellal	1159	106-23-0	Monoterpene aldehyde	Sweet, floral, rose	1
2	(*Z*)-citral (neral)	1255	106-26-3	Monoterpene aldehyde	Sweet, citrus, lemon	1
3	(*E*)-citral (geranial)	1287	141-27-5	Monoterpene aldehyde	Citrus, lemon	1
4	β-caryophyllene	1442	87-44-5	Sesquiterpene, bicyclic	Spicy, woody, citrus	1
5	germacrene-D	1504	37839-63-7	Sesquiterpene, monocyclic	–	0
6	β-caryophyllene oxide	1615	1139-30-6	Sesquiterpene oxide	Woody, spicy	0
7	Rosmarinic acid		20283-92-5	Caffeic acid ester	–	1

CAS chemical abstracts service registry Number. co-el—coelution of authentic reference substances. All compounds were purchased by Sigma-Aldrich, Taufkirchen, Germany or Carl Roth, Karlsruhe, Germany. Number 1–6 were semi-quantified by GC-FID. Number 7 was analyzed by HPLC

station Quedlinburg, Germany was located at the northern foreland of Harz Mountains. Altitude: 140 m, black earth, valuation index of field: 91–97, annual depth of rainfall and average annual temperature 2009: 683 mm, + 9.59 °C 2010: 751.2 mm, + 7.88 °C and 2011: 324 mm, + 10.01 °C.

Methods

Determination of ploidy level

Measuring of relative DNA amount of nuclei occurred by flow cytometry. The measurement followed the procedure described in Kittler et al. (2015).

Determination of essential oil content

For the collection distillation was performed at PytoLab Gmbh Co. KG, Vestenbergsgreuth, Germany in 2010 and 2011 following Ph. Eur. 6.0, 2.8.12 (Ph. Eur. 6 2008). All genotypes were analysed separately in a double assay and both repeats averaged. A sample consisted of the leaves from six plants. The content of essential oil is displayed in % (v/m), which conforms mL/100 g air-dried leaf material.

Determination of essential oil composition

The composition of the essential oil was analysed by gas chromatography (GC, 6890 N, Agilent Technologies, U.S.) equipped with flame ionisation detector and HP-5 capillary column 50 m × 0.32 mm ID × 0.52 µm film thickness. Injector temperature was 250 °C and detector temperature was 280 °C. The oven temperature started at 60 °C and then programmed to 120 °C at a rate of 3 °C/min, then to 250 °C at a rate of 8 °C /min and held at final temperature for 10 min. The carrier gas was hydrogen at 2 mL/min constant flow. The sample volume injected was 1 µL and the split rate was 1:20. The essential oil obtained by distillation was diluted in isooctane 1:1000 before injection. The relative amounts (norm-%) of individual components are based on the peak areas. The components of essential oil were determinated by MS identification, retention time and co-elution of authentic references (Table 1). The used spectra databases were NIST and WILEY. All essential oil probes were analysed with two analytical replications. The targeted semi-quantification of essential oil components was focussed on the six compounds mentioned in Table 1.

Determination of rosmarinic acid

Air-dried and crushed balm leaves (approx. 2 g) were powdered for 7 min at 80 s^{-1} using a mixer mill (MM2, Retsch) and a steel ball of 8 mm diameter. Ground leaf material (50 ± 1 mg) was weighed into a 2–mL polypropylene tube and 1.5 mL 50% (v/v) aqueous ethanol was added. The mixture was thoroughly vortexed for 20 s and sonicated for 10 min at 50 °C. After centrifugation (10 min, 12.000×*g*, 22 °C), the supernatant was transferred to a 10—mL volumetric flask. The remaining residue was extracted twice again with 1.5 mL 50% (v/v) aqueous ethanol as described above. The resulting extracts were combined and their volume adjusted to 10 mL using 50% (v/v) aqueous ethanol. An aliquot of the resulting solution was filtered into a vial using a syringe filter of 0.45 µm pore size and stored at 6 °C until analysis. Rosmarinic acid analyses were performed on an AGILENT 1100 Series HPLC system comprising a degasser (G1322A), binary pump (G1312A), autosampler (G1329A), autosampler thermostat (G1330A), column compartment (G1316A) and diode array detector (G1315A). Extracts (injection volume 5 µL) were separated on an Accucore C18 column (3 × 150 mm, 2.6 µm particle size, Thermo Scientific) using 0.1% (v/v) aqueous formic acid and methanol as eluent A and B, respectively. The following binary gradient programme at a flow rate of 500 µL/min was used: 0 – 20 min, linear from 20 to 100% B; 20 – 24, isocratic, 20% B. The column temperature was maintained at 40 °C. Rosmarinic acid was detected at 320 nm with a spectral bandwidth of 4 nm. ChemStation software (version B.03.02) was applied for controlling the instrument, data acquisition and quantitative analysis. Rosmarinic acid was quantified using an external standard calibration (calibration range 10–5000 ng on column). A linear calibration model was used resulting in R^2>0.999.

