The use of RAPD markers for detecting genetic similarity and molecular identification of chamomile (*Chamomilla recutita* (L.) Rausch.) genotypes

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S u m m a r y

The objectives of this study was to assess the of genetic similarity and identification of 13 wild species and 7 cultivars of chamomile using RAPD markers. 53 RAPD primers were screened, only 12 produced polymorphic and repeatable fragments. In total, all primers used produced 157 fragments out of which 149 were polymorphic. The RAPD-based genetic similarity was estimated. Genetic similarity matrix was applied for cluster analysis through UPGMA method. On the dendrogram, only genotypes from Austria, Czech Republic as weel as genotypes collected in area of Lublin were grouped together. The remaining genotypes from the same area were located in different groups. Present study demonstrated that RAPD markers provided a practical and effective method not only to evaluate the genetic similarity and relationships but also to identify chamomile genotypes.

Key words: *Chamomilla recutita (L.) Rausch., chamomile genotypes, genetic similarity, medicinal plant, RAPD*

INTRODUCTION

Common chamomile (*Chamomilla recutita* (L.) Rausch.) is one of the most favored and widely used herb plant throughout the world. Due to its antiseptic and therapeutic [1], antispasmodic [2], antimicrobial [3] and anti-inflamatory [4]

use, chamomile has many applications in pharmacy, nutritional and sanitary industrials. The diverse pharmaceutical proprieties are mainly due to essential oils like chamazulene, (-)-α-bisabolol and different flavonoids. Flowers of chamomile contain apigenine which is used as a hair color [5]. Chamomile plant adapts well to different soil conditions. Wild growing population of chamomile can be found in trodden, weed and dump conditions. The plant helps to improve the acid soils by cautions absorption [6].

Recognition of the genetic diversity is the base of breeding programs [7] and suitable for selection of plant genotypes. In recent years DNA base molecular markers have been used for the description of genetic differences between germplasms in many plant species [8-13]. The randomly amplified polymorphic DNA (RAPD) method described by Williams et al. [14] provides a faster and less expensive alternative to other molecular markers like RFLP or AFLP and also helps to estimate diversity, monitoring of genetic erosion, removing duplicates from germplasm collection [15]. The RAPD markers have been successfully used for study of genetic diversity of different medicinal plants such as coneflower (*Echinacea* Moench) [16, 17], mint (*Mentha* L.) [18, 19], foxgloves (*Digitalis* L.) [20, 21] summer savory (*Satureja hortensis*) [22], kenaf (*Hibiscus cannabinus* L.) [23]. The number of studies performed with molecular markers on chamomile is very limited. There is only a few publications concerning the use of molecular markers to estimate genetic diversity among chamomile genotypes on the molecular level [24, 25]. The aim of this paper was the study the genetic similarity, relationships and identification of different chamomile accessions revealed by RAPD markers.

MATERIALS AND METHOD

Plant material and DNA extraction

The 20 genotypes of *Chamomilla recutita*: 7 cultivars and 13 wild species were analyzed (tab. 1). Four wild genotypes were collected in area of Lublin, Poland, other genotypes came from gene banks: Gatersleben (Germany), Canada and Czech Republic. DNA was isolated from leaves of young plants in two replications for every genotype, following the CTAB method described by Doyle and Doyle [26].

Table 1.

Chamomile accessions used in molecular study and their origin

No.	genotype and cultivars	origin of genotype and cultivars	gene bank
1	PL 1	Poland	
2	PL 2	Poland	
3	PL 3	Poland	
4	PL 4	Poland	
5	MAT 26	Austria	IPK Gatersleben
6	MAT 5	Austria	IPK Gatersleben
7	MAT 24	Belgium	IPK Gatersleben
8	MAT 2	Germany	IPK Gatersleben
9	CZE 1	Germany	Gene Bank RICP Prague-Ruzyne
10	MAT 16	Bulgaria	IPK Gatersleben
11	CN 43728	Poland	Plant Gene Resources of Canada
12	CN 43727	Hungary	Plant Gene Resources of Canada
13	MAT 19	Korea	IPK Gatersleben
14	CZE 2 (Bochemia)	Czech Republic	Gene Bank RICP Prague-Ruzyne
15	MAT 15 (Pohorelicky Velkokvety)	Czech Republic	IPK Gatersleben
16	MAT 17 (Bodegold)	Germany	IPK Gatersleben
17	MAT 18 (Quedlinburger Großblütige)	Germany	IPK Gatersleben
18	MAT 20 (ital. Camomilla commune)	Italy	IPK Gatersleben
19	MAT 10 (Krajovy)	Unknown	IPK Gatersleben
20	PL 5 (Zloty Lan)	Poland	

