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ORIGINAL PAPER

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# MORPHOLOGICAL AND GENETIC DIVERSITY AMONG PEPPERMINT (*Mentha × piperita* L.) CULTIVARS

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**ABSTRACT**

The study determined the morphological and genetic diversity among nine cultivars of peppermint (*Mentha × piperita* L.): 'Almira', 'Asia', 'Chocolate', 'Citaro', 'Granada', 'Grapefruit', 'Multimentha', 'Swiss' and 'Variegata'. The leaves of the peppermint cultivars were characterized by substantial variation in morphology and size. The leaves of 'Multimenth', 'Grapefruit' and 'Swiss' were largest, and those of 'Swiss' were considerably elongated. The 'Almira' cultivar had the smallest leaves. Although similar leaf morphology was observed in 'Asia', 'Citaro' and 'Chocolate', in 'Grapefruit' and 'Multimentha' and in 'Swiss' and 'Variegata', no two cultivars were the same in this respect. Differentiation of tested peppermint cultivars were also confirmed at genetic level. Genetic diversity among tested cultivars ranged from 0.388 to 0.846. The most different were cultivars Almira and Citaro.

**Key words:** DNA polymorphism, leaf morphology, RAPD

## INTRODUCTION

Peppermint (*Mentha × piperita* L.) is a perennial aromatic herb native to Europe, cultivated in the northern USA, Canada, Asia, and many other parts of the world. A hybrid of spearmint (*M. spicata* L.) and water mint (*M. aquatica* L.), peppermint grows particularly well in areas with soil of high water-holding capacity. It is best known for its flavour and fragrance properties; peppermint leaves (fresh and dried) and the essential oil extracted from the leaves are used in many cosmetic, pharmaceutical and food products [Işcan et al. 2002, McKay and Blumberg 2006, Kiełtyka-Dadasiewicz et al. 2016]. As a pharmaceutical raw material peppermint leaves have been described in the *European Pharmacopoeia* and pharmacopoeias in many countries, but none of these specify which varieties or cultivars may be used [European Pharmacopoeia 2014, Pharmacopoeia Polonica 2014, British Pharmacopoeia 2015]. Yet phytochemical analysis indicates significant heterogeneity between peppermint cultivars [Ludwiczuk et al. 2015]. In recent years many new cultivars of peppermint have been created, with diverse morphological, flavour and utility features [Kiełtyka-Dadasiewicz et al. 2016].

In many cases, due to the high similarity of genotypes, it is difficult to distinguish cultivars using morphological and physiological methods. Sometimes also isozyme analyses are insufficient for the cultivars identification. [Reynders and Bollereau 1994]. In these cases molecular markers provide the best way to estimate of genetic diversity. DNA markers are independent of the confounding effects of environmental features, can be used at a very early stage of plant development, they are cheap and easy to apply [Waugh and Powell 1992, Kabir et al. 2014]. One of the most recently method used to estimate

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genetic diversity is RAPD (Randomly Amplified Polymorphic DNA). This fingerprinting technique provides an unlimited number of markers which can be used for various purposes [Williams et al. 1990, Gupta and Varshney 2013]. RAPD markers have been widely used in diverse plant species for assessment of genetic variation in populations and species, fingerprinting, and the study of phylogenetic relationships among species and subspecies [Gupta 1999]. This technique has become an increasingly popular tool in genetic studies because it is technically straightforward and inexpensive [Emadpour et. al. 2009]. *Mentha* species have been assessed for genetic relationships and cultivar identification [Fenwick and Ward 2000, Khanuja et al. 2000, Grisi et al. 2006, Smolik et al. 2007, Šarić-Kundalić et al. 2009].

The aims of presented study were identification and characterization of differences in leaf morphology and estimation of genetic diversity among 9 peppermint (*Mentha × piperita* L.) cultivars characterised by different biochemical compounds [Ludwiczuk et al. 2016].

## MATERIALS AND METHODS

### Plant materials

The study was performed on 9 cultivars of *Mentha × piperita* L.: 'Almira', 'Asia', 'Chocolate', 'Citaro', 'Granada', 'Grapefruit', 'Multimentha', 'Swiss' and 'Variegata'. Selected cultivars were previously characterised by Ludwiczuk et al. [2016] as a different in respect of essential oils and antibacterial activity against *Staphylococcus epidermidis*. The plants were taken from the collection of the Garden of Cosmetic Plants and Raw Materials, Research and Science Innovation Centre, located in Wola Zadybska in the Lubelskie region of Poland (51°45'N, 21°51'E). The plants were grown on lessive soil which was slightly acidic ($pH_{KCl}$ 6.1).