Statistical analysis

The statistical software package Statistica 7.1 from StatSoft (Tulsa, USA) was used for the calculation of the principal component analysis (PCA) and the

cluster analysis (agglomerative method, single linkage, euclidic distances). The heat map was created with the Multi Experiment Viewer TM4 from the MEV Development Team (www.tm4.org). Descriptive statistic was carried out with SPSS 16.0 (IBM, USA).

Results

Evaluation of ploidy level by flow cytometry

All 68 accessions of the collection were measured for their relative amount of DNA and ploidy level was deduced (electronical supplement Table 1). Of the genotypes 62 were diploid (2n = 2x = 32) and six were triploid (2n = 3x = 48): BLBP75, BLBP78, BLBP88, BLBP111 (73B), BLBP112 (75B), BLBP113 (78B). No tetraploid accessions were found.

Content of essential oil

The genotypes of the collection generated in the second cut of 2010 essential oil contents between MIN = 0.03, MEDIAN = 0.21 and MAX = 0.33% and in the second cut of 2011 MIN = 0.01, MEDIAN = 0.20 and MAX = 0.35% (electronical supplement Table 1). The mean essential oil content of all genotypes for the second cut was 0.2% in 2010 and 0.19% in 2011 (Fig. 1). A lemon-like scent was determined in 62 out of 68 genotypes. In both years, genotypes BLBP75, BLBP78, BLBP85, BLBP88, BLBP94, BLBP111, BLBP112 and BLBP113 showed very low contents of essential oil. These accessions except BLBP85 and BLBP94 had a soap-like offscent.

Composition of essential oil

For the essential oil of all 68 genotypes the following main components were characterised: citronellal, (*E*)-citral, (*Z*)-citral, citronellol, β-caryophyllene, germacrene D, and β-caryophyllene oxide. Substances with a concentration less than 1% were defined as minor components. The number of these mostly non-identified substances and their concentration differed but always ranged as minor components. There were up to 30 such substances and they were grouped as "sum of unknown substances". The concentrations ranged for citronellal from 1.32 to 59.95%, (*E*)-citral from 0.78 to

