AGRONOMY SCIENCE

VOL. LXXX (4) 2025

https://doi.org/10.24326/as.2025.5594

Department of Genetics and Horticultural Plant Breeding, Institute of Plant Genetics, Breeding and Biotechnology, University of Life Sciences in Lublin, Akademicka 15 Street, 20-950 Lublin, Poland

* e-mail: magdalena.dyduch@up.lublin.pl

JACEK GAWROŃSKI[1] https://orcid.org/0000-0001-9342-5005

MAGDALENA DYDUCH-SIEMIŃSKA[1]* https://orcid.org/0000-0002-6549-7391

Molecular identification and genetic diversity assessment of *Mentha* genotypes using SCoT and ISSR DNA markers

Identyfikacja molekularna i ocena zróżnicowania genetycznego genotypów mięty z wykorzystaniem markerów DNA – SCoT i ISSR

Abstract. The aim of this study was to molecularly identify and assess the genetic diversity of 12 *Mentha* genotypes using, for the first time, two types of DNA markers simultaneously – ISSR (inter simple sequence repeats) and SCoT (start codon targeted). Selected genotypes representing various *Mentha* species and varieties were analyzed to determine the level of genetic similarity and phylogenetic relationships between them. The level of polymorphism obtained for ISSR markers was 71%, while for SCoT it was 88.7%. The obtained data were analyzed, allowing for the assessment of the level of genetic similarity and the construction of dendrograms illustrating the genetic structure of the studied population. Studies indicate that the use of SCoT markers enables the identification of the following genotypes: *Plectranthus amboinicus* (Lour.) Spreng, *Mentha pulegium* L., *Mentha spicata* L. cv. Moroccan, *Mentha suaveolens* Ehrh. var. *variegata* (pineapple mint), *Mentha spicata* L., and *Mentha longifolia* L. ISSR markers, due to the generation of only monomorphic and polymorphic bands, do not allow for the direct identification of any of the studied genotypes. Data from both marker systems indicate significant genetic diversity among the analyzed genotypes, which may be important for breeding programs and the conservation of genetic resources of the *Mentha* genus.

Keywords: DNA polymorphism, genetic diversity ISSR *Mentha*, molecular markers, SCoT, UPGMA methods

Citation: Gawroński J., Dyduch-Siemińska M., 2025. Molecular identification and genetic diversity assessment of *Mentha* genotypes using SCoT and ISSR DNA markers. Agron. Sci. 80(4), 23–37. https://doi.org/10.24326/as.2025.5594

INTRODUCTION

The family *Lamiaceae*, representing the sixth largest group among angiosperms, comprises over 245 genera and approximately 7,886 species with a cosmopolitan distribution. Members of this family are characterized by a high content of essential oils and a wide diversity of secondary metabolites. Owing to these phytochemical properties, numerous *Lamiaceae* species have significant applications in traditional and modern medicine, pharmacology, cosmetics, and aromatherapy, as well as in horticulture for their ornamental and aromatic value [Sofyalıoğlu et al. 2025]. The genus *Mentha*, belongs to the *Lamiaceae* includes more than a dozen wild species and numerous hybrids and cultivated varieties with diverse functional properties [Silva et al. 2006, Mkaddem et al. 2009] and is one of the most important genera with approximately 30 identified species distributed in Europe, Asia, America, Australia, and temperate regions [Dorman et al. 2013]. The systematics of the genus *Mentha* is very complex and still not fully unified. Predisposition of mint to open pollination between wild and cultivated varieties have contributed to the development of numerous cultivars and interspecific hybrids [Jędrzejczyk and Rewers 2018]. The chromosome number in plants of the genus *Mentha* is usually x = 12, but it varies depending on the species and may be: monoploid (basic) number: x = 9, x = 10, x = 12, x = 18, x = 25; while the ploidy level (2n) may be equal to: 2x, 4x, 6x, 8x, 10x [Lawrence 2006]. Within this genus, in addition to variability in the basic chromosome number, polyploidy, and aneuploidy, there is also polymorphism in morphology, the composition of essential oils, and secondary metabolites, depending on the environmental conditions in which the plants are grown. Therefore, morphological, biochemical markers, and even karyological analysis are often unreliable in distinguishing *Mentha* species [Jędrzejczyk and Rewers 2018]. Because, as Devi et al. [2022] report the genus *Mentha* has undergone frequent interspecific hybridization between wild and cultivated populations, which leads to varying basic chromosome count. To distinguish genotypes, DNA markers should be used. In the case of the studied species, the diversity analysis was performed using SRAP [Malik et al. 2019], ISSR [Smolik et al. 2007, Rodrigues et al. 2013, Jędrzejczyk and Rewers 2018, Choupani et al. 2019, Soilhi et al. 2020, Moshrefi-Araghi et al. 2021, Devi et al. 2022, Çelik et al. 2024], RAPD [Khanuja et al. 2000, Ibrahim 2017, Kiełtyka-Dadasiewicz et al. 2017, Ahmad et al. 2018, Panjeshahin et al. 2018], SSR [Vining et al. 2019, Fukui et al. 2022], SCoT [Khan et al. 2017, Salama et al. 2019, Heylen et al. 2021] markers. Molecular marker techniques generate reliable and reproducible results without being influenced by environmental factors. In the analysis of genetic similarity, it is recommended to use two types of primers to scan a large area of the genome. SCoT exploits the gene regions flanking the ATG initiation codon [Collard and Mackill 2009], while ISSR markers can amplify the flanking regions of the target microsatellite [Sabboura et al. 2016]. Therefore, in this study, for the first time, the ISSR and SCoT marker systems were used simultaneously for the studied set of genotypes. Information on the genetic diversity and relationships among different species in *Mentha* genus may produce new insights and give a better understanding of the distribution of genetic diversity and is necessary for germplasm collection, conservation and breeding program. The aim of this study was to conduct molecular identification of various mint genotypes. Molecular analyses were conducted to develop rapid and precise species identification, determine interspecific and intraspecific variability within the genus, and describe relationships between genotypes.

