Morphological and genetic variability of chosen *Mentha* species

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S u m m a r y

The objects of the studies were 4 species of mint: (*Mentha aquatica* L.), peppermint (*Mentha* x *piperita* L.), spearmint (*Mentha spicata* var. *crispa* L.) and a variety of pineapple mint (*Mentha suaveolens* Ehrh.) - 'Variegata'. Phenotype differences between the studied species were determined on the basis of morphometric measurements of the length of the longest sprout, the plant diameter, the number of sprouts as well as length and width of the leaf. To determine the genotype differences, an ISSR-PCR technique was used. The ISSR reactions were carried out using 20 microsatellite primers and their products were separated in 2% agarose gel. It was found that the studied genotypes of mint differ significantly from one another, both in relation to the analyzed morphologic and genetic features. For genotypes characterized in the experiment, a number of mono- and polymorphic products were amplified and among them there were a series of species specific ISSR products.
Phylogenetic similarity of the compared species varied from 53.3% between *M.* x *piperita* and *M. aquatica* to 82.2 % between *M. suaveolens* 'Variegata' and *Mentha spicata* var. *crispa.*

Key words: Mentha sp., biodiversity, phenotypic variability, genetic variability, ISSR

INTRODUCTION

Getting acquainted with interspecific and intraspecific diversity within *Mentha* L. (*Lamiaceae*) species is not an easy task. The main reason for this is variability observed both in morphology [1], cytology [2, 3] as well as genetics [4-6] and chemistry - concerning oil composition.

Mint oil obtained from mint plants is synthesized and gathered in the so-called glandular hairs and its quantity and quality depend on the cultivated genotype. Three species play a significant role in its production in the world: Japanese mint (*M. arvensis* var. *piperascens*), curled spearmint (*Mentha spicata* var. *crispa*) and corn mint (*M. pulegium*), wheras in Poland it is peppermint (*M.* x *piperita*) [7].

The aim of this study was to determine morphological and genetic diversity of the four mint accessions examined in the experiment: water mint, peppermint, curled spearmint and pineapple mint.

MATERIAL AND METHODS

Research material was obtained from the collection of medicinal plants of the Department of Vegetable Crops, Agricultural University in Szczecin and was composed of four accessions of mint: water mint (*M. aquatica* L.), peppermint (*M.* x *piperita*), curled spearmint (*M. crispa* L.) and pineapple mint (*Mentha suavedens* Ehrh.) 'Variegata'. Morphological variability of the mentioned species was determined at the Vegetable Department, whereas genetic variability at the Department of Horticultural Plant Breeding in the Agricultural University in Szczecin.

Morphological variability was determined in a field experiment. For each characterized species 16 cuttings, constituting 8-10 cm long and 3-5 mm thick underground runners with at least 3 nodes of 40 x 20 cm spacing and at the depth of 4-5 cm. In the course of vegetation, biometric measurements were carried out in 7-10 day intervals determining the length of the longest shoot (cm), the plant diameter (cm), a number of shoots and the length as well as width of a leaf (cm). For statistical calculations the computer program AWAR was used, whereas the NIR values were calculated by means of the Tukey test for the significance level of 0.05.

Genotype variability. The differences between the examined genotypes of mint were described by the ISSR-PCR technique in accordance to method described by Ziętkiewicz et al. [8]. DNA was isolated from fresh mint leaves and the amplification of DNA (Mastercycler – Eppendorf) was carried out using 20 microsatelite primers (Genset Oligos – USA, Table 1) in three replication for each primer. PCR products were separated in a 2% agarose gel in a SubCell GT (Bio-Rad) system. Then they were visualized with ethidium bromide (5 $mg \cdot cm^{-3}$) on a UV-21 transilluminator and finally photographed (Polaroid DS-34).

Table 1.

ISSR primer sequences and results of experiments performed in four of *Mentha* accessions with ISSR markers.