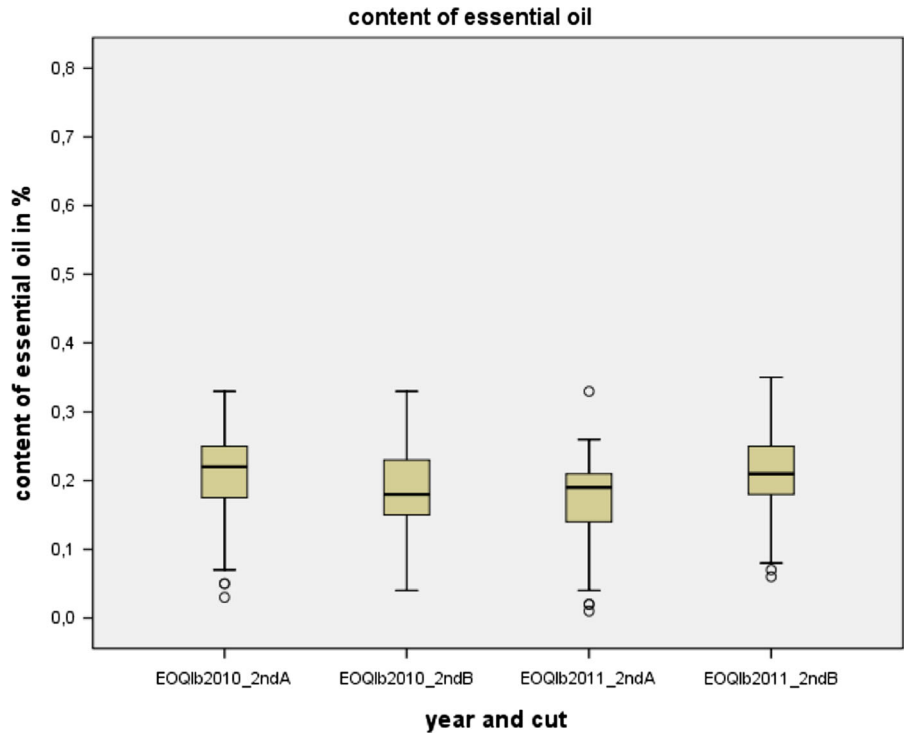

Fig. 1 Content of essential oil (EO) at trial location Quedlinburg (Qlb) of 68 genotypes of *Melissa officinalis* for the second cut 2010 and 2011, two repeats (**a**, **b**), Boxes: interquartile range, including 50% of the values; bar: median; whiskers: maximum and minimum value, excluding outliers; circle: aberration

Table 2 Minimum, maximum, median and s^2 values for content of rosmarinic acid and content and composition of essential oil (second cut 2010 and 2011) of 68 balm and lemon balm genotypes (*Melissa officinalis*) specified for chemotypes (ct.) citral and ct. ß-caryophyllene-oxide are shown for content of rosmarinic acid, content of essential oil and composition of essential oil

Chemotype	Content of rosmarinic acid	Content of essential oil	Citronellal	(*Z*)-citral	(*E*)-citral	ß-caryophyllene	Germacrene D	ß-caryophyllene-oxide	Sum of unknown substances
ct. citral, 62 accessions, second cut 2010									
Minimum	3.67	0.01	3.58	15.67	21.65	1.24	0.00	0.39	7.51
Maximum	7.55	0.35	45.17	34.27	45.65	5.09	0.49	4.28	27.42
Median	4.91	0.20	25.61	25.10	33.96	2.59	0.00	0.90	10.38
s^2	0.67	0.06	12.81	5.41	7.61	1.18	0.25	1.15	5.71
ct. citral, 62 accessions, second cut 2011									
Minimum	4.92	0.04	3.80	6.79	10.95	1.17	0.00	0.45	5.66
Maximum	8.07	0.33	59.95	32.80	44.94	8.87	1.93	6.21	38.00
Median	6.65	0.21	38.43	17.22	24.03	3.04	0.00	0.88	12.94
s^2	0.61	0.06	9.02	3.71	4.87	0.91	0.09	0.79	2.27
ct. β—caryphyllene oxide, 6 accessions, second cut 2010									
Minimum	4.39	0.03	1.32	0.50	0.78	4.70	2.01	29.61	34.39
Maximum	6.23	0.10	6.90	3.02	4.54	10.16	8.52	54.07	43.35
Median	5.09	0.07	2.99	1.43	2.23	9.51	5.50	38.90	37.52
s^2	0.49	0.02	1.57	0.82	1.24	2.01	2.39	9.17	2.91
ct. β—caryphyllene oxide, 6 accessions, second cut 2011									
Minimum	6.48	0.02	1.98	0.98	1.54	7.70	1.79	18.83	31.56
Maximum	7.95	0.11	7.26	5.47	8.45	18.64	13.61	46.98	44.39
Median	7.49	0.07	4.24	4.05	5.36	14.07	7.07	28.10	36.82
s^2	0.57	0.03	1.56	1.70	2.54	3.47	3.80	8.40	3.37

45.65%, (*Z*)-citral from 0.50 to 34.27%, β-caryophyllene from 1.17 to 18.64%, germacrene D from 0 to 13.61% and β-caryophyllene oxide from 0.39 to 54.07% (electronical supplement Table 2).