RAPD analysis

The PCR reactions were performed according to the RAPD method described by Williams et al. [14] with minor modification. Reaction mixtures contained 1 x PCR Buffer (10 mM Tris pH 8.8, 50 mM KCl, 0.08% Nonidet P40) (Fermentas, Lithuania), 160 μM of each dNTP, 530pM oligonucleotide primer, 1.5 mM $MgCl_2$, 70 ng of template DNA, 0.5 U Taq DNA Polymerase (Fermentas, Lithuania) in a final reaction mixture of 15 μl. Amplifications were carried out in Biometra T1 thermal cycler programmed for 3 min in 94°C of initial denaturation, 44 cycles: 94°C – 45 s, 37°C – 45 s, 72°C – 45 s, with final extension at 72°C for 7 min. A negative control was added in each run. In order to check reproductability the selected primers were tested two times on the same sample.

Amplification products were separated by electrophoresis on 1.5% agarose gels containing 0.1% EtBr. Fragments were visualized under UV transiluminator and photographed using PolyDoc System. GeneRuler™ 100bp DNA Ladder Plus was used to establish molecular weight of the products.

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Data analysis

The RAPD products were scored as present (1) or absent (0) on the photographs. Only bright and reproductible products were scored. Unique RAPD markers were employed to identify chamomile genotypes.

The level of polymorphism of the primer (polymorphic products/total products) and relative frequency of polymorphic products (genetic resources where polymorphic products were present/ total number of genetic resources) [27] were calculated.

Resolving power of the primer was calculated using the formula: Resolving power (Rp) = Σ Ib (band informativeness). Band informativeness was calculated for each band scored individually by the primer. Ib=1–[2(0,5–p)], p is the proportion of the occurrence of bands in the genotypes out of the total number of genotypes. Resolving power of primers is a very useful parameter for the molecular diagnosis of any species from the mixed population [28].

Banding patterns (the different combination of bands obtained for each primer) were designated by the number of the respective primers. Relative frequency of banding patterns were also calculated.

Genetic pairwise similarities (SI-similarity index) between studied genotypes were evaluated according to Dice's formula after Nei and Li [29]. A cluster analysis was conducted using the distance method UPGMA (Unweighted Pair-Group Method with Arithmetic Mean) in the NTSYS program [30].

RESULTS AND DISCUSSION

Chamomile genotypes were analyzed using 53 RAPD primers (Operon Technologies), out of which 12 produced high number of polymorphic and repeatable fragments. In total primers produced 157 fragments. The number of fragments ranged from 6 to 19, with an average 13.08 per primer as well as 70.5 bands per genotype. Among the 12 primers G2 had the lowest primer diversity 84.2%, five primers scored to 100%. Most of them did not generate any monomorphic products, only G2 generated two and U225, J5, G7, D7, A18 and G5 generated only one.

Among total 157 amplified products 149 (94.9%) were polymorphic. The number of polymorphic bands amplified by single primer ranged from 6 to16 with an average of 12.41 per primer and 7.45 per genotype (table 2). Solouki et al. [25] used RAPD markers to estimate genetic diversity between 25 populations of *Matricaria chamomilla*. Authors used 29 RAPD primers which gave 369 bands, out of which only 55 were monomorphic. The number of bands per primer obtained by Solouki et al. [25] varied from 8 to 22, the average of the polymorphic bands generated per primer was less than that obtained in this study and valued 10.84. Molecular weigh of polymorphic products obtained by Solouki et al. [25] ranged from 400 to 1500 bp and was lower than that received in present study where molecular weigh of polymorphic products ranged from 200 to 2600 bp.

Table 2.

Characteristic of selected RAPD primers

primer no.	sequence 5`–`	amplified products	polymorphic products	primer diversity (%)	frequency of polymorphic products	resolving power of the primer	number of banding patterns
A18	AGG TGA CCG T	7	6	85.7	0.57	8	12
D7	TTG GCA CGG G	15	14	93.3	0.54	15.2	20
D16	AGG GCG TAA G	16	16	100	0.44	14.2	20
G2	TGC TGC AGG T	19	16	84.2	0.39	15	19
G3	CCA GTA CTT C	10	10	100	0.42	8.4	19
G5	AAC CCG GGA A	9	8	88.8	0.47	8.5	18
G7	CCT CTA GAC C	11	10	90.9	0.24	3.4	13
J5	CTC CAT GGG G	16	15	93.7	0.39	12.4	15
M11	GTC CAC TGT G	9	9	100	0.41	6.9	12
U225	CGA CTC ACA G	17	16	94.1	0.41	13.3	20
U532	TTG AGA CAG G	15	15	100	0.41	12.3	20
X6	TCC GAG TCT G	13	13	100	0.42	11.1	20
Total		157	149	94.9			
Per primer		13.08	12.41				
Per genotype		7.85	7.45				