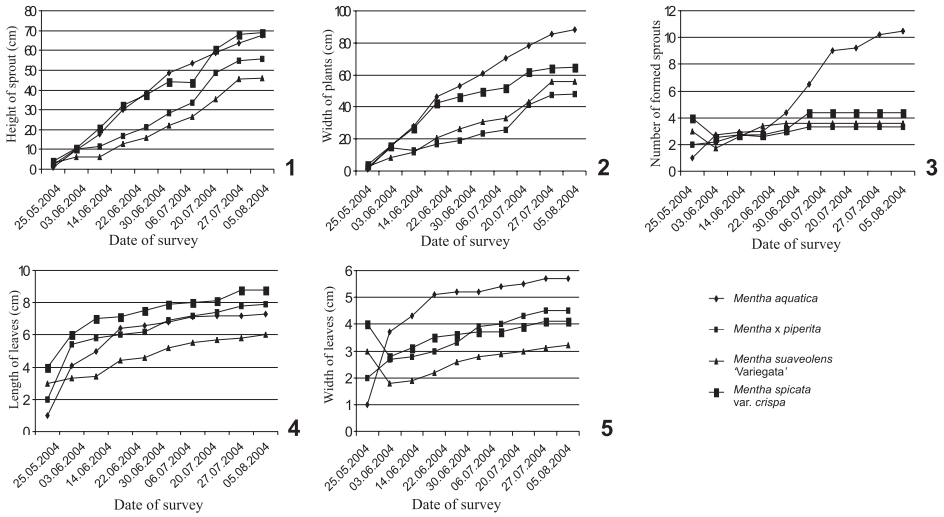
primer number	sequence 5' – 3'	length of amplification products (bp)	number of generated products	mean of ISSR products amplified for genotypes	number and percentage of products			
					monomorphic	%	polymorphic	%
806	(TG)8A	1851–921	16	4.0	8	50	8	50
809	(AG)8YG	1291–143	20	5.0	18	90	2	10
816	(AG)8YC	1265–977	12	3.0	8	66	4	34
817	(AG)8YG	874–103	26	6.5	24	92	2	8
820	(GA)8YC	1825–731	20	5.0	4	20	16	80
821	(GA)8YC	1576–686	18	4.5	8	45	10	55
848	(CA)8GC	749–655	6	1.5	4	67	2	33
873	(GACA)4	1685–1019	16	4.0	4	25	12	75
		Σ	134	33.5	78	-	56	-
		mean	16,7	4.2	9.7	58.2	7.0	41.8

Electrophoregrams were scanned and digital pictures were analyzed by means of the computer program „Diversity one" 1.3 (Pharmacia LKB). Each amplicon that was amplified using ISSR primers was coded in a binary form by 0 or 1 for absence or presence in each accession. To infer phylogenetic relationships, the 0/1 matrix was used to calculate genetic similarity and then employed to construct unweighted pair-group method with arithmetic means UPGMA, dendrogram using software packages Diversity one 1.3 (Pharmacia LKB). Molecular weight of each amplicon was calculated using the same software packages.

RESULTS

Morphological variability. In the analysis of morphological features of the examined mint species it was stated that the tallest shoots (69 cm) were characteristic for *Mentha spicata* var. *crispa,* and the shortest (45.9) were those of *M. suaveolens* 'Variegata' (Fig. 1). The average height of the two remaining mints (*M. aquatica* and *M.* x *piperita*) amounted to 67.5 and 55.6 cm, respectively. The largest diameter was observed in water mint (88.5 cm), and the smallest (48.5 cm) in peppermint. In curled spearmint and in pineapple mint it equaled 65 and 56 cm, respectively. At the early stage of growth the mint species had a similar number of shoots on a plant (from 1 to 4), but the quickest rate of growth was that of *M. aquatica*, in which 10 shoots were observed at the end of the vegetation period. *Mentha spicata* var. *crispa* had the longest leaves (8.8 cm), while the shortest leaves (7.3 cm) were in *M. aquatica*. In the remaining two species, i.e. *M.* x *piperita* and *M. suaveolens* 'Variegata', the leaves were 7.9 and 6.0 cm long, respectively. The widest leaves (5.7 cm) were observed in water mint (*M.*