Hierarchical cluster analysis and heat map

A hierarchical cluster analysis was carried out with the content of essential oil and the relative amounts of the essential oil components: citronellal, (*E*)-citral, (*Z*)-citral, β-caryophyllene, germacrene D, β-caryophyllene oxide and non identified substances as sum of remaining, mostly unknown minor substances ("sum of unknown substances"). Citronellol was excluded because of the very low contents in all accessions. For calculation analytic data from hydro-distillations of the second cut 2010 and 2011 (electronical supplement Table 2) were used. In the resulting dendrogram accessions are clearly divided into two groups (Fig. 2). The smaller group consist of six genotypes which were all triploid [BLBP75, BLBP78, BLBP88, BLBP111 (73B), BLBP112 (75B), BLBP113 (78B)] and the bigger group includes 62 genotypes, all diploid.

After standardisation of essential oil content and concentrations of essential oil components hierarchical cluster analysis was diagrammed as a heat map (Fig. 3). In the heat map two main groups were found. The first includes content of essential oil and essential oil components citronellal, (*E*)-citral and (*Z*)-citral. The second group is characterised by β-caryophyllene, germacrene D, β-caryophyllene oxide and non identified substances.

In the second cut the citral-component as the sum of the citronellal and diastereomeres (*E*)-citral, (*Z*)-citral can represent up to 85% of the essential oil. In connection with higher essential oil content the citral-

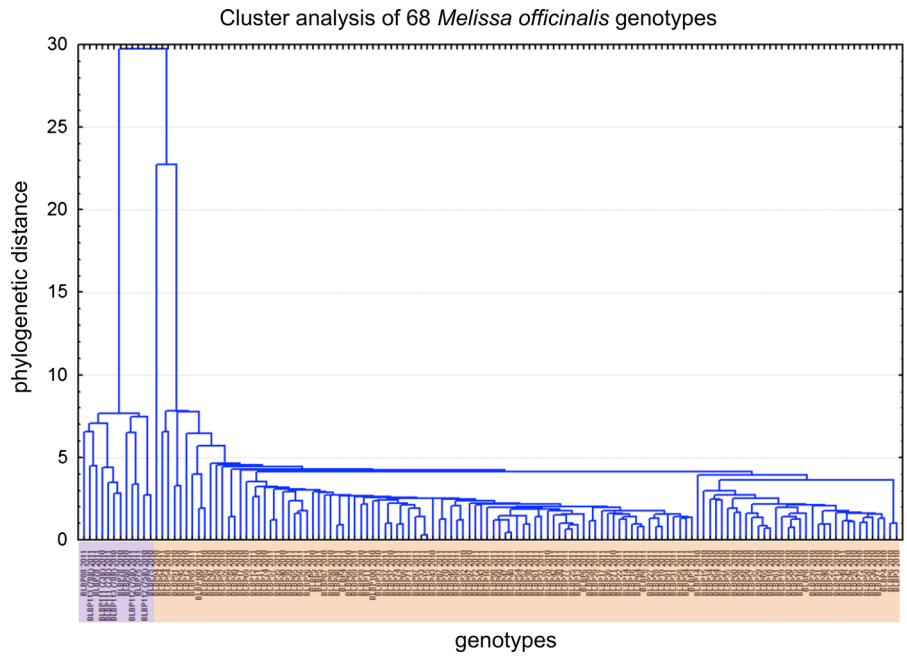

Fig. 2 Cluster analysis for content and relative amount of essential oil components [citronellal, (*Z*)-citral, (*E*)-citral, β-caryophyllene, germacrene D, β-caryophyllene oxide and non identified substances] of 68 *Melissa officinalis* genotypes. Citronellol was excluded. Data for second cut 2010 and 2011 are mean values of two replications. Orange: bigger group, 62 genotypes; Violet: smaller group, 6 genotypes

components characterises the first group. This group is established as chemotype (ct.) citral. The concentrations of essential oil components in this group reached for citronellal 3.58–59.95%, (*E*)-citral 10.95–45.65%, (*Z*)-citral 6.79–34.27%, β-caryophyllene 1.17–8.87%, germacrene D 0–1.93%, β-caryophyllene oxide 0.39–6.21% (Table 2, Fig. 4A). As chemotype citral 62 of 68 accessions were defined.