MATERIALS AND METHODS

DNA extraction

Samples of healthy leaves from 10 randomly selected plants grown at the Experimental Farm of the Department of Vegetable and Medicinal Plants of the University of Life Sciences in Lublin (51°14'53"N, 2°34'13"E) were collected. DNA was isolated in two replications, for every listed in table 1 genotypes. DNA was extracted following the CTAB method described by Doyle and Doyle [1987]. The DNA concentration was determined using Thermo Scientific Nanodrop spectrophotometer. Test samples were diluted to a final concentration 25 ng μl^{-1}.

Table 1. The list of genotypes

Species	Code
Mentha suaveolens Ehrh. var. *variegata* (pineapple mint)	G1
Mentha suaveolens Ehrh.	G2
Mentha pulegium L.	G3
Mentha longifolia L.	G4
Plectranthus amboinicus (Lour.) Spreng. (Mexican mint)	G5
Mentha piperita L. var. *officinalis* Sole f. *rubescens* Camus	G6
Mentha spicata L.	G7
Mentha spicata var. *crispa* cv. Persian	G8
Mentha spicata L. cv. Moroccan	G9
Mentha rotundifolia L. Huds.	G10
Mentha piperita L. var. *citrata* Ehrh.	G11
Mentha spicata L. var. *crispa*	G12

ISSR analysis

A total of 30 ISSR primers (Sigma-Aldrich) were initially screened, of which 13 produced clear, reproducible banding patterns. These primers were selected for subsequent analyses. PCR amplification was performed in a final reaction volume of 15 µL, containing 130 µM dNTPs, 1.5 mM $MgCl_2$, 1 U of Taq DNA polymerase, 1× reaction buffer, 470 pM primer, and 60 ng of genomic DNA. Amplification reactions were carried out in a gradient thermal cycler (Biometra GmbH) using the following program: an initial denaturation step at 94°C for 7 min; followed by 35 cycles of denaturation at 94°C for 30 s, primer annealing at the primer-specific annealing temperature (Tm) for 30 s, and extension at 72°C for 2 min; with a final extension step at 72°C for 7 min.

SCoT analysis

Fifteen 18-base primers selected from 25 arbitrary primers were used for PCR amplification. DNA amplification of SCoT markers was carried out in a final volume of 10 µl containing 0.5 U of Taq DNA Polymerase (Fermentas), 0.8 µl of oligonucleotide primer (0.8 µM), 1 µM dNTPs, 1 × PCR buffer with $MgCl_2$, and 25 ng of genomic DNA as a template. Amplification was performed in a gradient thermal cycler (Biometra GmbH) with the following reaction conditions: initial predenaturation at 94°C for 3 min, followed by 35 denaturation cycles at 94°C for 1 min, annealing at 50°C for 1 min, and extension at 72°C for 2 min. The final extension was carried out for 5 min at 72°C with the holding temperature of 4°C.

DNA data analysis

PCR products were separated on 1.5% agarose gels stained with ethidium bromide and electrophoresed at a constant voltage of 3 V cm^{-1} until the bromophenol blue tracking dye reached the end of the gel. The gel was visualized in a UV transilluminator and photographed using GeneSnap ver. 7.09 (SynGene) gel documentation system. NZYDNA Ladder III (NZYtech; ISSR) and MassRuler DNA Ladder Mix (Thermo Scientific; SCoT) were used to establish molecular weight of the products. ISSR and SCoT amplification products that were clearly distinguishable and reproducible were scored. Bands were recorded in a binary format: presence (1) or absence (0). A band was considered monomorphic if it was present in all analyzed individuals. Polymorphic bands were those observed only in specific genotypes, while specific bands referred to those uniquely present in a single individual. The unique combination of bands produced by each primer was defined as its banding pattern.

Statistical analysis

The similarity coefficient between the studied genotypes in the ISSR and SCoT analysis was assessed according to the Dice formula [Nei 1979]. Cluster analysis was conducted using the UPGMA (unweighted pair-group method with arithmetic mean) distance method implemented in the PAST software [Hammer 2001]. PAST software was also used for the principal component analysis (PCA).

RESULTS AND DISCUSSION

ISSR-PCR amplification

In this study, a total of 136 allelic bands were detected that could be assessed using 13 different ISSR markers in 12 *Mentha* genotypes. Of these, 97 were found to be polymorphic, representing 71 percent polymorphism. Thirty-nine monomorphic bands were also detected, representing an average of three bands per primer. Primer ISSR12 generated the maximum number of bands (14), while primers ISSR3 and ISSR8 generated the minimum number (8). Band sizes ranged from 350 to 3800 bp (tab. 2). No specific bands were identified in the sample. Therefore, the marker sequences used in the study did not allow identification of any of the studied genotypes. However, according to Jędrzejczyk and

Rewers [2018], ISSR markers are useful in identifying *Mentha* species. The ISSR markers used by Çelik et al. [2024] to analyze various mint genotypes generated 100% polymorphic bands, which, similarly to our study, make identification impossible. In the study by Sabbour et al. [2016], no specific bands were identified. The primers used generated only polymorphic bands. The studies by the cited authors show significant differences in the average number of bands generated by primer, which in the case of the first author was 17.5 and the second 3.42. Our results are situated between these values (10.5). This may be due to the sequence of markers used and the genetic diversity of the plant material analyzed. The average polymorphism of 71% obtained in our study should be considered moderate, especially in the context of the study by Çelik et al. [2024], where it reached 100%. However, it was higher compared to the value of 65% reported by Choupani et al. [2019]. A high value of this parameter is observed when, among all amplicons, those that can be considered polymorphic predominate, and this most often happens in the analysis of a genetically diverse group of genotypes.