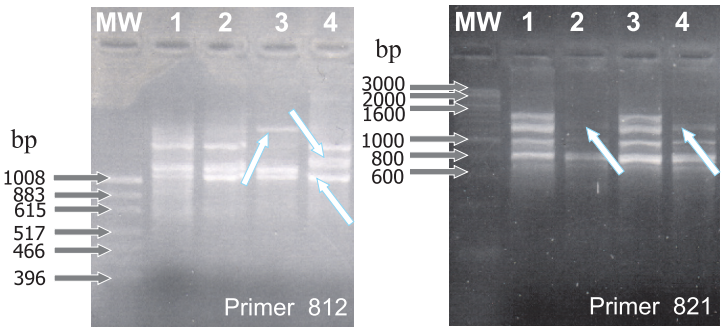
aquatica), whereas the narrowest leaves (3.2 cm) were noticed in *M. suaveolens* 'Variegata'. In other mints (*M.* x *piperita* and *Mentha spicata* var. *crispa*) the value of these features amounted to 4.5 and 4.1, respectively. The largest mass of fresh herb from a plant (360.2 g, on average) was in *M. suaveolens* 'Variegata', the smallest (163.2 g, on average) - *M.* x *piperita*.



1 - The average height of the longest sprout, 2 - The average width of the plant, 3 - The average number of formed sprouts in the plant, 4 - The average length of leaves, 5 - The average width of leaves,

Figure 1. Values of selected morphometric features studied in the experiment of the four accessions of mint

Genotype variability. The results of the analysis confirmed genetic differences between compared mint species. Out of 20 microsatelite primers used for the ISSR reaction, distinct amplicons were generated in the reactions from 8 ones (806, 809, 816, 817, 820, 821, 848 and 873) (Table 1, Fig. 2). On the whole, in the reactions carried out, 134 ISSR products were amplified, of which 78 (52.8 %) were monomorphic and 56 (41.8 %) polymorphic (Table 1). On average, in the reaction with one primer 4 *loci* were amplified. The most ISSR products (26) were amplified in the reactions with primer 817, the least (6) - with starter 848. The most polymorphic products of the ISSR reaction (5) were generated with starter 820. These were the products of the following length: ~1825, ~1600, ~1434, ~1164 and ~731 bp. One polymorphic *locus* was amplified with each of the following primers: 806, 817 and 848.



MW - weight marker, 1 - *Mentha spicata* var. *crispa* 2 - *Mentha aquatica,* 3 - *Mentha suaveolens* 'Variegata', 4 - *Mentha* × *piperita*

Figure 2. Electrophoregrams of ISSR products amplified on DNA templates of four Mentha accessions. White arrows mark ISSR polymorphic products

For all the studied mint accessions, genotype specific ISSR *loci* were amplified. Their length and primers amplifying them are listed in Table 2.

Table 2.

Polymorphic products of ISSR-PCR amplification of analyzed *Mentha* accession.

accession	lenght of amplification products [bp]
Mentha spicata var. *crispa*	$806_{[921]}$, $820_{[939]}$, $873_{[1068]}$
Mentha aquatica	$821_{[1050]}$, $873_{[1685]}$
Mentha suaveolens 'Variegata'	$806_{[1527]}$
Mentha x piperita	$806_{[1851, 1222, 1090]}$

UPGMA analysis was carried out to classify the mint accessions and to verify the ability of ISSR markers to reveal genetic similarity (Fig. 3). The similarity at the level of 53.3 % was observed between *M.* x *piperita* and *M. aquatica*, while that of 82.2 % was between *M. suaveolens* 'Variegata' and *Mentha spicata* var. *crispa*. The phylogenetic similarity between *M. suaveolens* 'Variegata' and *M.* x *piperita* equalled 77.6 %, whereas between *Mentha spicata* var. *crispa* and *M. aquatica* it was 75.8%.

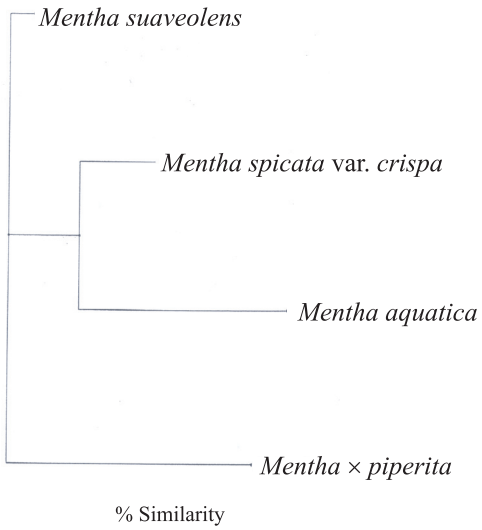


Figure 3. UPGMA dendrograms, representing genetic relationships among the four Mentha accessions analysed by ISSR markers

DISCUSSION

Mentha comprises a large number of species that are found in different latitudes. Despite such large biodiversity, this species is relatively well known, regarding both to genetic and morphological aspects. However, a modifying effect of the environment on mint plants is often observed. It can lead to the isolation of local forms [7].