In the collection genotypes exist with the main component β-caryophyllene oxide. They belong to the second group in connection with relative content of β-caryophyllene, germacrene D and non identified substances. This group is established as chemotype β-caryophyllene oxide. The concentration of essential oil components for chemotype β-caryophyllene oxide where β-caryophyllene 4.7–18.64%, germacrene D 1.79–13.61% and β-caryophyllene oxide 18.38–54.07% (Table 2, Fig. 4B). For the genotypes of this chemotype traces of citral-components could be detected. As chemotype β-caryophyllene oxide, six of 68 genotypes were defined which had no lemon scent [BLBP75, BLBP78, BLBP88, BLBP111 (73B), BLBP112 (75B), BLBP113 (78B)].

The six genotypes of chemotype β-caryophyllene oxide are identical with the smaller group of hierarchical cluster analysis and the triploid group.

Content of rosmarinic acid

In the collection the rosmarinic acid content ranged for the second cut in the year 2010, from MIN = 3.67%, MEDIAN = 4.94% to MAX = 7.55%. Replication A of diploid genotype of *M. officinalis* BLBP8 reached 3.67% and, replication A of diploid genotype of *M. officinalis* BLBP22 7.55%. In the year 2011 the values for the second cut ranged from MIN = 4.92%, MEDIAN = 6.68% to MAX = 8.07%. Replication B of diploid genotype of *M. officinalis* BLBP33 reached 4.92%, replication A of diploid genotype of *M. officinalis* BLBP52 8.07% (electronical supplement Table 1). The mean values of 2010 for replication A were 5.26% and for replication B 4.86%. For both replications in 2011 the mean values were higher: A 6.82% and B 6.54%. The mean and median values do not differ (Fig. 5).

Discussion

Content of essential oil

The content of essential oil varies in a great range between accessions, cuts and years. Askari and Sefidkon (2004) and Bomme et al. (2002) reported values between 0.14 and 0.25%. Bahtiyarca Bagdat