Table 2. Starter sequence and assessment of the number and type of products generated with the ISSR primers used in the study

Number of starter	Starter sequence	Number of PCR products				Polymorphism (%)	Range (bp)
		total	polymorphic	monomorphic	specific		
ISSR 1	VBV$(AC)_7$*	11	10	1	0	90.9	400–3800
ISSR 2	BDB$(CA)_7$	10	10	0	0	100.0	500–3200
ISSR 3	HBH$(CT)_7$	8	4	4	0	50.0	500–1500
ISSR 4	GCV$(TC)_7$	11	8	3	0	72.7	400–2300
ISSR 5	BDV$(AG)_7$	11	5	6	0	45.5	400–1500
ISSR 6	HVHTG$(TTG)_4$T	9	7	2	0	77.8	400–1400
ISSR 7	BDBCA$(CCA)_4$C	10	7	3	0	70.0	500–1400
ISSR 8	BDM$(CAG)_5$	8	4	4	0	50.0	400–2300
ISSR 9	$(GAA)_6$	11	11	0	0	100.0	400–2800
ISSR 10	$(ATG)_6$	11	7	4	0	63.6	400–3200
ISSR 11	$(TGG)_6$	11	7	4	0	63.6	350–1500
ISSR 12	$(GATA)_5$	14	8	6	0	57.1	400–2000
ISSR 13	$(GACA)_5$	11	9	2	0	81.8	350–1500
Total		136	97	39	0	–	350–3800
Mean		10.46	7.46	3.0	–	71.0	–

*V – not T; B – not A; H – not G; D – not C

The genetic similarity coefficient between the studied genotypes calculated using the Dice method ranged from 0.56 to 0.88 (tab. 3). As a result of clustering the genotypes, a dendrogram was generated which can be divided into three main clusters (fig. 1a). The first two have only one genotype each: *M. spicata* var. *crispa* and *Mentha piperita* var. *citrata*, respectively. In cluster III, comprising a total of 10 genotypes, two subclusters should be distinguished: A and B. Subcluster III A consisted of *M. suaveolens* and *M. suaveolens* var. *variegata* (for which the similarity coefficient was 0.79), joined by *Plectranthus amboinicus*. Subcluster III B contained the largest group of objects (7). The first clusters within it were formed by *M. spicata* var. *crispa* Persian and *M. piperita* var. *officinalis*, and then *M. spicata*, *M. longifolia, M. spicata* cv. Maroccan, *M. pulegium*, and finally *M. rotundifolia* were added. According to Vining et al. [2022], *M. spicata* is a hybrid whose parental forms are *M. suaveolens* and *M. longifolia*. Dendrogram analysis indicates that these forms are located in different subclusters of cluster III – III A and III B, respectively. At the same time, *M. spicata* and *M. spicata* cv. Maroccan are located in subcluster III B, indicating their greater similarity to the paternal form. Gobert et al. [2002], Choupani et al. [2019] point out the high similarity between *M. longifolia* and *M. spicata* based on their studies. The study by Naseem et al. [2024] indicates the joint clustering of the *M. spicata* hybrid and the parent forms *M. suaveolens* and *M. longifolia*. Taxonomy defines *M. piperita* as a hybrid between *M. spicata* and *M. aquatica*, and according to Momeni et al. [2006] shows greater similarity to *M. spicata* than to *M. aquatica*. This is confirmed by the results of our study, as both these genotypes were grouped together, and the genetic similarity coefficient was 0.88. A similar relationship between the studied objects is indicated by Soilhi et al. [2020]. According to Choupani et al. [2019], *M. piperita* and *M. longifolia* are highly similar, and our findings support this view with a genetic similarity value of 0.85. However, it should be noted that *M. piperita* is grouped into separate clusters, which was also observed by Çelik et al. [2024]. The authors suggest that this may be due to backcrossing. Relationships between *Mentha* accessions were assessed using PCA (fig. 2). For ISSR markers, principal component analysis (fig. 2a) revealed that the variance of the first two components accounted for 50.39% of the total variance. The first axis, accounting for 35.05% of the variance, separated genotypes G12, G11, and G5, G2, and G1, of which the first two formed single clusters in the dendrogram, while the remaining three formed cluster IIIa from the remaining genotypes. The second axis, accounting for 15.34% of the variance, essentially separated genotypes G4 and G7 from the remaining five (G3, G6, G8, G9, and G10), which constituted cluster IIIB in the dendrogram. The results of the PCA analysis are consistent with the results of the cluster analysis. PCA also confirms the results obtained by UPGMA clustering for SCoT markers (fig. 2b). The first axis, explaining 40.60% of the variance, separated genotypes G3, G5, and G11 from the others except G1, while the second axis, accounting for 16.89% of the variance, partially divides the objects of cluster IV dendrogram and genotype G3.