### Morphological analysis

Morphological analysis of leaves was conducted in 2014 and 2015. The results are reported as the arithmetic mean of the two years. Each year measurements were made four times at weekly intervals (twice before flowering, at the bud stage, and at the beginning of the flowering stage) as replications. On each occasion 4 middle leaves were taken from 10 plants of each cultivar. The length of the petiole and the length and width of the leaf blade were measured. The results were used to calculate the leaf blade shape index. Morphological qualitative traits (leaf shape, margin, bases and colour) were defined according to Tsukaya [2006] and Leaf Architecture… [1999]. The numerical results were analysed statistically by analysis of variance at a significance level of 0.05. The Pearson correlation coefficient between the length and width of the leaf blade and the length of petiole and blade were determined. Calculations were carried out in Statistica 9.0 and Excel 7.0.

### DNA preparation

Genomic DNA was extracted from fresh and young tissue using GeneMATRIX Plant & Fungi DNA Purification Kit (EURx). The quantity and quality of the isolated DNA was assessed using NanoDrop 2000 Spectrophotometer. Additionally DNA concentration was determined by electrophoresis on a 1.5% agarose gel by comparison with a molecular weight standard MassRuler™ DNA Ladder (Thermo Fisher Scientific). The samples were then adjusted to equal DNA concentrations of 20 ng/ml.

### RAPD analysis

PCR reactions were performed according to the RAPD method described by Williams et al. [1990] with modifications. Reaction mixtures contained 1 x PCR Buffer (10 mM Tris pH 8.8, 50 mM KCl, 0.08% Nonidet P40) (Thermo Fisher Scientific), 160 µM of each dNTP, 530 pM oligonucleotide primer, 1.5 mM $MgCl_2$, 70 ng of template DNA and 0.5 U Taq DNA Polymerase (Thermo Fisher Scientific) in a final reaction mixture of 15 µl. Amplification was carried out in a Biometra T1 thermal cycler programmed for 3 min at 94°C of initial denaturation, 44 cycles: 94°C – 45 s, 37°C – 45 s and 72°C – 45 s, with a final extension at 72°C for 7 min. A negative control was added in each run. To verify reproducibility, the primers were tested twice on the same sample.

Amplification products were separated by electrophoresis on 1.5% agarose gels containing 0.1% EtBr (1.5 h, 120 V). Fragments were visualized under a UV transilluminator and photographed using the PolyDoc System. GeneRuler™ 100bp DNA Ladder Plus (Thermo Fisher Scientific) was used to establish the molecular weights of the amplification products.

### Data analysis

RAPD products were scored as present (1) or absent (0) from the photographs. Only bright and reproducible products were scored. The level of polymorphism of the primer (polymorphic products/ total products) and relative frequency of polymorphic products (genotypes where polymorphic products were present/ total number of genotypes) [Belaj et al. 2001] were calculated. The resolving power of the primer was calculated using the following formula: Resolving power (Rp) = Σ Ib (band informativeness). Band informativeness was calculated individually for each band scored by the primer: Ib = 1-[2(0.5-p)], where p is the proportion of occurrence of bands in the genotypes out of the total number of genotypes [Prevost and Wilkinson 1999]. Polymorphic information content (PIC) was calculated by applying the following simplified formula [Anderson et al. 1993]: PIC = 2fi(1-fi), where fi is the percentage of the $i^{th}$ amplified band present.

Genetic pairwise similarities (SI-similarity index) between genotypes were evaluated according to Dice's formula after Nei and Li [1979]. A cluster analysis was conducted using the distance method UPGMA (Unweighted Pair-Group Method with Arithmetic Mean), and clustering was verified by bootstrapping (1000 rep.). PCA analysis was performed using PAST software. Statistical analysis was performed in PAST software [Hammer et al. 2001].

## RESULTS AND DISCUSSION

In the case of plants producing oil glands, especially on the leaves, leaf area (which depends on leaf length and width) is an important factor in the productivity of essential oil [Maffei et al. 1994]. The results of our study indicate substantial biometric variation in the leaves of the peppermint cultivars analysed (tab. 1 and fig. 1).