Relative frequency of polymorphic bands ranged from 0.05 (polymorphic band present only in one genotype of the 20 studied mones) to 0.95 (polymorphic band absent only in one genotype of 20 studied ones). The DNA polymorphic average frequency generated by single primer was 0.42, varying from 0.24 (G7) to 0.57 (A18). The average frequency of polymorphic products was previously calculated for tea genotypes [31]. Relative frequency of polymorphic products obtained by Chen et al. [31] was similar to average frequency obtained in present study and amounted to 0.47.

From 20, 8 genotypes could be identified by means of the presence of 11 unique RAPD markers and absence of 4 unique markers generated by 6 primers: G7, D7, G2, U532, U225 and J5 (table 3). Genotype MAT5 could be identified by four unique markers, including the presence of G7 (9), U225 (5), J5 (13) and the absence of D7 (1). Meanwhile, two unique markers could be used to identify PL1, PL2, MAT26 and MAT2 genotypes. Genotypes MAT24, MAT16 and MAT19 could be identified using only one unique marker. Chen et al. [31] gained 32 unique RAPD markers which could identify all analyzed tea genotypes.

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Table 3.

Unique RAPD markers used for identification of chamomile genotypes

No.	identified *Chamomilla* genotypes	unique marker	criteria
1	MAT5	G7 (9), U225 (5), J5 (13)	presence
		D7 (1)	absence
2	PL1	G (7)	presence
		U532 (13)	absence
3	PL2	U532 (1)	presence
		J5 (16)	absence
4	MAT26	G7 (8), D7 (15)	presence
5	MAT2	U225 (2)	presence
		G2 (16)	absence
6	MAT24	G2 (18)	presence
7	MAT16	G2 (5)	presence
8	MAT19	G7 (8)	presence

The selected primers yielded 208 banding patterns. The number of banding patterns ranged from 12 to 20, with an average of.3 per primer. Relative frequency of the banding patterns varied from 0.05 to 0.3. Average frequency of the banding patterns was very low (0.1). Belaj et al. [27] calculated relative frequency of banding patterns for olive (*Olive europea* L.), which varied from 0.02 to 0.88 and, similar to this described in present study, average frequency of banding patterns was very low (0.2).

The resolving power of the 12 RAPD primers ranged from 3.4 for primer G7 to 15.2 for primer D7 (tab. 2). Usually, primers with high resolving power are used for the molecular diagnosis of any species from the mixed population [28]. Three of the RAPD primers D7, G2 and D16 possessed high resolving power values 15.2, 15, 14.2 respectively and were able to distinguish all 20 genotypes, and could be potentially used for identifying chamomile genotypes from any mixed population of chamomile. In the past, a similar approach has been successfully used for molecular diagnosis of potato cultivars [28] , *Rhus* species [32], fig cultivars [33], and *Jatropha* genotypes [34].

The genetic similarity matrices were produced based on RAPD using the Dice's coefficient. RAPD based genetic similarity was estimated between 0.460 and 0.832. The mean genetic similarity was calculated at 0.602. Wagner et al. [24] estimated genetic similarity between chamomile population with high and low content of (-)-α-bisabolol. Genetic similarity based on RAPD markers was similar to genetic similarity obtained in present study and ranged from 0.52 to 0.91. Genetic similarity matrix was applied for cluster analysis through UPGMA method (fig. 1). The 20 genotypes could be grouped into two major groups.

Group A contained 18 genotypes and could be subdivided into three minor subgroups A1, A2 and A3. Subgroup A1 contained four wild genotypes from Poland collected from Lublin ara. Subgroup A2 contained 9 genotypes: 5 wild species from different countries and 4 cultivated genotypes from Germany and Czech Republic. Subgroup A3 included three cultivated genotypes ital. Camomilla commune, Zloty Lan, Krajovy. Two wild genotypes from Belgium and Germany showed much difference in their molecular characteristic and stand alone far from the other genotypes forming the A group. The B major cluster contained two genotypes from Austria. Solouki et al. [25] found, similar like in present study that the genetic diversity was not in accordance to the geographical diversity. In present study only genotypes from Austria, Czech Republic and genotypes collected in area of Lublin grouped together. The rest of genotypes from the same area are located in different groups (fig. 1).