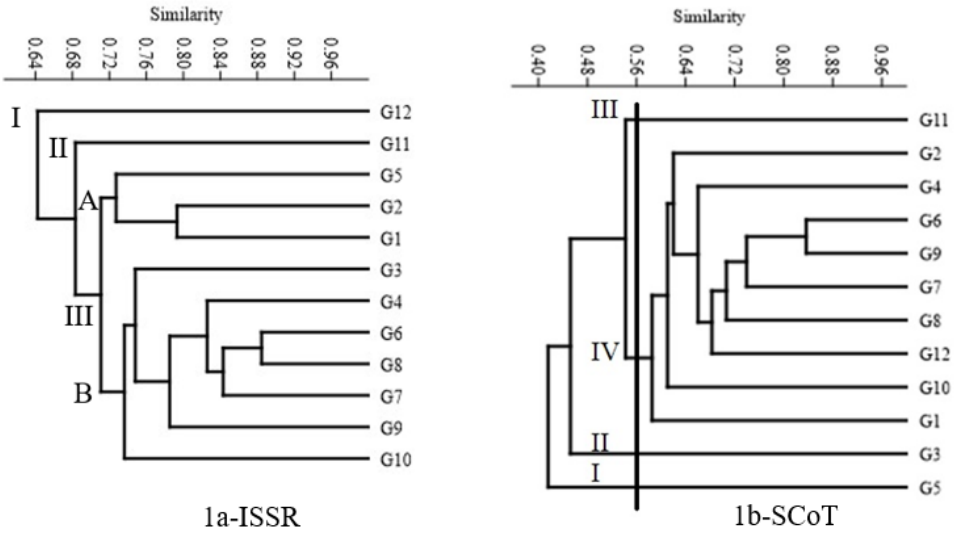


Fig. 1. UPGMA dendrogram of genetic similarity generated by ISSR (1a) and SCoT (1b) markers for 12 *Mentha* genotypes

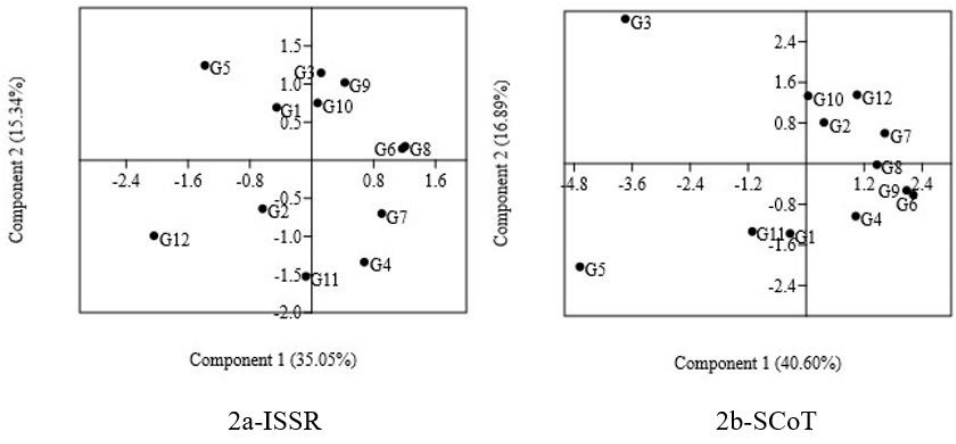


Fig. 2. Principal component analysis of 12 *Mentha* accessions with ISSR (2a) and SCoT (2b) markers

Table 3. Matrix of genetic similarity between the studied genotypes obtained on the basis of ISSR below the diagonal and SCoT above diagonal markers

	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
G1	1.00	0.55	0.40	0.61	0.47	0.63	0.55	0.59	0.60	0.58	0.55	0.56
G2	0.79	1.00	0.46	0.62	0.36	0.64	0.63	0.65	0.57	0.57	0.47	0.62
G3	0.73	0.67	1.00	0.39	0.37	0.41	0.48	0.42	0.43	0.54	0.41	0.58
G4	0.69	0.80	0.70	1.00	0.48	0.71	0.62	0.67	0.67	0.60	0.54	0.63
G5	0.76	0.69	0.70	0.65	1.00	0.45	0.36	0.43	0.42	0.40	0.43	0.40
G6	0.76	0.73	0.80	0.85	0.69	1.00	0.73	0.71	0.84	0.63	0.59	0.70
G7	0.74	0.70	0.71	0.82	0.67	0.86	1.00	0.70	0.75	0.57	0.57	0.72
G8	0.76	0.73	0.77	0.81	0.65	0.88	0.82	1.00	0.71	0.66	0.49	0.61
G9	0.72	0.69	0.76	0.76	0.73	0.80	0.78	0.80	1.00	0.62	0.65	0.70
G10	0.70	0.74	0.74	0.70	0.67	0.74	0.72	0.78	0.74	1.00	0.45	0.62
G11	0.70	0.63	0.71	0.73	0.60	0.70	0.75	0.73	0.63	0.65	1.00	0.56
G12	0.68	0.71	0.66	0.68	0.68	0.64	0.65	0.57	0.64	0.59	0.56	1.00