**Table 1.** Leaf dimensions of analysed peppermint cultivars

| Peppermint cultivar | Length of leaf blade (mm) | Width of leaf blade (mm) | Petiole length (mm) | Shape index | Correlation coefficients | |
|---|---|---|---|---|---|---|
| | | | | | A | B |
| 'Almira' | $25.5^{f}$ ±2.1 | $14.5^{e}$ ±0.9 | $26.4^{g}$ ±2.2 | $1.76^{b}$ | 0.894* | 0.235 |
| 'Asia' | $43.1^{e}$ ±1.0 | $30.6^{c}$ ±1.6 | $70.4^{d}$ ±1.7 | $1.41^{cd}$ | 0.755* | –0.073 |
| 'Citaro' | $56.4^{c}$ ±1.5[#] | $30.5^{c}$ ±1.2 | $81.6^{bc}$ ±2.2 | $1.85^{b}$ | 0.913* | –0.296 |
| 'Chocolate' | $41.8^{e}$ ±1.6 | $23.0^{d}$ ±0.9 | $89.4^{b}$ ±1.6 | $1.82^{b}$ | 0.933* | –0.255 |
| 'Granada' | $48.3^{de}$ ±1.7 | $35.9^{bc}$ ±1.6 | $86.1^{b}$ ±3.6 | $1.35^{d}$ | 0.847* | 0.450 |
| 'Grapefruit' | $66.1^{b}$ ±2.3 | $45.4^{a}$ ±3.0 | $53.6^{e}$ ±2.5 | $1.46^{c}$ | 0.919* | –0.282 |
| 'Multimentha' | $64.8^{b}$ ±1.8 | $47.6^{a}$ ±2.0 | $73.9^{cd}$ ±3.8 | $1.36^{d}$ | 0.954* | 0.359 |
| 'Swiss' | $75.5^{a}$ ±2.3 | $36.6^{b}$ ±1.5 | $41.8^{f}$ ±1.0 | $2.06^{a}$ | 0.690* | 0.548* |
| 'Variegata' | $51.8^{cd}$ ±1.1 | $23.5^{d}$ ±1.9 | $108.4^{a}$ ±1.7 | $2.21^{a}$ | 0.859* | 0.696* |

Explanatory notes: [#] mean values ± standard deviation; values designated by different lowercase letters are significantly different ($P < 0.05$); Pearson's correlation coefficients between: A – length and width of leaf blade; B – petiole length and length of leaf blade; * significant correlation

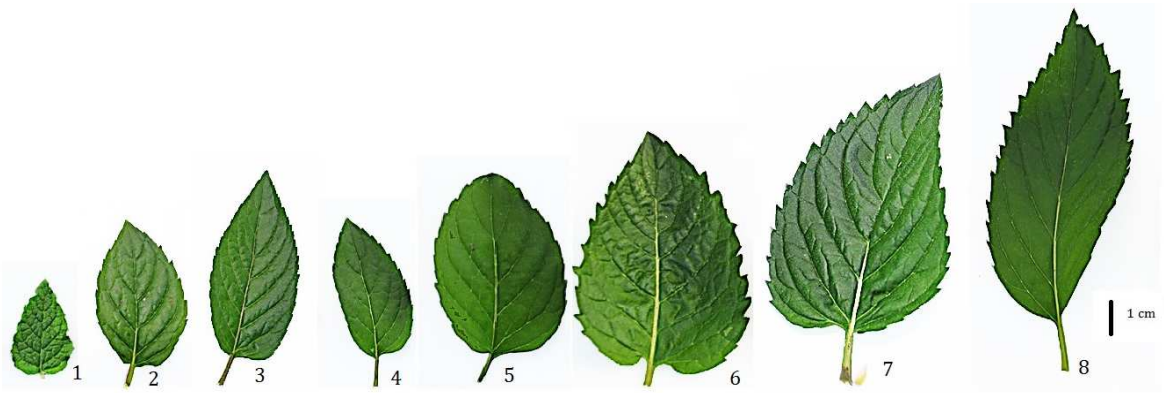



**Fig. 1.** The leaves of peppermint (*Mentha* × *piperita* L.) cultivars: 1 – 'Almira', 2 – 'Asia', 3 – 'Citaro', 4 – 'Chocolate', 5 – 'Granada', 6 – 'Grapefruit', 7 – 'Multimentha', 8 – 'Swiss' (phot. M. Dadasiewicz)