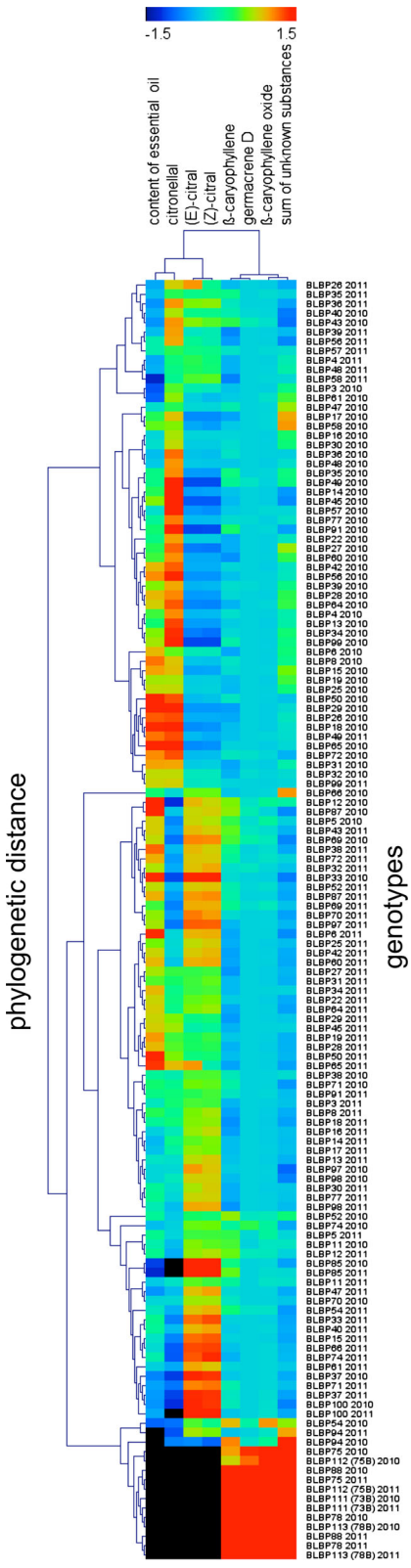

Fig. 3 Heat map for amount and relative concentration of essential oil components [citronellal, (*Z*)-citral, (*E*)-citral, β-caryophyllene, germacrene D, β-caryophyllene oxide and non identified substances, without citronellol] of 68 *Melissa officinalis* accessions. The relative concentrations were standardised before hierarchical cluster analysis (HCL). Colour code: deep blue/black (low/absent) − 1.5 to red (high) + 1.5

and Cosge (2006) reported values between 0.01 and 0.25%. Bomme et al. (2002) also stated that values up to 0.8% could only be realised under Spanish conditions. He cited the data of Adzet et al. (1992a), which describes the improvement by selection of balm varieties with an average content 0.3% essential oil to cultivars with an average of more than 0.5% and extreme values up to 0.68% and more. But also Basker and Putievsky, (1978) depict values between 0.6 and 0.7% in their evaluation of two cultivars. Mrlianova et al. (2002) analysed 16 accessions and reported contents from 0.06 – 0.16%.

The content of essential oil in the evaluated collection ranged from 0.01 – 0.35%. The data confirms the values of the literature. The range shows a strong dependence from biotic and abiotic conditions, different harvesting years and genetic make-up of the genotypes. Especially the chemotype ct. β-caryophyllene oxide had very low contents of essential oil (Table 2). The data could not be related to other literature, because those chemotypes are not explicitly mentioned. The data suggest that the β-caryophyllene oxide chemotype had very low content of essential oil. Authors of previous evaluations reported very low contents of essential oil in the first cut, which were harvested shortly before flowering (Bomme et al. 2002).

Composition of essential oil

The composition of essential oil for the genotypes showed an individual pattern of ingredients. Tavares et al. (1996) state the composition of (*E*)-citral + (*Z*)-citral 48%, citronellal 39.47% and β-caryohyllene with 2.37% and Bahtiyarca Bagdat and Cosge (2006) 39% citronellal, 33% citral (citronellol, Linalool, (*E*)-citral and geraniol (*Z*)-citral). We determined the components (*E*)-citral, (*Z*)-citral, citronellal, citronellol, germacrene D, β-caryophyllene and β-caryophyllene-oxide and found different chemotypes in the collections. Every genotype had its own pattern of metabolites (Fig. 3). That is the reason why we suggest ranges for the main components like Sharafzadeh et al. (2011) and Azizi et al. (2009). A major shift between (*E*)-citral, (*Z*)-citral, citronellal to germacrene or β-caryophyllene could not be investigated. There is no transition from ct. citral to ct. β-caryophyllene. If an accession showed a special chemotype, it could not be transferred to another