G1 – Mentha suaveolens Ehrh. var. variegata (pineapple mint); G2 – Mentha suaveolens Ehrh.; G3 – Mentha pulegium L.; G4 – Mentha longifolia L.; G5 – Plectranthus amboinicus (Lour.) Spreng. (Mexican mint); G6 – Mentha piperita L. var. officinalis Sole f. rubescens Camus; G7 – Mentha spicata L.; G8 – Mentha spicata var. crispa cv. Persian; G9 – Mentha spicata L. cv. Moroccan; G10 – Mentha rotundifolia L. Huds.; G11 – Mentha piperita L. var. citrata Ehrh.; G12 – Mentha spicata L. var. crispa

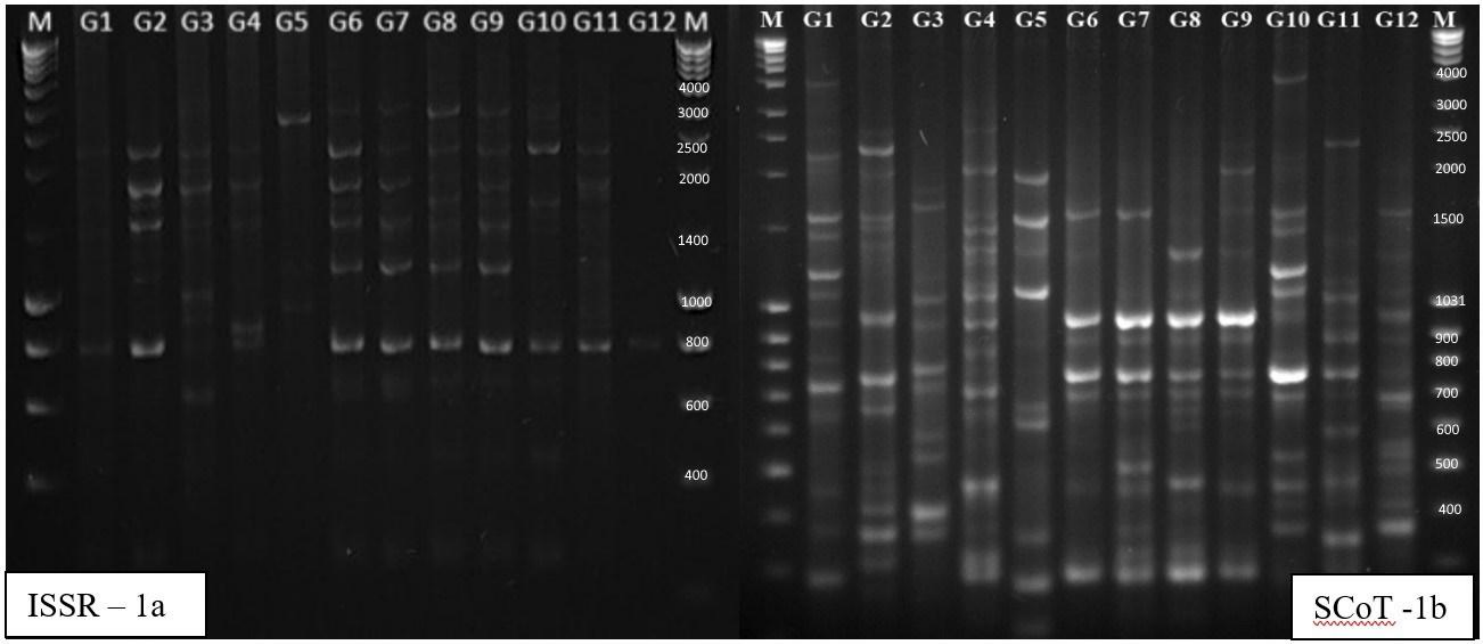
SCoT-PCR amplification

Molecular markers are used to assess genetic diversity, study population structure, and investigate relatedness between individuals. A relatively new marker technique used in DNA research is the start codon targeted (SCoT) polymorphism method, developed by Collard and Mackill [2009]. It utilizes dominant and repetitive markers based on the region surrounding the ATG translation initiation codon. This method is based on the polymerase chain reaction (PCR) using single primers, 18 nucleotides in length. In this study, fifteen SCoT primers were analyzed, and their sequences, along with the generated products, are listed in table 4. All primers produced a total of 157 bands, averaging more than 10 bands per primer. The percentage of polymorphism obtained ranged from 75% to 100%, averaging 88.7% for the fifteen primers mentioned above. A slightly lower value of this parameter at 56.8% was observed in 4 mint species by Salama et al. [2019]. Specific products generated by ten primers (SCoT 2, SCoT 3, SCoT 4, SCoT 5, SCoT 7, SCoT 9, SCoT 10, SCoT 12, SCoT 14, and SCoT 15) were also detected. A total of 14 products were obtained, with an average of 1.4 bands for primers amplifying these products. Primers SCoT 8, SCoT 9, and SCoT 15 generated monomorphic products. All the above-mentioned primers produced only one band of this type. The size of the obtained products ranged between 350 and 4200 bp. The usefulness of SCoT markers in the characterization of mint species is indicated by Salama et al. [2019] and Heylen et al. [2021]. Salama et al. [2019] identified specific amplicons generated by some primers allowing the identification of the species *M. sativa*, *M. spicata*, *M. piperita* and the subspecies *M. longifolia* subsp. *typhoides* and *schimperi*. The possibility of identifying *M.* × *piperita* cultivars with this marker system was indicated by Khan et al. [2017], and with the RAPD marker system by Kiełtyka-Dadasiewicz et al. [2017]. This is confirmed by the results of this study, which enable the identification of six of the twelve genotypes tested, which include: *Plectranthus amboinicus* – using primers SCoT 2, 3, 4, 10 and 14 generating a total of 6 specific fragments, *Mentha pulegium* L. using primer SCoT 7, *Mentha spicata* L. cv. Moroccan using primers SCoT 5 and 9, *Mentha suaveolens* Ehrh. var. *variegata* (pineapple mint) using starter SCoT 2 and 4, *Mentha spicata* L. using starter SCoT 12, and *Mentha longifolia* L. using starter SCoT 15 (tab. 5).