The results of biometric measurements of the examined species showed their large phenotype diversity. The longest shoots (81.5 cm), the largest diameter of a plant (88.5 cm), the fastest growth of shoots (10.5) and the largest width of leaves (5.7) were characteristic for *M. aquatica* species, while the characteristic feature of *M. crispa* was the longest leaf (8.8). The obtained results of the morphological studies are approximate to the ones described by Rumińska [7] and Broda et al. [9].

Using the ISSR-PCR technique, genetic differences between four mint species: *M.* x *piperita*, *M. aquatica*, *M. suaveolens* 'Variegata' and *Mentha spicata* var. *crispa* were determined. Out of 20 microsatelite primers used for ISSR reactions, 134 ISSR products were amplified of only 8 primers. 78 (52.8%) of these products turned out to be monomorphic, whereas 56 (41.8%) polymorphic. In the ISSR reactions, specific ISSR products were amplified. They can constitute specific molecular markers.

The indication of large genetic unification of mint varieties used for the production of oil in the USA, basing their theses on the results of the studies was carried out by means of the RAPD technique [4]. Shasany et al. [6] determined genetic diversity (80 RPPD primers) of 15 mint genotypes (C19-C30, C32 and C34)

from the collection of National Bank of Genes in India (CIMAP). The authors studied differentiation both between the taxons and within them. The results of the studies confirmed the usefulness of the RAPD technique for genotyping mint.

In the present work the genetic similarity varied from 53.3% between *M.* x *piperita* and *M. aquatica* to 82.2% - between *M. suaveolens* 'Variegata' and *Mentha spicata* var. *crispa*. Similar results of the studies concerning phylogenetic relationships were reported by [1, 4].

CONCLUSIONS

1. The mint accessions chosen for the research: *M. x piperita*, *M. suaveolens* 'Variegata', *M. crispa* and *M. aquatica* showed a diverse morphological structure. The examined species were differentiated by 8 out of 20 ISSR primers used in the experiment.
2. 134 ISSR products were amplified with their contribution, at the same time identifying 56 polymorphic products, among them genotypically specific ISSR-PCR products.

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ZRÓŻNICOWANIE MORFOLOGICZNE I GENETYCZNE WYBRANYCH GATUNKÓW MIĘTY (*MENTHA SP.*)

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S t r e s z c z e n i e

Obiektem badawczym były 4 gatunki mięty: mięta nawodna (*Mentha aquatica* L.), mięta pieprzowa (*Mentha* x *piperita* L.), mięta kędzierzawa (*Mentha spicata* var. *crispa* L.) oraz odmiana mięty ananasowej (*Mentha suaveolens* Ehrh.) - 'Variegata'. Różnice fenotypowe między gatunkami badanymi w doświadczeniu określono na podstawie pomiarów morfometrycznych, mierząc: długość najdłuższego pędu, średnicę rośliny, liczbę pędów oraz długość i szerokość liścia. Do określenia różnic genotypowych zastosowano technikę ISSR-PCR. Reakcje ISSR prowadzono z zastosowaniem 20 starterów mikrosatelitanych, a ich produkty rozdzielano w 2% żelu agarozowym. Stwierdzono, że badane genotypy mięty istotnie różnią się między sobą, zarówno pod względem analizowanych cech morfologicznych, jak i genetycznych. Dla genotypów charakteryzowanych w doświadczeniu amplifikowano szereg produktów mono- i polimorficznych, a wśród nich szereg specyficznych gatunkowo produktów ISSR. Podobieństwo filogenetyczne między porównywanymi gatunkami wahało się od 53,3% między *M.* x *piperita* a *M. aquatica* do 82,2% między *M. suaveolens* 'Variegata' a *Mentha spicata* var. *crispa*.

Słowa kluczowe: Mentha sp., bioróżnorodność, zmienność fenotypowa, zmienność genotypowa, ISSR