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Could the variety influence the quantitative and qualitative outcome of lemon balm production?

Krisztina Szabó[a,*], Mahmoud Malekzadeh[a], Péter Radácsi[a], Márta Ladányi[b], Péter Rajhárt[a], Katalin Inotai[a], Szilvia Tavaszi-Sárosi[a], Éva Németh[a]

[a] Department of Medicinal and Aromatic Plants, Corvinus University of Budapest, Hungary
[b] Department of Biometrics and Agricultural Informatics, Corvinus University of Budapest, H-1118 Budapest, Villányi Str. 29-43. Hungary

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ABSTRACT

Intraspecific variability of Lemon balm is hardly known. In order to reveal the genetic potential of the varieties available for producers, five *Melissa officinalis* genotypes were compared in a pot experiment. Morphological and yield characteristics were remarkably different for the variety 'Gold Leaf'. Considering their dry mass of shoots/plant the tested accessions represented a series with slightly increasing dry yield as follows: 'Gold Leaf'(44.6 g/plant), 'Lemona' (72.8 g/plant), 'Lorelei'(80.2 g/plant), 'Quedlinburger Niederliegende'(84.6 g/plant), and finally 'Soroksár' (102.8 g/plant). The essential oil content was the highest for variety 'Lorelei' (0.298 mL/100 g dry weight), while the remaining 4 varieties grouped into the same statistical category with lower essential oil content. The essential oil composition based on the data showed only slight quantitative differences. Main compounds were geranial, neral and citronellal for each of the 5 varieties. The parameters (total phenolic content (TPC), total flavonoid content (TFC), total hydroxycinnamic derivatives (THA), rosmarinic acid (RA)) representing different phenolic compounds or groups of compounds showed some differences among the varieties, however, there was not a single variety in which all parameters would have been outstanding. TPC results varied between 359 and 426 mg gallic acid equivalent/g dry weight, but did not show significant difference among the accessions. The highest TFC was measured in the case of variety 'Lorelei' (0.948 mg isoquercitroside equivalent/g dry weight). Total hydroxycinnamic derivatives accumulated in the highest ratio in variety 'Gold Leaf' (8.06% dry weight), however, the main compound RA were measured in variety 'Lorelei' in the highest ratio (3.01% dry weight). Ferric reducing antioxidant power (FRAP) was the highest in variety 'Soroksár' (309.1 ascorbic acid equivalent/g dry weight), however this genotype was not among the best ones when TPC, TFC, THA or RA content of varieties were compared.

1. Introduction

Lemon balm (*Melissa officinalis*) is a perennial bushy plant, native of Western Asia and the Eastern Mediterranean region, cultivated in many countries throughout Europe. The aim of its cultivation is either the production of essential oil or to produce its leaf drug *Melissae folium* containing the biologically active main compound rosmarinic acid as it is officially described in several pharmacopoeias. Since both types of its secondary metabolites are extremely important for human use, attempts were made to study the *genetic potential* of the species.

On the one hand, the essential oil belongs to the more precious oils in the higher price class. Therefore, in addition to the genetic potential of different accessions of *M. officinalis* the question of adulteration or replacement of subsp. *officinalis* brings into focus the two other known subspecies (subsp. *inodora*, subsp. *altissima*), as well (Miceli et al., 2006; Marongiu et al, 2004; Patora et al., 2003). In respect of essential oil accumulation further modifier aspects were examined such as ecological conditions, different stages of plant growth, intraspecific variability, drying and storage. So far one knows, that the essential oil content of *M. officinalis* usually vary between 0.05–0.52% (Patora et al., 2003; Sari and Ceylan 2002; Saeb and Gholamrezaee 2012; Manukyan and Schnitzler, 2006; Basta et al., 2005; Seidler-Lozykowska et al., 2013; Argyropoulos and Müller, 2014a), and rarely reaches 0.8% what can be achieved only by means of genetic improvement (Adzet t. Ponz et al., 1991). On the other hand, much less is known about the genetic potential of the species concerning its main phenolic compounds including rosmarinic acid or the influencing factors that modify their economically feasible production. Few results were published con-

* Corresponding author. Fax: +36 14826330.
E-mail address: Szabo.Krisztina@kertk.szie.hu (K. Szabó).

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cerning the effect of phenophase, intraspecific variability, water stress or primary processing (Tóth et al., 2003; Oniga et al., 2010; Manukyan, 2011; Argyropoulos and Müller, 2011; Argyropoulos and Müller 2014b,c; Szabó et al., 2009; Hohmann et al., 1999; Atanassova et al., 2011). Summing up the former results, rosmarinic acid can be found between 3 and 5.7% in the plant. The content of hydroxicinnamic derivatives varies between 4 and 12%; and the total flavonoid content is usually between 0.2 and 0.5%.

One has a general picture of the genetic potential of the species especially for both types of its secondary metabolites. However, the results of the description and comparison of different accessions can usually be used later on only by the actual research group by whom the experiment was carried out. Therefore, the aim of this paper is to provide a comprehensive evaluation of some commercially available genotypes including morphological, yield and active ingredient characteristics.