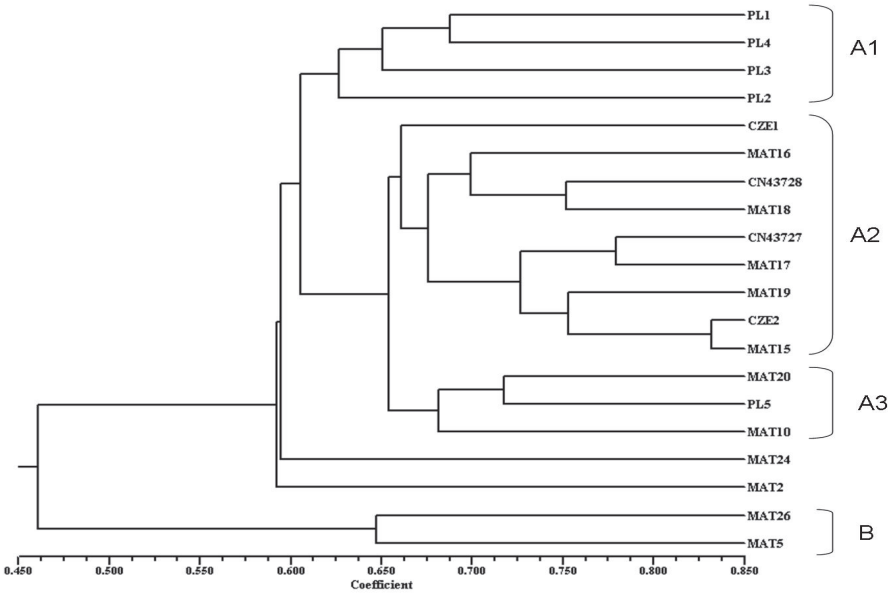

Figure 1.
Dendrogram of chamomile genotypes constructed using UPGMA method, shows the presence of three groups: A1 (PL1, PL4, PL3, PL2), A2 (CZE1, MAT16, CN43728, MAT18, CN43727, MAT17, MAT19, CZE2, MAT15), A3 (MAT20, PL5, MAT10) and separateness of the two wild species: MAT24, MAT2 and B group (MAT26, MAT5)

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CONCLUSIONS

1. Present study demonstrated that RAPD markers provided a practical and effective method not only to evaluate the genetic similarity and relationships, but also to identify chamomile genotypes (tab. 3).

2. Analyzed chamomile genotypes were characterized by high genetic similarity. The similarity was not correlated with origin of analyzed genotypes. Genotypes from the same area located in different groups on the dendrogram.

3. Analyzed chamomile genotypes could be identified from any mixed population of chamomile using three RAPD primers D7, G2 and D16 which possessed high resolving power values.

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WYKORZYSTANIE MARKERÓW RAPD DO OKREŚLENIA PODOBIEŃSTWA GENETYCZNEGO ORAZ MOLEKULARNEJ IDENTYFIKACJI GENOTYPÓW RUMIANKU POSPOLITEGO *(CHAMOMILLA RECUTITA* (L.) RAUSCH.)

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

Celem badań było określenie podobieństwa genetycznego oraz identyfikacja 13 dzikich gatunków i 7 odmian rumianku pospolitego za pomocą markerów RAPD. Spośród 53 starterów wybrano 12, które amplifikowały polimorficzne i powtarzalne produkty, łącznie uzyskano 157 fragmentów, z których 149 było polimorficznych. Podobieństwo genetyczne oszacowano w oparciu o markery RAPD. Analizę skupień wykonano metodą średnich połączeń UPGMA. Na uzyskanym dendrogramie tylko genotypy pochodzące z Austrii, Czech oraz zebrane w okolicach Lublina ulegały wspólnemu skupieniu. Reszta analizowanych genotypów pochodzących z tych samych regionów ulegała skupieniu w różnych grupach. Przedstawione badania wykazują, że metoda RAPD jest dobrą metodą nie tylko do oszacowania podobieństwa genetycznego, ale także do identyfikacji genotypów rumianku pospolitego.

Słowa kluczowe: *Chamomilla recutita (L.) Rausch, rumianek, podobieństwo genetyczne, rośliny lecznicze, RAPD*