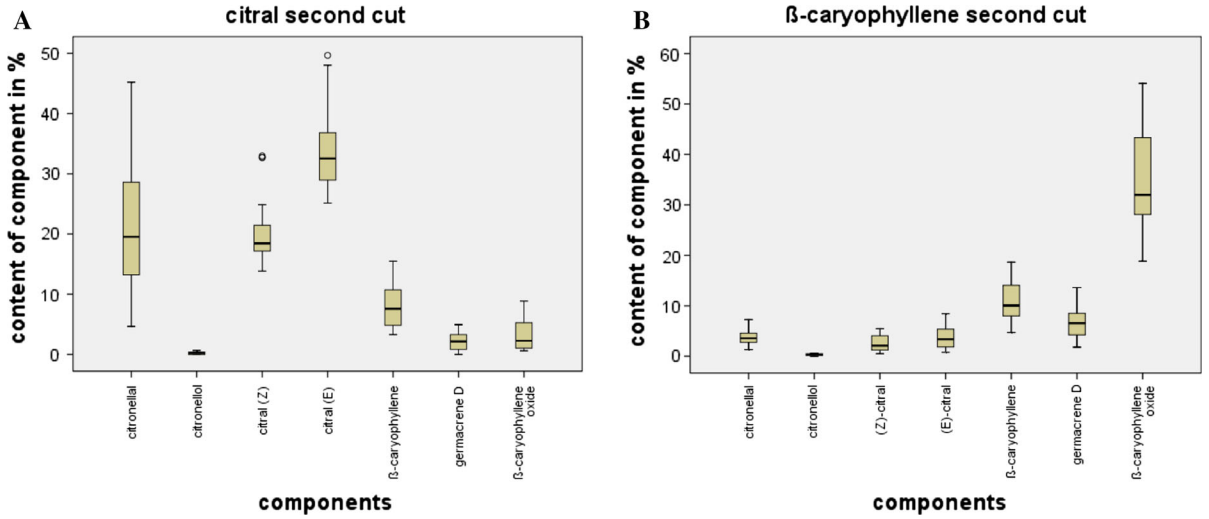

Fig. 4 Composition of essential oil of 68 genotypes for separate chemotypes: ct. citral: 62 genotypes (**A**) and ct. β-caryophyllene oxide: 6 genotypes (**B**). Values for two repeats of second cut 2010 and 2011; Boxes: interquartile range, including 50% of the values; bar: median; whiskers: maximum and minimum value, excluding outliers; circle: aberration

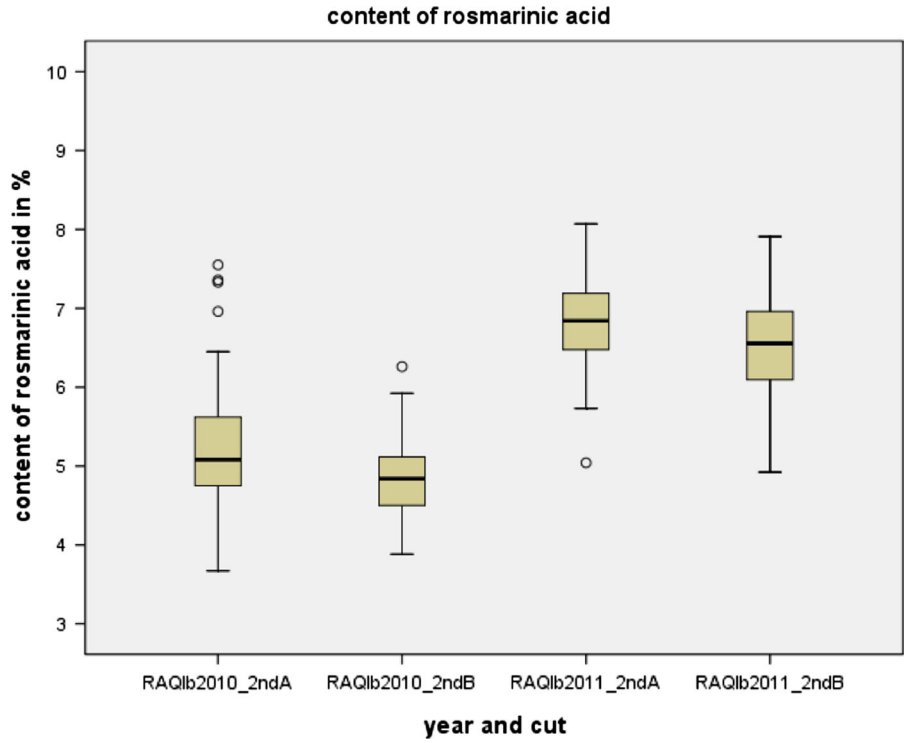

Fig. 5 content of rosmarinic acid (RA) for the years 2010 and 2011, second cut (2nd) for two repeats (**A**, **B**) at trial location Quedlinburg (Qlb)Boxes: interquartile range including 50% of the values; Bar: median; Whiskers: maximum and minimum value, excluding outliers; Circle: outliers; No extreme values are in the data sets

chemotype. Literature mentions changes between (*E*)-citral, (*Z*)-citral and citronellal. Hefendehl (1970) observed ranges from 8.7–96.6% citral and citronellal 0.9–39%. He suggested that young leaves show a high content of citral, while older leaves show a higher content of citronellal. He assumed the age of leaves could be a reason for that phenomenon or that the content and composition of essential oil could be a matter of leaf position. Because of this it is not justified

to subdivide ct. citral for amount of (*E*)-citral, (*Z*)-citral and citronellal (Fig. 4).