The genetic similarity coefficient assessed according to the Dice formula between 12 studied genotypes is presented in table 3. The similarity ranged from 0.36 to 0.84. The highest similarity occurred between *Mentha piperita* L. var. *officinalis* Sole f. *rubescens* Camus and *Mentha spicata* L. cv. Moroccan while the lowest between *Mentha suaveolens* and *Plectranthus amboinicus* and *Plectranthus amboinicus* and *Mentha spicata* L.

Table 4. Starter sequence and assessment of the number and type of products generated with the SCoT primers used in the study

Number of starter	Starter sequence	Number of PCR products				Polymorphism (%)	Range (bp)
		total	polymorphic	monomorphic	specific		
SCoT 1	CAACAATGGCTACCACCA	11	11	0	0	100	650–3500
SCoT 2	CAACAATGGCTACCACCC	12	10	0	2	83.3	450–4200
SCoT 3	CAACAATGGCTACCACCT	9	8	0	1	88.8	850–2700
SCoT 4	CAACAATGGCTACCACGT	13	11	0	2	84.6	550–3200
SCoT 5	CAACAATGGCTACCAGCC	8	7	0	1	87.5	650–4200
SCoT 6	ACGACATGGCGACCGCGA	17	17	0	0	100.0	250–3500
SCoT 7	ACCATGGCTACCACCGAC	9	7	0	2	77.7	550–3200
SCoT 8	ACCATGGCTACCACCGAG	10	9	1	0	90.0	550–3200
SCoT 9	ACCATGGCTACCACCGCC	8	6	1	1	75.0	350–4200
SCoT 10	ACCATGGCTACCACCGGC	10	8	0	2	80.0	350–2400
SCoT 11	CCATGGCTACCACCGCCG	9	9	0	0	100.0	450–3500
SCoT 12	CCATGGCTACCACCGGAG	10	9	0	1	90.0	350–2300
SCoT 13	ACGACATGGCGACCAGCG	10	10	0	0	100.0	550–2500
SCoT 14	ACGACATGGCGACCACGT	9	8	0	1	88.8	450–2500
SCoT 15	CCATGGCTACCACCGGCA	12	10	1	1	83.3	450–2700
Total		157	140	3	14	–	350-4200
Mean		10.5	9.3	0.2	0.9	88.7	–



M – standard of DNA fragment size; G1 – *Mentha suaveolens* Ehrh. var. *variegata* (pineapple mint); G2 – *Mentha suaveolens* Ehrh.; G3 – *Mentha pulegium* L.; G4 – *Mentha longifolia* L.; G5 – *Plectranthus amboinicus* (Lour.) Spreng. (Mexican mint); G6 – *Mentha piperita* L. var. *officinalis* Sole f. *rubescens* Camus; G7 – *Mentha spicata* L.; G8 – *Mentha spicata* var. *crispa* cv. Persian; G9 – *Mentha spicata* L. cv. Moroccan; G10 – *Mentha rotundifolia* L. Huds.; G11 – *Mentha piperita* L. var. *citrata* Ehrh.; G12 – *Mentha spicata* L. var. *crispa*

Fig. 3. Fingerprints of twelve *Mentha* genotypes using ISSR 2 (1a) and SCoT 6 (1b) primer

Table 5. SCoT markers specific to *Mentha*

Species names	SCoT primer	Specific band size (bp)
M. suaveolens Ehrh. var. *variegata* G1	SCoT2	4200
	SCoT4	1500
Mentha pulegium L. G3	SCoT7	1700
	SCoT7	800
Mentha longifolia L. G4	SCoT15	1200
Plectranthus amboinicus (Lour.) Spreng G5	SCoT2	1400
	SCoT3	2700
	SCoT4	650
	SCoT10	1200
	SCoT10	450
	SCoT14	1400
Mentha spicata L. G7	SCoT12	1700
Mentha spicata L. cv. Moroccan G9	SCoT5	650
	SCoT9	2500

The analysis of genetic similarity generated using SCoT markers was presented in the form of a dendrogram (fig. 1b). The dendrogram intersection at 0.56, which is the average value for all analyzed genotypes, allows for the identification of four distinct clusters. Three of these clusters include single genotypes: *Mentha piperita* var. *citrata*, *Mentha pulegium*, and *Plectrantus amboinicus* – Mexican mint, the latter of which was the most distant from all the others. In cluster IV, composed of nine genotypes, the first cluster was formed by *M. piperita* var. *officinalis* and *M. spicata* cv. Moroccan. Subsequently, the remaining genotypes were added to this cluster, and finally *M. suaveolens* var. *variegata*. However Khan et al. [2017] in their study of genetic diversity using SCoT markers did not group *Mentha piperita* and *Mentha spicata* genotypes into separate clusters, but rather combined them with *M. cardiaca* and *M. viridis* into one large cluster. The genotypes present in cluster IV were characterized by a high level of similarity ranging from 0.55 to 0.84. Among them, *M. spicata* and *M. longifolia* were used by Salama et al. [2019] in the analysis using SCoT markers and by Ibrahim [2017] using RAPD markers. The authors report similarity between *M. spicata* and *M. longifolia* at 0.45 and 0.44, respectively, for the marker systems used. This is reflected in the results of this study, as these species formed one cluster, and the similarity value was even higher than the reported results and amounted to 0.62. In turn, the study by Vining et al. [2019] using SSR markers indicate a joint grouping of *M. suaveolens* and *M. longifolia* genotypes. This relationship is confirmed by the cluster IV observed in our study, which includes both aforementioned species. Phylogenetic tree analyses conducted by Thakur et al. [2016] indicate grouping of *M. piperita* and *M. citrata* in one cluster and *M. spicata* in a separate one. Genetic similarity determined by SCoT markers within the *M. spicata* species, represented by three genotypes, should be considered high, as it ranged from 0.70 to 0.75. This was reflected