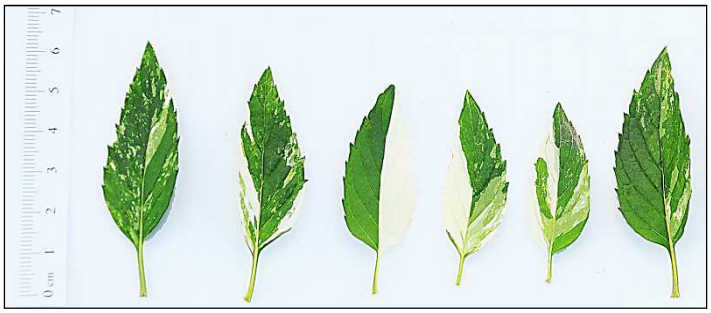


**Fig. 2.** Examples of leaves of the peppermint cultivar 'Variegata' (phot. M. Dadasiewicz)

**Table 2.** Morphological leaf features of analysed peppermint cultivars

| Peppermint cultivar | Leaf margin | | | Leaf shape | Leaf base | Leaf colour |
|---|---|---|---|---|---|---|
| | type | tooth shape | teeth per cm | | | |
| 'Almira' | serrate/irregular | corrugated | 2.4 | triangular | cordate | green |
| 'Asia' | serrate | CC/FL | 2.7 | ovate | truncate | dark green |
| 'Citaro' | serrulate | ST/FL | 3.1 | ovate | truncate | green |
| 'Chocolate' | finely serrate | ST/FL | 2.9 | ovate | truncate | dark green with purple markings |
| 'Granada' | finely serrate | ST/FL | 2.6 | oval | rounded | light green |
| 'Grapefruit' | serrate | CV/FL | 2.5 | ovate | cordate | green |
| 'Multimentha' | serrate | ST/FL | 2.1 | ovate | cordate | dark green |
| 'Swiss' | serrate | CC/FL | 2.2 | elliptic | convex | green |
| 'Variegata' | serrate* | ST/FL | 2.6 | elliptic | convex | irregular, bicolour white and green |

Explanatory notes: CC – concave, ST – straight, FL – flexuous, CV – convex; * sometimes 2 orders

The longest leaf blades were noted for the 'Swiss' cultivar (75.5 mm) and the shortest for 'Almira' (25.5 mm). This cultivar also had the narrowest blades. Peppermint leaves can be more or less elongated, as indicated by the leaf shape index, i.e. the length-to-width radio. The larger this parameter, the more the leaf is elongated. The length-to-width ratio in the leaves of the peppermint cultivars ranged from 1.35 to 2.21. The most elongated leaves were noted for the cultivars 'Variegata' and 'Swiss', and the least for 'Granada' and 'Multimentha'. The leaves of 'Multimentha' and 'Grapefruit' were similar in size, but the shape index was greater for 'Grapefruit'. Statistical analysis showed a positive correlation between the length and width of the leaf blade for all tested cultivars. Moreover, in the case of 'Variegata' and 'Swiss', a significantly positive correlation was found between the length of the leaf blade and petiole. Šarić-Kundalić et al. [2009] also observed considerable morphological diversity between different species, varieties and cultivars of mint. However, the peppermint leaves in their study were smaller than in most of the varieties we analysed (2.6–4.6 cm length and 1.2–1.6 cm width of leaf blade) [Šarić-Kundalić et al. 2009]

Leaves of cultivars of the same species may have different morphological features [Marotti et al. 1996, Klimko et al. 2015]. Table 2 presents the morphological features of the leaf blade of the peppermint cultivars analysed (*Mentha* × *piperita* L.). Differences were observed between cultivars in leaf margin, leaf shape, leaf base and colour (tab. 2).

All of the cultivars analysed have leaf margins with teeth, but the shape of the teeth was varied (tab. 2). The serrate and finely serrate types of leaf margin were most common. 'Citaro' had serrulate leaf margins and its teeth were closest together (3.1 per cm). Only 'Almira' had a significantly different tooth shape, which was difficult to define because the leaf margin was irregular, undulating and corrugated, sometimes with spinose tooth apices (fig. 1). Tooth apices were simple (except for 'Almira', which had spinose tooth apices) and of one order, except for the 'Variegata' cultivar, with 2 orders in some cases.

The cultivars can be classified as follows on the basis of leaf blade shape and leaf base:

– ovate with truncate base ('Asia', 'Citaro' and 'Chocolate');

– ovate with cordate base ('Grapefruit' and 'Multimentha'); these also have the same type of leaf margin, but different tooth shape;

– elliptic with convex base ('Swiss' and 'Variegata'); these also have the same type of leaf margin, but different tooth shape.

The remaining cultivars are distinctive: 'Almira' has triangular leaves with a cordate base, and 'Granada' has oval leaves with a rounded base.

**Table 3.** Selected RAPD primers used in the study

| No. | Primer | Primer sequence |
|---|---|---|
| 1 | C-05 | GATGACCGCC |
| 2 | C-19 | GTTGCCAGCC |
| 3 | E-04 | GTGACATGCC |
| 4 | J-13 | CCACACTACC |
| 5 | N-06 | GAGACGCACA |
| 6 | T-01 | GGGCCACTCA |
| 7 | T-05 | GGGTTTGGCA |
| 8 | T-06 | CAAGGGCAGA |
| 9 | T-07 | GGCAGGCTGT |
| 10 | T-12 | GGGTGTGTAG |
| 11 | T-13 | AGGACTGCCA |
| 12 | T-14 | AATGCCGCAG |
| 13 | T-15 | GGATGCCACT |
| 14 | T-16 | GGTGAACGCT |
| 15 | T-20 | GACCAATGCC |