The handbook of essential oil (Baser and Buchbauer 2010) state that phytochemical polymorphism is often the case between different plant organs. In *Origanum vulgare* subsp. *hirtum* even different oil glands of the same plant showed a polymorphism (Johnson et al. 2004). Hose et al. (1997) also showed that the composition in essential oil glands of *M. officinalis* changes during ontogenesis. This is mentioned for other species (*Salvia sclarea* L., *Satureja hortensis* L., *Salvia officinalis* L.) as well (Grassi et al. 2004; Johnson et al. 2004; Novak et al. 2006; Schmiderer et al. 2008).

Of the 68 evaluated genotypes 62 belong to ct. citral and conform to Pharmacopoeia Europaea (Ph. Eur. 8 2014). The described variation offers the required basis for breeding programs to increasing essential oil content. The breeding process can be expedited by using haploid induction as reported for balm (Kästner et al. 2016).

Content of rosmarinic acid

For the pharmaceutical use next to essential oil, rosmarinic acid is the substance of interest, because of its proven pharmaceutical effects. Until 2008 rosmarinic acid had to be calculated by a photometric method as the sum of all hydroxycinnamic acid derivatives (Ph. Eur. 6 2008). Since 2009 the Pharmacopoeia Europaea changed to calculate specific rosmarinic acid content by using high performance liquid chromatography (HPLC) (Ph. Eur. 6 2009). Krüger et al. (2010) compared HPLC and photometry methods to evaluate the content of rosmarinic acid in 2009. The determined values ranged between 2.8 and 9%, HPLC method and 7.4 and 15.5% sum of hydroxycinnamic acid derivatives. The content of rosmarinic acid is an important quality requirement and a raised content is a desired aim of breeding programs. The aim of the evaluation was first time characterization of large sets of lemon balm and balm for their content of rosmarinic acid according to the HPLC method. All tested 68 accessions fulfil the requirements of Ph. Eur. for rosmarinic acid. For most accessions from this collection the sum of hydroxycinnamic acid derivatives were measured by photometric method (Bomme et al. 2008). There is no correlation between this data and the presented data because of the insufficient correlation between both methods (Krüger et al. 2010) and the high impact of the year and the ontogenetic status of measured plants. The presented data are valuable contribution for characterization of rosmarinic acid in the species *M. officinalis* and offer in connection with status of ploidy (Kittler et al. 2015) and amount and composition of essential oil a prerequisite for taxonomical studies inside the species.

Lemon balm produces a high amount of rosmarinic acid in comparison with other species of family Lamiaceae. This can be used for special breeding programme to create lines with stable very high yield of rosmarinic acid. The better winter hardiness and higher fresh mass production of triploid balm accessions can be used for production of rosmarinic acid even they are not conforming to Ph. Eur.

Conclusions

The screening of 68 balm and lemon balm genotypes showed that every genotype had its own pattern of essential oil, which shifted in ranges as well in quantity as in quality. The presented results suggest the existence of two different chemotypes in the tested collection of *M. officinalis*. We declare the ct. citral and ct. β-caryophyllene oxide. In this study the chemotypes coincide with the ploidy level. The genotypes of ct. citral were always diploid and the genotypes of ct. β-caryophyllene oxide were triploid. To verify this appearance more non-citral accessions need to be characterised. Content of rosmarinic acid is a quality requirement of the Ph. Eur. But there is also a rising demand for lemon balm with high content of rosmarinic acid. The data of rosmarinic acid evaluation in connection with ploidy level and amount and composition of essential oil can contribute to taxonomical studies inside the species *M. officinalis*.

The presented results could be used for generation of a core collection for *M. officinalis*. The evaluation results of 28 M. *officinalis* accessions which include 10 accessions of ct. germacrene D and 15 of ct. citral (Kittler et al. 2018) should also be include in the selection of the core collection to reach the maximum of variability for characterized traits. Candidate accessions should be tested more intensive again.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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