in the formation of the second cluster on the dendrogram between *M. spicata* and *M. spicata* cv. Maroccan. Furthermore, the same cluster included *M. suaveolens* and *M. longifolia*, which, according to Vining et al. [2022], are parental forms of this species, and their direct similarity was estimated at 0.62. Therefore, using SCoT markers allows for the assessment of genetic diversity within the studied genus, allowing for effective differentiation of even forms with close phylogenetic relationships.

CONCLUSION

Genetic improvement of *Mentha* should integrate both morphological and molecular data, as accessions with similar morphology often differ at the molecular level. Molecular diversity databases are thus valuable tools for breeding and analysing novel intra and interspecific hybrids. SCoT markers, due to their primer design based on conserved start codon regions, are predominantly distributed within gene rich regions on both DNA strands, enhancing their relevance for functional genomic studies. Unlike ISSR markers, which target repetitive non-coding regions, SCoT markers are more likely to amplify coding sequences, including regions associated with pseudogenes and transposable elements, providing greater insight into gene-associated polymorphisms. Additionally, the efficiency of SCoT markers is influenced by the precise distance between primer binding sites, contributing to their reproducibility and specificity. These advantages make SCoT a more informative and functionally relevant tool compared to ISSR for assessing genetic diversity and structure *Mentha* genus. Selected primers generating unique bands can be used to precisely identify specific genotypes.

REFERENCES

Ahmad İ., Khan S.U., Khan A. et al., 2018. Reassessment of *Mentha* species from Kunhar River catchment using morphological and molecular markers. Anadolu J. Aegean Agric. Res. Inst. 28(1), 6–12.

Choupani A., Shojaeiyan A., Maleki M., 2019. Genetic relationships of Iranian endemic mint species, *Mentha mozaffariani* Jamzad and some other mint species revealed by ISSR markers. BioTechnologia 100, 19–28. https://doi.org/10.5114/bta.2019.83208

Çelik C., Seraj N.A., Yasak S. et al., 2024. Molecular characterization and genetic relationships in different mint (*Mentha* L.) species with ISSR marker technique. Biol. Bull. 51(4), 959–968. https://doi.org/10.1134/S106235902360616X

Collard B.C., Mackill D.J., 2009. Start codon targeted (SCoT) polymorphism: a simple novel DNA marker technique for generating gene-targeted markers in plants. Plant Mol. Biol. Rep. 27, 86–93. https://doi.org/10.1007/s11105-008-0060-5

Devi A., Iqbal T., Ahmad Wani I. et al., 2022. Assessment of variability among morphological and molecular characters in wild populations of mint [*Mentha longifolia* (L.) L.] germplasm. Saudi J. Biol. Sci. 29, 3528–3538. https://doi.org/10.1016/j.sjbs.2022.02.013

Dorman H.D., Koşar M., Kahlos K. et al., 2013. Antioxidant properties and composition of aqueous extracts from *Mentha* species, hybrids, varieties, and cultivars. J. Agric. Food Chem. 51, 4563–4569. https://doi.org/10.1021/jf034108k

Doyle J.J., Doyle J.L., 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. 19, 11–15.

Fukui Y., Saito M., Nakamura N. et al., 2022. Classification of Southeast Asian mints (*Mentha* spp.) based on simple sequence repeat markers. Breed. Sci. 72, 181–187. https://doi.org/10.1270/jsbbs.21058

Gobert V., Moja S., Colson M. et al., 2002. Hybridization in the section *Mentha* (Lamiaceae) inferred from AFLP markers. Am. J. Bot. 89, 2017–2023. https://doi.org/10.3732/ajb.89.12.2017

Hammer Ø., Harper D.A.T., Ryan P.D., 2001. Past: Paleontological statistics software package for education and data analysis. Palaeontol. Electron. 4, 9.

Heylen O.C.G., Debortoli N., Marescaux J. et al., 2021. A revised phylogeny of the *Mentha spicata* clade reveals cryptic species. Plants 10, 819. https://doi.org/10.3390/plants10040819

Ibrahim H.M., 2017. Assessment of genetic diversity and relationships of five *Mentha* species using RAPD marker. Curr. Sci. Int. 6, 271–277.

Jędrzejczyk I., Rewers M., 2018. Genome size and ISSR markers for *Mentha* L. (Lamiaceae) genetic diversity assessment and species identification. Ind. Crops Prod. 120, 171–179. https://doi.org/10.1016/j.indcrop.2018.04.062

Khan N., Singh S., Singh Dhawan S., 2017. Development of species-specific SCoT markers and analysis of genetic diversity among *Mentha* genotypes. Int. J. Innov. Sci. Engineer. Technol. 4, 2348–7968.

