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GENETIC DIVERSITY AMONG CULTIVATED AND WILD CHAMOMILE GERMPLASM BASED ON ISSR ANALYSIS

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Abstract. *Chamomilla recutita* (L.) Rausch. is a wide known herbal plant which has many medical attributes and find applications in pharmacy, nutritional and sanitary industries. Estimating genetic diversity in population is very important to protect variety of chamomile species. The objective of this study was characterization of chamomile germplasm using ISSR markers. Among 20 screened ISSR primers, only 5 produced polymorphic and repeatable fragments. In total primers produced 48 fragments out of which 41 (85.4%) were polymorphic. The average PIC value for the amplification products was 0.340. Based on ISSR markers the genetic similarity matrices were produced. The mean genetic similarity was calculated at 0.653. Present study demonstrated that ISSR markers provided a practical and effective method to evaluate the genetic similarity and relationships of chamomile genotypes. Analyzed chamomile genotypes were characterized by quite high genetic similarity; it suggested that there is necessity to find new sources of genetic diversity in chamomile in wild populations.

Key words: *Chamomilla recutita* (L.) Rausch., genetic variability, Inter Simple Sequence Repeat (ISSR), molecular markers

INTRODUCTION

In recent years polymerase chain reaction (PCR) based molecular markers such as RAPD (*Random Amplified Polymorphic DNA*), ISSR (*Inter Simple Sequence Repeat*) or AFLP (*Amplified Fragment Length Polymorphism*) were explored to study genetic diversity of many plant species [Anthony et al. 2004, Chen et al. 2005, Hagidimitriou et al. 2005, He et al. 2009].

ISSR analysis was found to be the most economical among PCR based markers. The ISSR has many advantages like low quantities of template DNA, no need of sequence data for primer construction, random distribution throughout the genome, generation of

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many informative bands per reaction and reproducible production [Zietkiewicz et al. 1994, Nagoaka and Ogihara 1997]. ISSR has been shown to provide a powerful, rapid, simple and reproducible tool used in marker assisted selection, DNA fingerprinting, evolution and molecular ecology [Nybom 2004, Zhao et al. 2007, Gajera et al. 2010, Zhang and Dai 2010]. ISSR is also used to assess genetic diversity and identify differences between closely related cultivars in many species [González et al. 2002]

Chamomile (*Chamomilla recutita* (L.) Rausch.) is a wide known herbal plant which has many medical attributes such as antiseptic, antispasmodic, antimicrobial and anti-inflamatory [Letchamo and Marquard 1993, Manifesto et al. 2001, Pourohit and Vyas 2004, Franke and Schilcher 2007]. Many important attributes caused that chamomile has applications in pharmacy, nutritional and sanitary industries. Because of this fact estimating genetic similarity and assessment of diversity in population is very important to protect variety of chamomile species. Looking a new sources of diversity among wile genotypes and introduced this diversity into cultivars could enable to obtain plants characterized by higher content of specific for chamomile substances. Molecular markers are very suitable method for estimating genetic diversity among accessions. The number of studies performed with molecular markers on chamomile is very limited. There are only a few publications concerning the use of molecular markers to estimate genetic diversity among chamomile genotypes or to develop markers for some important traits in chamomile genotype [Wagner et al. 2005, Solouki et al. 2008, Okoń and Surmacz-Magdziak 2011]. The aim of the present paper was to estimate genetic diversity in chamomile using ISSR molecular markers and characterization of available chamomile germplasm as well as identification of new diversity in wild genotypes of chamomile.

MATERIALS AND METHOD

Plant material and DNA extraction. 15 genotypes of chamomile: 7 cultivars and 8 wild accessions originated from different European countries, listed in Table 1 were analyzed. Seeds of analyzed genotypes were provided with three different gene banks. Seeds of cultivar 'Złoty łan' were kindly supply by Chair of Industrial and Medicinal Plant University of Life Science in Lublin. DNA was isolated from 20 young plants in two replications, for every genotype. DNA was extracted following the CTAB method described by Doyle and Doyle [1987].

ISSR analysis. PCR were performed according to the ISSR method described by Zietkiewicz et al. [1994] with minor modification. Reaction mixtures contained 1 × PCR Buffer, 130 μM of each dNTP, 470 pM oligonucleotide primer, 1.5 mM $MgCl_2$, 60 ng of template DNA, 1U of Taq DNA Polymerase (Fermentas) in a final reaction mixture of 15 μl. Amplification were carried out in Biometra T1 thermal cycler programmed for 7 min in 94°C of initial denaturation, 3 cycles: 94°C – 54 s, 72°C – 45 s, 3 cycles: 94°C – 53 s, 72°C – 45 s and 32 cycles: 94°C – 52 s, 72°C – 45 s with final extension at 72°C for 7 min. 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 (Ethidium bromide). Fragments were visualized under UV transiluminator and photographed using PolyDoc

Table 1. Chamomlie accessions used in molecular study and their origin

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

System. GeneRuler^{TM} 100bp DNA Ladder Plus was used to establish molecular weight of the products.

