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## Morphological-, chemical- and RAPD-PCR evaluation of eight different *Ocimum basilicum* L. gene bank accessions



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### ABSTRACT

In our study eight different *Ocimum basilicum* L. gene bank accessions were characterized by morphological evaluation, essential oil composition and RAPD molecular markers. Morphological traits were described using the standardized descriptor list of UPOV. The composition of the essential oil distilled from dried leaves was determined by gas chromatography/mass spectrometry (GC–MS). Fresh young leaves were used to extract genomic DNA for the RAPD-PCR evaluation. From the morphological aspects two groups could be distinguished with the method of cluster analysis. From the chemical aspect two different chemical types can be distinguished, according to the main components. The first one is a linalool type ('Arvada', 'Dark Opal', 'Genovese', 'Lengyel', 'Rit-Sat') and the second one is a linalool–methyl chavicol type ('A-1', 'Mittelgroßblättriger Grünes', 'Piros'). Based on RAPD-PCR analysis the accessions formed two clusters. The purple-leaved accessions ('Piros', 'Dark Opal') were located in group A, and the remaining ones ('Genovese', 'Arvada', 'Lengyel', 'Rit-Sat', 'Mittelgroßblättriger Grünes', 'A-1') were classified into group B. 'Rit-Sat' and 'Genovese' were not separated, based on their band pattern.

It is shown by the result of the morphological, chemical and RAPD-PCR analysis that the data obtained by different methods not necessarily correlated with each other, except the accessions of 'Genovese' and 'Dark Opal', in which cases all characteristics show a good distinction. We observed that the cultivar 'Genovese' and the 'Rit-Sat' accession represent quite similar quality and the 'Arvada' accession of American origin is not really separated from the European ones by the complex evaluation.

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## 1. Introduction

*Ocimum basilicum* L. is one of the most important species amongst the member of the *Ocimum* genus. Its aromatic leaves, flowering tops and essential oils are used in food industry (flavouring foods and beverages), in perfum-

ery (e.g. hair dressings, perfumes, soaps, dental creams, mouth washes) and in traditional medicine (Holm, 1999; De Masi et al., 2006). It has antibacterial, antifungal, insecticidal, and hepatoprotective activity and contains antimicrobial substances, also. It has antioxidative- and antiulcerogenic effect by the literature references (Holm, 1999). The plant accumulates essential oil in high amount (monoterpenes, sesquiterpenes and phenylpropanoids), which can be found in the organs of the plant in different amount.

The genus *Ocimum* (Lamiaceae) includes annual and perennial herbs and shrubs native to the tropical and subtropical regions of Asia, Africa, Central and South

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America (Paton et al., 1999). Based on extensive taxonomical study made by Paton et al. (1999) it can be stated that 65 species belong to the *Ocimum* genus, only and many of the related species can be classified into other genera. Large morphological and chemical variability exists in the genus *Ocimum* (Carović-Stanko et al., 2010a,b; Carović-Stanko et al., 2011a,b; Grayer et al., 2002; Labra et al., 2004; De Masi et al., 2006; Telci et al., 2006), which are influenced by several environmental factors.

For morphological characterization International Union for the Protection of New Varieties of Plants (Anonymous, 2003) have been invented a descriptor list for plant cultivars; which is the result of the agreement of the scientific community of this field.

The composition is modified furthermore by morphogenesis, essential oil extraction method, drying and storage. Thus, chemotaxonomical approach based on essential oil composition of *O. basilicum* generates several discussions among experts. Therefore morphological and chemical traits as characterization features for gene bank accessions are not exclusively useable. Also, for breeding purposes deeper knowledge on relationships among existing materials is needed.

To make a better distinction between *Ocimum* taxa the traditional evaluation methods has to be completed by involving molecular markers (which are not influenced by the environmental factors) (Carović-Stanko et al., 2011a,b; Labra et al., 2004).