Straumite et al. [2015] reported that the colour of mint leaves depends on the content of chlorophyll and carotenoids. Tarhan et al. [2010] demonstrated that peppermint leaf colour changes on drying. The colour of the fresh leaves of the peppermint cultivars analysed ranged from light green ('Granada') to green ('Almira', 'Citaro' 'Grapefruit' and 'Swiss') to dark green ('Asia' and 'Multimentha'), or dark green with purple markings in the case of the 'Chocolate' cultivars. Similar colouring of mint leaves of differ-

ent species and varieties was observed by Grisi et al. [2006] and Erum et al. [2012]. One of the cultivars ('Variegata') had irregular, bi-colour green and white leaves (fig. 2).

In the present study, nine *M.× piperita* cultivars were analysed by the RAPD method. Initially three randomly selected cultivars were used to screen 50 RAPD primers (Operon Technologies). Among the primers tested only 15 amplified polymorphic and repeatable fragments (tab. 3, phot. 1). In total, the selected primers generated 120 fragments. The number of amplicons ranged from 3 to 13, with an average of 8 per primer, and 13.33 bands per genotype. Khanuja et al. [2000] analysed *Mentha* species using 60 RAPD primers which produced 630 bands, of which 93.5% were polymorphic. The number of fragments obtained with single primers was higher than in the present study, ranging from 11 to 19, with an average of 10.5 bands per primer. Kabir et al. [2014] used the RAPD method to assess genetic diversity of *Mentha* species. The authors selected 9 primers which generated 60 bands, on average 6 amplicons per primer. Fenwick and Ward [2001] also used RAPD markers to evaluate genetic diversity of *Mentha* species. The authors used 24 RAPD primers which produced 133 amplicons, of which 104 were polymorphic. The mean numbers of amplification products per primer obtained by the authors were lower than in our study and scored 5.5%. A high number of bands in *Mentha* analysis by RAPD was obtained by Soheila et al. [2006], who selected 31 RAPD primers, which produced 617 bands. The number of bands generated by a single primer was higher than in the present study, ranging from 1 to 32 with an average of 19.9 per primer.

**Table 4.** Characteristics of selected RAPD primers

| No. | Primer | Number of products | | | Primer diversity (%) | Frequency of polymorphic products | Resolving power of the primer | PIC |
|---|---|---|---|---|---|---|---|---|
| | | total | polymorphic | monomorphic | | | | |
| 1 | C05 | 12 | 11 | 1 | 91.67 | 0.4 | 9.56 | 0.28 |
| 2 | C19 | 8 | 8 | 0 | 100 | 0.42 | 6.67 | 0.39 |
| 3 | E04 | 6 | 4 | 2 | 66.67 | 0.70 | 8.44 | 0.30 |
| 4 | J13 | 9 | 8 | 1 | 88,89 | 0.42 | 7.56 | 0.28 |
| 5 | N06 | 7 | 6 | 1 | 85.71 | 0.40 | 5.56 | 0.32 |
| 6 | T01 | 13 | 13 | 0 | 100 | 0.46 | 12.00 | 0.37 |
| 7 | T05 | 9 | 9 | 0 | 100 | 0.52 | 9.33 | 0.35 |
| 8 | T06 | 4 | 3 | 1 | 75 | 0.58 | 4.67 | 0.22 |
| 9 | T07 | 10 | 10 | 0 | 100 | 0.39 | 7.78 | 0.41 |
| 10 | T12 | 6 | 5 | 1 | 83.33 | 0.61 | 7.33 | 0.33 |
| 11 | T13 | 9 | 9 | 0 | 100 | 0.46 | 8.22 | 0.35 |
| 12 | T14 | 7 | 6 | 1 | 85.71 | 0.27 | 3.78 | 0.21 |
| 13 | T15 | 9 | 8 | 1 | 88.89 | 0.43 | 7.78 | 0.35 |
| 14 | T16 | 8 | 7 | 1 | 87.50 | 0.31 | 4.89 | 0.25 |
| 15 | T20 | 3 | 2 | 1 | 66.67 | 0.52 | 3.11 | 0.26 |
| Total | | 120 | 109 | 11 | 1320.04 | 6.89 | 106.68 | 4.67 |
| average/ primer | | 8 | 7.27 | 0.73 | | | | 0.31 |
| average/ genotype | | 13.33 | 12.11 | 1.22 | | | | 0.52 |