Data analysis. ISSR products were scored as present (1) or absent (0) from the photographs. Only bright and reproducible products were scored. Unique ISSR markers were employed to identify chamomile genotypes.

Polymorphic information content (PIC) was calculated by applying the simplified formula [Anderson et al. 1993]: PIC = 2fi(1-fi), where fi is the percentage of the ith amplified band present.

Genetic pairwise similarities (SI-similarity index) between studied 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) in the program NTSYS [Rohlf 2001].

RESULTS AND DISSCUSION

Chamomile genotypes were analyzed using 20 ISSR primers, out of which 5 produced polymorphic and repeatable fragments. In total, primers produced 48 fragments, out of which 41 (85.4%) were polymorphic. The number of polymorphic bands amplified by single primer ranged from 4 to10 with an average 8.2 per primer and 2.7 per genotype (tab. 2). Size of the obtained polymorphic products ranged from 190 to

2200bp. Rahimmalek et al. [2009] used ISSR markers to detect genetic polymorphism in *Thymus daenesis*. Authors chose 15 primers which produced 256 bands, of which 228 (88.9%) were polymorphic. Li et al. [2010] employed ISSR markers to investigate the genetic variability among *Panax ginseng* genotypes. They used 6 selected primers which produced 95 polymorphic bands. Size of the amplified DNA bands was very similar to obtained in the present paper and ranged from 180 to 2000 bp. ISSR primers were also used to assess the genetic diversity in castor (*Ricinus communis* L) genotypes [Gajera et al. 2010]. The 5 selected primers produced 47 bands of which 32 were polymorphic, which an average of 6.4 polymorphic bands per primer. The average percentage of polymorphism obtained by authors was lower than obtained in the present study and reached 68.1%. Sun et al. [2010] analyzed two genotypes of *Schisandra* using ISSR markers. They also found that only a few among tested primers amplified polymorphic products. Author obtained 99 amplicons, out of which 99.32% were polymorphic. These all analyses show that ISSR markers amplify high level of polymorphic bands, even using only a few primers.

Table 2. Characteristic of selected ISSR primers

Primer	Sequence 5`– 3`	Amplified Products	Polymorphic Products	PIC value
SR 14	$(GA)_7YG$	12	10	0.235
SR 16	$(GA)_8C$	12	10	0.275
SR 33	$(AG)_8T$	6	4	0.450
SR 36	$(AC)_8CG$	8	7	0.300
SR 37	$(AC)_8C$	10	10	0.440
Total		48	41	
Per primer		9.6	8.2	
Per genotype		3.2	2.7	

The average PIC value for the amplification products was 0.340 (tab. 2). The highest PIC value showed primer SR 33 (0.450) and the lowest SR 14 (0.235). Similar PIC values (0.314) were obtained by Rahimmalek et al. [2009]. Gajera et al. [2010] obtained higher PIC values which varied from 0.87 to 0.92, with an average of 0.88.

The genetic similarity matrices were produced based on ISSR markers using the Dice's coefficient. ISSR based genetic similarity was estimated between 0.522 and 0.862. The mean genetic similarity was calculated at 0.653. Wagner et al. [2005] estimated genetic similarity among cultivated chamomiles using RAPD and AFLP markers. They identified high level of similarity ranged from 0.68 to 0.95 regarding RAPDs, and from 0.74 to 0.88 for AFLPs. Okoń and Surmacz-Magdziak [2011] analyzed wild and cultivated chamomile genotypes using RAPD method. They found that estimated genetic similarity for cultivated genotypes was quite high. Wild genotypes were character-

ized by higher genetic diversity. Soluoki et al. [2008] analyzed 25 populations of chamomile collected from Iran, Italy, Hungary and Germany. Genetic similarity estimated between analyzed populations was low, and ranged from 0.15 to 0.63, with a mean of 0.35. Analysis conducted by Wagner et al. [2005], Okoń and Surmacz-Magdziak [2011] and results presented in this paper shown that chamomile cultivars characterized high level of similarity. Because of this fact there is necessity to find new sources of diversity in chamomile. Research conducted by Soluoki et al. [2008] showed that the good sources for new diversity could be wild genotypes collected in natural environmental habitats. Okoń and Surmacz-Magdziak [2011] estimated genetic similarity of chamomile genotypes, some of these genotypes were collected from natural habitats. These genotypes were different from other wild genotypes and chamomile cultivars and located in one group on the dendrogramme and this confirms that these genotypes could be good sources of news diversity. Introducing new diversity into cultivars is very helpful in receiving cultivars characterized by higher content of important substances or better agronomic traits.