DNA markers are regarded as one of the best technique to determinate genetic diversity, due to the high polymorphism, stability and independency from environmental

factors. Contrary to the fact that *O. basilicum* is a worldwide used spice the existing literature background on its DNA markers is not as wide as for other species. Random amplified polymorphic DNA (RAPD) (Williams et al., 1990) is one of the numerous techniques. RAPD have been used in experiments of genetic diversity in various studies (Carović-Stanko et al., 2010a; De Masi et al., 2006; Singh et al., 2004; Vieira et al., 2003). Other, more sophisticated, sequence-based markers either are available only in silico (Gupta et al., 2010) or they require high investments which unfortunately are not available in everyday gene bank techniques (De Maitta et al., 2011). The advantage of RAPD is its simplicity and low cost, and DNA sequence information is not required for the analysis (Khanuja et al., 1998; Powell et al., 1996). Its disadvantage is its low reproducibility.

The purpose of our research was to compare and characterize different gene bank accessions of *O. basilicum* based on their morphological-, chemical- and molecular traits.

## 2. Material and methods

### 2.1. Plant material

Eight *O. basilicum* accessions (Fig. 1) have been involved into the investigation. The gene bank accessions and their origin are listed in (Table 1). Seeds were sown in green house and were grown in the field of Medical Plant Research Station at Soroksár in 2012 (Corvinus University of Budapest). The planting date was 30.05.2012. The spacing was 50 cm × 30 cm. Plants for analysis were

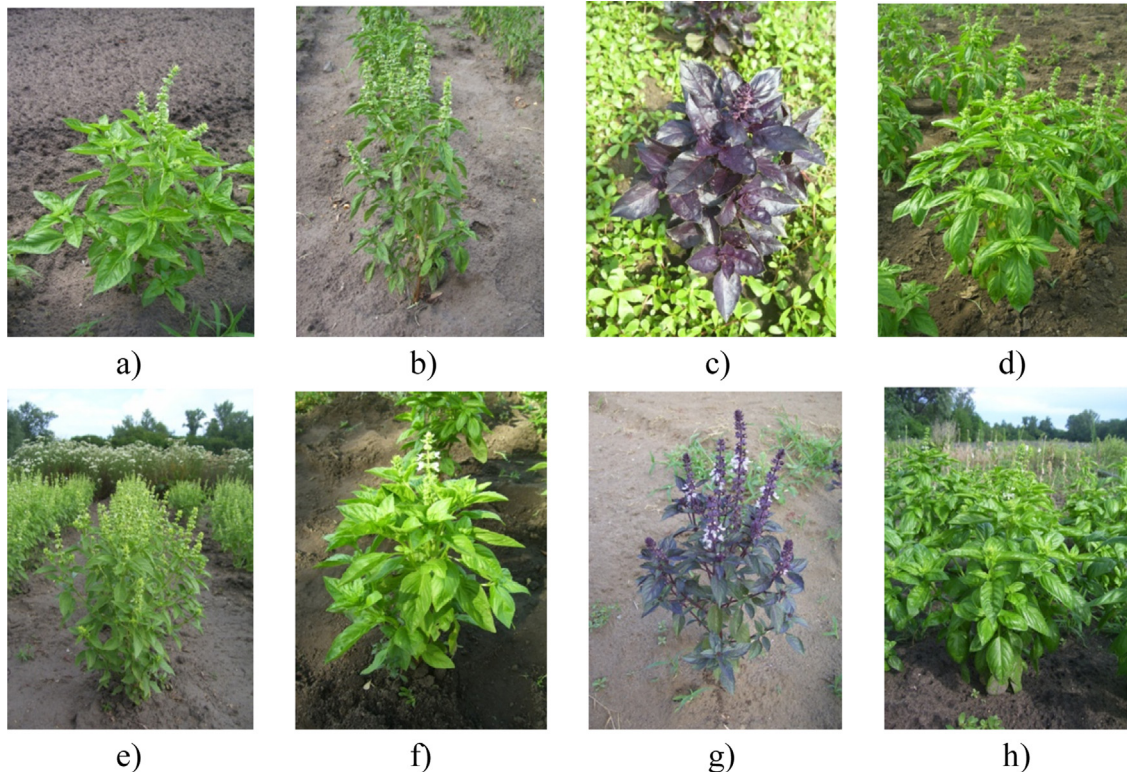


Fig. 1. (a) 'A-1', (b) 'Arvada', (c) 'Dark Opal', (d) 'Genovese', (e) 'Lengyel', (f) 'Mittelgroßblättriger Grünes', (g) 'Piros', and (h) 'Rit-Sat'.

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