**Table 5.** Unique RAPD markers identify for *M.* × *piperita* cultivars

| Cultivar | Unique markers | | | | | |
|---|---|---|---|---|---|---|
| 'Citaro' | $T16_{400}$ | $T01_{350}$ | $T05_{850}$ | $C05_{450}$ | | |
| 'Granada' | $T15_{350}$ | $C05_{700}$ | $C05_{750}$ | $C05_{1500}$ | | |
| 'Multimentha' | $T14_{400}$ | $T16_{400}$ | $J13_{700}$ | | | |
| 'Variegata' | $T15_{2500}$ | | | | | |
| 'Chocolate' | $T12_{1700}$ | | | | | |
| 'Almira' | $T14_{350}$ | $T16_{700}$ | $T13_{400}$ | $J13_{460}$ | $C05_{300}$ | $C19_{400}$ |
| 'Grapefruit' | $T14_{450}$ | $T14_{1300}$ | $T16_{2000}$ | $J13_{200}$ | $N06_{350}$ | |
| 'Asia' | $T01_{550}$ | | | | | |

**Table 6.** Similarity matrix for Dice coefficient of nine *M.* × *piperita* cultivars

| Cultivar | 'Citaro' | 'Granada' | 'Multimentha' | 'Swiss' | 'Variegata' | 'Chocolate' | 'Almira' | 'Grapefruit' | 'Asia' |
|---|---|---|---|---|---|---|---|---|---|
| 'Citaro' | 1.00 | | | | | | | | |
| 'Granada' | 0.430 | 1.00 | | | | | | | |
| 'Multimentha' | 0.411 | 0.700 | 1.00 | | | | | | |
| 'Swiss' | 0.535 | 0.558 | 0.596 | 1.00 | | | | | |
| 'Variegata' | 0.543 | 0.614 | 0.632 | 0.846 | 1.00 | | | | |
| 'Chocolate' | 0.484 | 0.645 | 0.758 | 0.659 | 0.735 | 1.00 | | | |
| 'Almira' | 0.426 | 0.535 | 0.551 | 0.418 | 0.436 | 0.450 | 1.00 | | |
| 'Grapefruit' | 0.388 | 0.702 | 0.641 | 0.589 | 0.629 | 0.626 | 0.458 | 1.00 | |
| 'Asia' | 0.467 | 0.683 | 0.764 | 0.644 | 0.701 | 0.822 | 0.491 | 0.684 | 1.00 |

Among the 120 amplified products obtained in the study using 15 RAPD primers, 109 (90.83%) were polymorphic. The number of polymorphic bands amplified by a single primer ranged from 2 to 13, with an average of 7.27 per primer and 12.11 per genotype (tab. 4). A similar percentage of polymorphic products, 93.5%, was obtained by Khanuja et al. [2000]. Soheila et al. [2006] demonstrated that RAPD primers amplified a high percentage of polymorphic products (98.5%). In our study 5 of the 15 primers used in the experiments showed 100% polymorphism. The lowest level of primer diversity was noted for primers E04 and T20 (66.67%). In results obtained by Soheila et al. [2006], 23 of 31 RAPD primers showed 100% polymorphism. Differences in numbers of products obtained by different authors may result from the use of different RAPD primers or different *Mentha* species in the experiments. Among analysed cultivars 8 could be identify by presence of unique RAPD markers. 11 primers initiate amplification of 25 unique markers. Cultivar Almira could be identify by 7 specific amplicons, Grapefruit by 5,