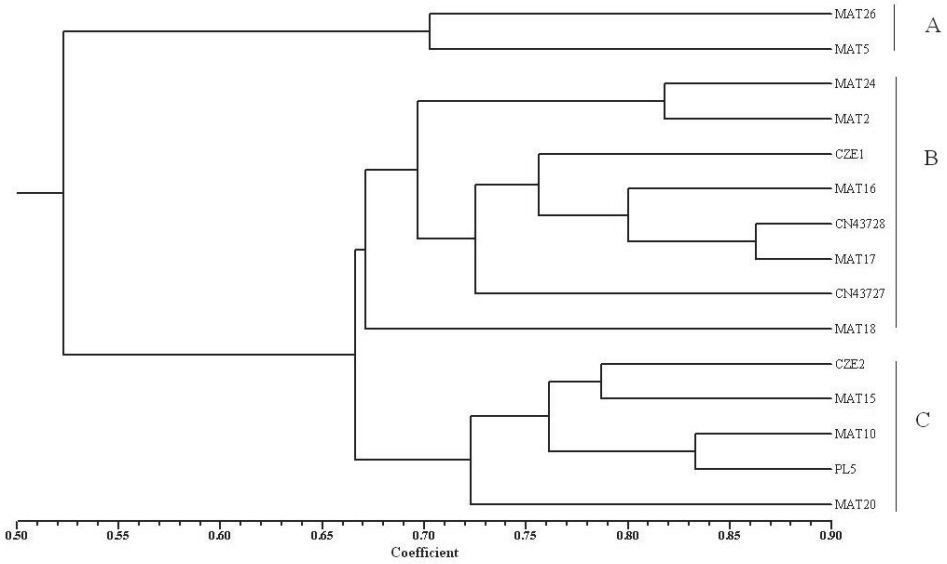

Fig. 1. Dendrogram of chamomile genotypes constructed using UPGMA method

In presented studies genetic similarity matrix was applied for cluster analysis through UPGMA method (fig. 1). The 15 analyzed genotypes were grouped into three major groups. Group A contained two wild genotypes from Austria. The B major cluster contained 6 wild genotypes of chamomile from Germany, Poland, Belgium, Bulgaria and Hungary and two cultivars Bodegold and Quedlinburger Großblütige from Germany. Third major cluster grupped 5 cultivars of chamomila: Bochemia, Pohorelicky Velkokvety, Krajowy, Złoty Łan and ital. Camomilla commune. On the dendrogram

almost all cultivars clustered together on the same group, and wild genotypes were located in another group. Two cultivars from Germany were located in B major group with wild genotypes. It suggested that cultivated genotypes from Germany represented different germplasm than cultivated chamomile genotypes from other countries. The wild genotypes from Austria were the most different and represented different germplasm.

CONCLUSIONS

1. Present study demonstrated that ISSR markers provide a practical and effective method to estimate the genetic diversity among chamomile genotypes both wild and cultivated

2. Analyzed chamomile genotypes characterized quite low genetic variability. Because of this fact there is necessity to find a new source of diversity in chamomile.

3. The most different from other genotypes were wild genotypes from Germany and Austria, and they can be used as new donors of diversity in cultivated chamomile.

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ZRÓŻNICOWANIE GENETYCZNE DZIKICH I UPRAWNYCH FORM RUMIANKU PRZY WYKORZYSTANIU MARKERÓW ISSR

Streszczenie. Celem przeprowadzonych badań była charakterystyka genotypów rumianku wykorzystując markery ISSR. Spośród 20 testowanych starterów ISSR jedynie 5 inicjo-

wało amplifikację polimorficznych i powtarzalnych produktów. Łącznie uzyskano 48 fragmentów, z których 41 (85,4%) było polimorficznych. Średnia wartość PIC dla uzyskanych produktów amplifikacji wynosiła 0,340. Wykorzystując markery ISSR, utworzono matryce podobieństwa genetycznego. Średnia wartość podobieństwa analizowanych genotypów wynosiła 0,653. Przeprowadzone badania potwierdzają przydatność metody ISSR do oceny podobieństwa genetycznego rumianku. Analizowane genotypy charakteryzowały się wysokim podobieństwem genetycznym, co wskazuje na konieczność poszukiwania nowych źródeł polimorfizmu wśród dzikich gatunków, w celu poszerzenia zmienności genetycznej uprawnych form rumianku.

Słowa kluczowe: *Chamomilla recutita* (L.) Rausch., podobieństwo genetyczne, ISSR

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