2. Materials and methods

2.1. Plant material

Seed materials of 4 genotypes of lemon balm (*M. officinalis* L.) were purchased from commercial companies (Table 1). Genotype 'Soroksár' without variety denomination was purchased from a Hungarian nursery as seedling in 2009 and maintained at the Research Station at Soroksár, Hungary. Seeds of this plot were collected in 2013 and used later on in the experiment with the name 'Soroksár'. Seeds of all varieties were sown on 1 April 2014 in greenhouse and pricking was done on 28 April 2014. The seedlings were planted into pots on 21 May 2014 (1plant/pot), with 5 individuals/genotype except genotype 'Gold Leaf', in the case of which two plants were replanted into one pot in order to ensure enough plant material for phytochemical analyses.

2.2. Growth conditions

Plants were grown in pots under transparent plastic roof to exclude the natural precipitation and to maintain a stable level of soil water capacity. The medium was a commercially available soil mixture (type Florasca Bio "B") consisted of 10% sand, 65% peat from the Fertő-Hanság area (Hungary) and 25% cattle manure compost. Characteristics of the medium are summarized in Table 2. Each pot was installed to an equal weight of 8 kg. Soil water capacity (SWC) was determined using the gravimetric method (Reynolds, 1970). According to this the plants were irrigated by measuring the pots' weight and filling the pots to a standard weight representing 70% saturation of soil water capacity. Checking of both SWC and irrigation were carried out 3 times per week.

Planting was made on 21 May 2014 and the harvest was carried out on 3 September 2014; when the plants were still in vegetative phase. Before the harvest, the most important morphological characteristics were recorded. After that all the plants were taken out from the pots and separated into shoot and root parts to determine the biomass production and prepare samples for laboratory analysis.

2.3. Physico-chemical characteristics

2.3.1. Morphological characteristics

Plant height was measured as the length of the longest shoot from the root neck to the tip of the shoot. In line with this, root length was determined as a distance from the root to the tip of the longest root. Plant diameter was measured as a natural horizontal expansion of the shoots. Besides, the number of main shoots on each individual was determined. These measurements were carried out in 5 replicates/variety. Leaf length and width were measured on randomly chosen fully expanded leaves in 10 replicates/variety.

2.3.2. Production

Immediately after lifting the plants from the pots, the shoot and root parts were measured for each individual to get the fresh mass of the organs. The plant parts were dried at room temperature till constant weight and the dry mass was registered. These measurements were carried out in 5 replicates/variety.

2.3.3. Phytochemical measurements

After drying all harvested individual shoot samples were mixed creating a bulk sample for each variety. Thick stem parts making up the lower two-third of the shoots were eliminated from the samples in order to ensure the proper powder state during grinding, practically resulting in *Melissae folium* powder. From the homogeneous mass sample, three replicates were made as biological replicates. In the case of each biological replicate, phytochemical measurements were done in 3 replicates. To determine the essential oil content three biological replicates were used without replicates. These samples were not grinded and they contained also the stem part (*Melissae herba*) in order to have enough material for essential oil distillation.

2.3.3.1. Determination of essential oil. Essential oil was extracted from a mixture of 10 g chopped aerial part of the plants (bulk sample of 5 individuals from each accession) by hydro-distillation (500 mL water) with a Clevenger-type apparatus for 2 h according to the method recommended in the VII. Hungarian Pharmacopoeia (Pharmacopoeia, 1986). The amount is expressed in mL/100 g dry weight (DW).

2.3.3.2. Analysis of the essential oil composition. GC–FID analysis was carried out using an Agilent Technologies 6890N instrument equipped with a HP-5 capillary column (length: 30 m, $d = 350\ \mu m$, film thickness: 0.25 μm), programmed as follows: initial temperature 50 °C, then at a rate of 4 °C min^{-1} up to 150 °C; and afterwards by a rate of 12 °C min^{-1} up to 220 °C. Injector and detector temperatures: 250 °C, carrier gas: helium (constant flow rate 0.5 mL min^{-1}); split ratio: 22.6:1.

GC–MS analysis was carried out from the essential oils using an Agilent Technologies 6890N GC equipped with an Agilent Technologies MS 5975 inert mass selective detector, by using a capillary column of HP-5MS (5% phenyl methyl siloxane), length: 30 m, $d = 250\ \mu m$, film thickness: 0.25 μm), programmed as follows: initial temperature 60 °C, then at a rate of 3 °C min^{-1} up to 240 °C. Carrier gas: helium (constant flow rate: 1 mL min^{-1}); injector: 230 °C, split ratio: 30:1, transfer line: 240 °C. Injectation: Agilent Technologies 7683B automatic injector. Injected quantity: 0.2 mL (10% hexane solution) Ionization energy was 70 eV. Compounds identification was made by comparing their mass spectra to librarian references (NIST and Wiley) and by calculating their linear retention indexes (LRI) according to the elution ranking of alkanes (C9–C20) using the generalized equation of Van den Dool and Kratz (1963).

2.4. Antioxidant properties

2.4.1. TPC

For the total phenolic content measurement, 1 g dried and powdered plant material was extracted by 100 mL hot distilled water and was allowed to stand for 24 h. Then the extracts were filtered and stored in freezer until the experiments took place.

The total phenolic content was determined by the modified method of Singleton and Rossi (1965). Sample solution of 0.5 mL was introduced into a test tube and then 2.5 mL Folin–Ciocalteau's