Khanuja S.P.S., Shasany A.K., Srivastava A. et al., 2000. Assessment of genetic relationships in *Mentha* species. Euphytica 111, 121–125. https://doi.org/10.1023/A:1003829512956

Kiełtyka-Dadasiewicz A., Okoń S., Ociepa T. et al., 2017. Morphological and genetic diversity among peppermint (*Mentha* × *piperita* L.) cultivars. Acta Sci. Pol. Hortorum Cultus 16, 151–161. https://doi.org/10.24326/asphc.2017.3.15

Lawrence B.M., 2006. Mint. The Genus Mentha. CRC Press, Boca Raton. https://doi.org/10.1201/9780849307980

Malik R.H., Shah S.M., Khan A.R. et al., 2019. Evaluation of sequence related amplified polymorphic markers for genetic characterization of *Mentha* species. Phillipine J. Crop Sci. 44, 71–76.

Mkaddem M., Bouajila J., Ennajar M. et al., 2009. Chemical composition and antimicrobial and antioxidant activities of *Mentha* (*longifolia* L. and *viridis*) essential oils. J. Food Sci. 74, M358–M363. https://doi.org/10.1111/j.1750-3841.2009.01272.x

Momeni S., Shiran B., Razmjoo K., 2006. Genetic variation in Iranian mints on the bases of RAPD analysis. Pak. J. Biol. Sci. 9, 1898–1904. https://doi.org/10.3923/pjbs.2006.1898.1904

Moshrefi-Araghi A., Nemati H., Azizi M. et al., 2021. Association of genetic structure and diversity in Iranian wild germplasms of *Mentha longifolia* L. based on phenotypical, biochemical, and molecular markers. Chem. Biodivers. 18, e2001044. https://doi.org/10.1002/cbdv.202001044

Naseem I., Khan M.A., Habib U. et al., 2025. Morphological profiling and DNA barcoding revealed genetic diversity and phylogeny of Mentha species cultivated in Pakistan. Genet. Resourc. Crop Evol. 72(3), 2977–2995. https://doi.org/10.1007/s10722-024-02140-x

Nei M., Li W., 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA 76, 5269–5273.

Panjeshahin Z., Sharifi-Sirchi G., Samsampour D., 2018. Genetic and morphological diversity of wild mint *Mentha longifolia* (L.) Hudson subsp. *noeana* (Briq.) Briq. in south- and southeastern Iran. *J. Med. Plants By-products* 7(1), 105–115. https://doi.org/10.22092/jmpb.2018.116741

Rodrigues L., van den Berg C., Póvoa O. et al., 2013. Low genetic diversity and significant structuring in the endangered *Mentha cervina* populations and its implications for conservation. Biochem. Syst. Ecol. 50, 51–61. https://doi.org/10.1016/j.bse.2013.03.007

Sabboura D., Yacoub R., Lawand S., 2016. Assessment of genetic relationships among *Mentha* species. Int. J. ChemTech Res. 9, 462–468. https://doi.org/10.1023/A:1003829512956

Salama A.M., Osman E.A., El-Tantawy A.A., 2019. Taxonomical studies on four *Mentha* species grown in Egypt through morpho-anatomical characters and SCoT genetic markers. Plant Arch. 19, 2273–2286.

Silva D., Vieira R., Alves R. et al., 2006. Mint (*Mentha* spp.) germplasm conservation in Brazil. Rev. Bras. Pl. Med. 8, 27–31.

Sofyalıoğlu E., Sevindik E., Gübeş İ. et al., 2025. Phylogenetic analysis of endemic *Sideritis* L. spp. (*Lamiaceae*) in Türkiye based on chloroplast *trn*L-F, *mat*K, and *rbc*L DNA sequences. Genet. Resourc. Crop Evol**.** 72(4), 4381–4391. https://doi.org/10.1007/s10722-024-02225-7

Smolik M., Rzepka-Plevnes D., Jadczak D. et al., 2007. Morphological and genetic variability of chosen *Mentha* species. Herba Pol. 53, 90–97.

Soilhi Z., Trindade H., Vicente S. et al., 2020. Assessment of the genetic diversity and relationships of a collection of *Mentha* spp. in Tunisia using morphological traits and ISSR markers. J. Hortic. Sci. Biotechnol. 95, 483–495. https://doi.org/10.1080/14620316.2019.1702482

Thakur V.V., Tiwari S., Tripathi N. et al., 2016. DNA barcoding and phylogenetic analyses of Mentha species using *rbc*L sequences. Ann. Phytomed. 5(1), 59–62

Vining K.J., Pandelova I., Hummer K. et al., 2019. Genetic diversity survey of *Mentha aquatica* L. and *Mentha suaveolens* Ehrh., mint crop ancestors. Genet. Resour. Crop Evol. 66, 825–845. https://doi.org/10.1007/s10722-019-00750-4

Vining K.J., Pandelova I., Lange I. et al., 2022. Chromosome-level genome assembly of *Mentha longifolia* L. reveals gene organization underlying disease resistance and essential oil traits. G3 Genes Genomes Genet. 12(8), jkac112. https://doi.org/10.1093/g3journal/jkac112

Source of funding: This research was funded by the statutory activity of the University of Life Sciences in Lublin (Grant No. SUBB.WRH.19.023.RiO).

Received: 1.09.2025
Accepted: 17.10.2025
Published: 31.12.2025