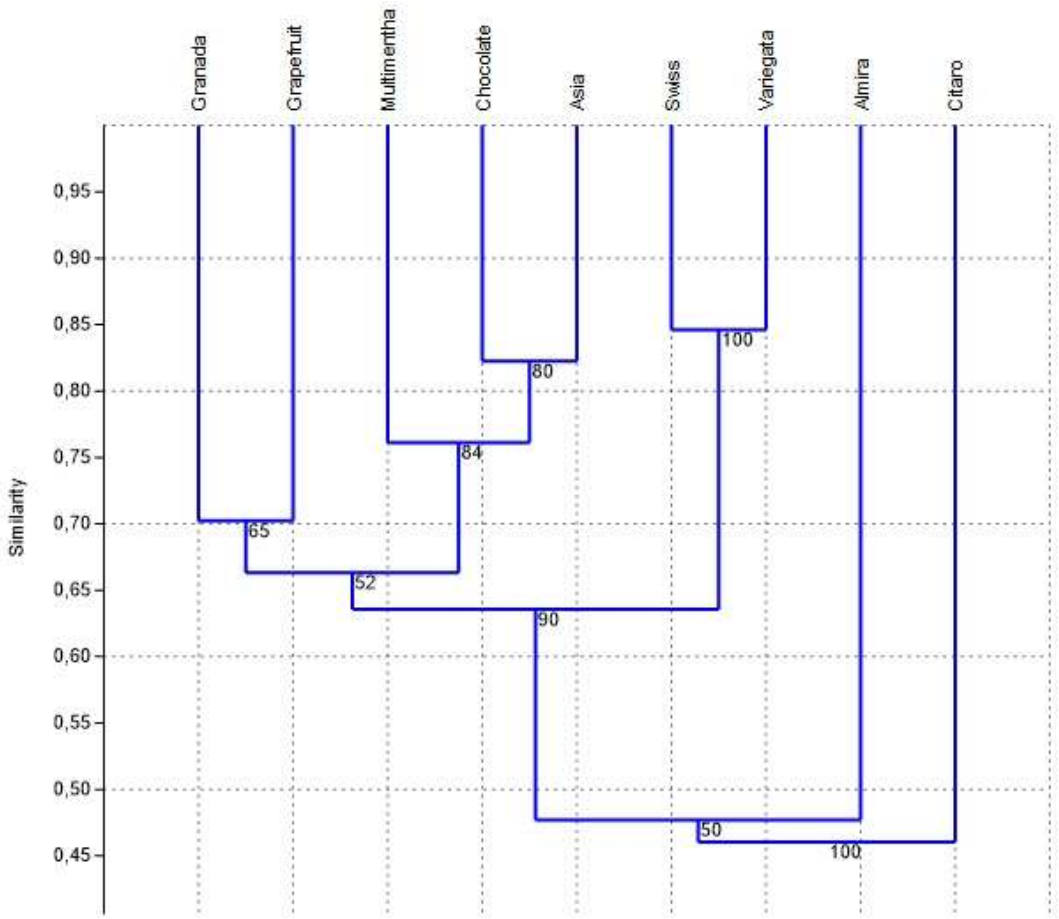



**Fig. 3.** UPGMA dendrogram of nine *M. piperita* cultivars based on RAPD primers

Citaro and Granada by 4 unique markers, cultivar Multimentha by 3, and cultivars Chocolate, Asia and Variegata by 1 specific primers (tab. 5).

The relative frequency of polymorphic products and the resolving power of the primers were calculated in the study. The relative frequency of polymorphic bands ranged from 0.11 (polymorphic band present in only one genotype of the 9 studied) to 0.89 (polymorphic band absent in only one genotype of the 9 studied). Overall the average frequency generated by a single primer was high (0.46), varying from 0.27 to 0.70 (tab. 4). These results suggest that the material was characterized by a high level of polymorphism. The average frequency of polymorphic products has also been calculated for tea [Chen et al. 2005], *A montana* [Okoń et al. 2014] and *M. chamomilla* [Okoń and Surmacz-Magdziak 2011]. The average values of these coefficients were similar to those obtained in the present study: 0.47, 0.58 and 0.42 respectively.

Primers with high resolving power are used for molecular diagnosis of a species from a mixed population [Prevost and Wilkinson 1999]. In our study the resolving power of the 15 RAPD primers ranged from 3.11 to 12.00 (tab. 4). Resolving power values obtained for the RAPD primers were able to distinguish all analysed cultivars, and could potentially be used to identify them from any mixed population. A similar approach has previously been used successfully for molecular diagnosis of *Rhus* species [Prakash et al. 2007], *Jatropha* genotypes [Tatikonda et al. 2009],

*A. montana* [Okoń et al. 2014] and *M. chamomilla* [Okoń and Surmacz-Magdziak 2011].

Genetic similarity matrices were produced based on RAPD using Dice's coefficient. RAPD-based genetic similarity was estimated between 0.388 for the 'Grapefruit' and 'Citaro' cultivars and 0.846 for 'Variegata' and 'Swiss' (tab. 6). The mean genetic similarity was calculated at 0.590. The lowest genetic similarity to the remaining genotypes was calculated for the 'Almira' (0.471) and 'Citaro' (0.460) cultivars, while the 'Asia' cultivar was the most similar to the other genotypes (0.657).

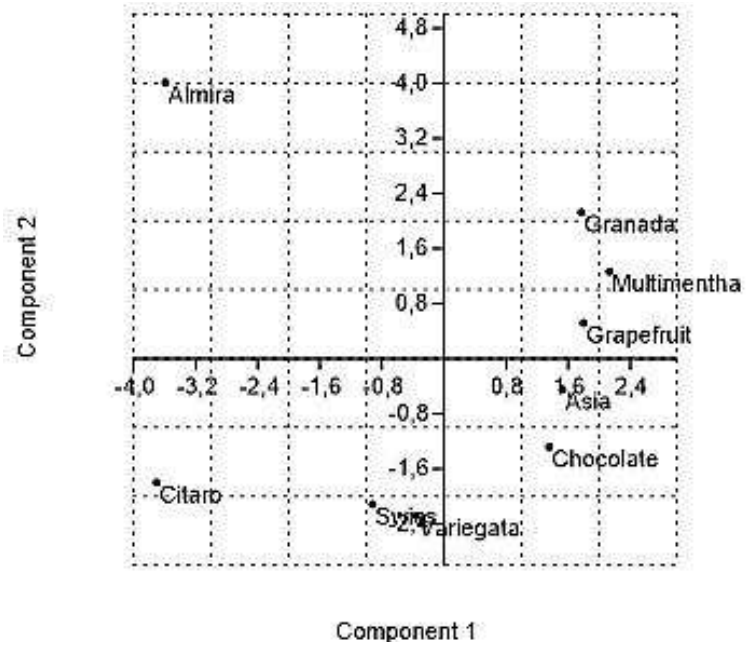
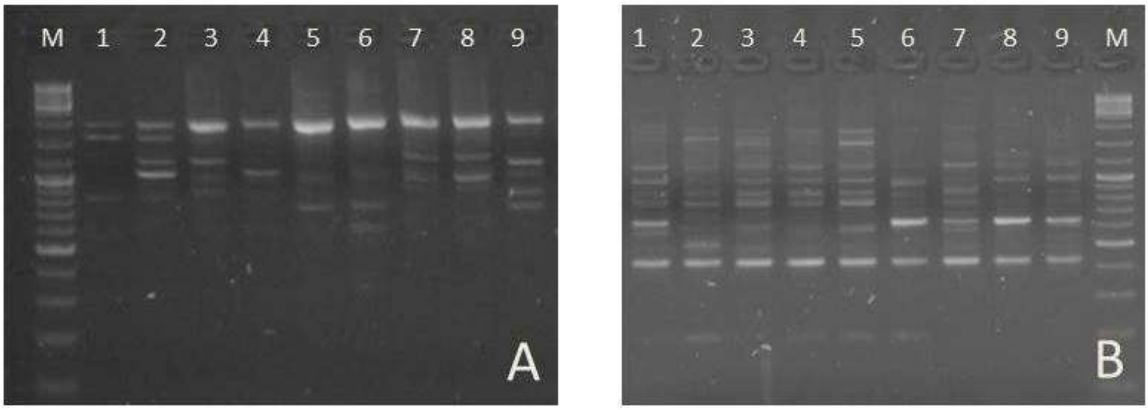



**Fig. 4.** Principal component analysis (PCA) of *M.* × *piperita* cultivars




**Phot. 1.** PCR amplification using A – T12 and B – T15 RAPD primers. M- GeneRuler$^{TM}$ 100bp DNA Ladder Plus (Thermo Fisher Scientific), 1– Citaro, 2 – Granada, 3 – Multimentha, 4 – Swiss, 5 – Variegata, 6 – Chocolate, 7 – Almira, 8 – Grapefruit, 9 – Asia

A genetic similarity matrix was used for cluster analysis by the UPGMA method (fig. 3). The nine *M.* × *piperita* cultivars were grouped into three major groups based on bootstrapping. Group A contained 2 cultivars, 'Granada' and 'Grapefruit'. In group B the cultivars 'Multimentha', 'Chocolate' and 'Asia' were grouped together. Group C included 'Swiss' and 'Variegata'. The 'Almira' and 'Citaro' cultivars showed the lowest similarity to all other genotypes and were located at the edge of the dendrogram.

Relationships between the *M. piperita* cultivars were revealed by principal component analysis (PCA) (fig. 4). PCA confirms the results obtained by UPGMA clustering. The cultivars formed three distinct groups, which correspond to groups A, B and C in the UPGMA dendrogram. The 'Citaro' and 'Almira' cultivars formed separate groups. For the RAPD data, the first three principal components explained 79.3% of the total variation, with PC-1, PC-2 and PC-3 accounting for 35.0, 32.0 and 12.3% of the total variation, respectively.

## CONCLUSIONS

1. The peppermint cultivars were characterized by diversity of morphological features and leaf size. No cultivars were found to have the same leaf shape and size.

2. The morphology of the 'Almira' and 'Granada' was clearly distinct from the other cultivars.

3. The mint cultivars showed fairly high genetic similarity. The highest genetic similarity was estimated between 'Variegata' and 'Swiss', and the lowest between the 'Almira' and 'Citaro' cultivars.

4. Our research shows that the peppermint cultivars tested are distinct, both morphologically and genetically, which suggests the need for further research into the chemical composition and pharmacological properties of the cultivars.

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