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MOLECULAR DIVERSITY ANALYSIS OF HUNGARIAN APRICOT (*Prunus Americana* L.) VARIETIES BASED ON INTER-PRIMER BINDING SEQUENCE (iPBS) MARKERS

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Abstract. In this study, iPBS markers were used for assessing genetic diversity and determining the relationship among apricot cultivars. Six apricot genotypes from Hungary were examined with 11 iPBS retrotransposon markers through PCR amplification. The results show that 86 amplified bands were scored with an average of 8.6 amplicons for each primer. The number of polymorphic bands ranges from 0 to 4, averaged at 1.6. The percentage of the polymorphic band varies from 0 to 40.0%, with an average of 18.6%. The polymorphic information content ranges from 0.00 to 0.17, with a mean of 0.06. The resolving power varies from 0.00 to 1.67, with an average of 0.63. iPBS markers can differentiate only the MK135 and Veecot genotypes. The remaining Magyar kajszi apricot genotypes could not be determined by using iPBS markers.

Keywords: PCR, apricot, iPBS, *Prunus Americana* L.

1 Introduction

The apricot (*Prunus Americana L.*) is a member of the *Rosaceae* family and the *Prunus* genus. Apricot is a diploid species with eight chromosomes ($2n$ = 16) [1]. This fruit cultivar is hermaphrodite and mostly self-fertile [2]. From prehistoric cultivation, apricots were suspected to be native to China [3, 4]. However, some argue that apricot originated from Armenia because of several hundred years of cultivation in this area [3, 5]. The origin of the 'apricot' name from Armenia is based on the early times; the Romans called the apricot tree Armeniaca (i.e., the tree of Armenia) [6]. There exist six subspecies of apricot: *P. brigantine*, *P. armeniaca*, *P. mume*, *P. dasycarpa*, *P. sibirica*, and *P. mandshurica* [1]. Depending on the diversity of climates, there are four major eco-geographical groups of apricots: the Central Asian group with five regional subgroups: Fergana, Zeravshan, Shakhrisyabz, Khorezm, and Kopet-Dag; the Iranian-Caucasian group; the European group with western, eastern, and northern subgroups, and the Dzhungar-Zailij group linked to the wild Tien-Shan group [7].

Most of the varieties cultivated in Hungary are from the Middle East and bring the original basic material for domestic apricot cultivation. In Hungary, apricot cultivation is restricted to some specific areas because of poor ecological adaptableness and sensitivity to frost damage.

According to STADAT [8], 10,623 tons of apricot were produced in 2020, and the average yield was 1,790 kg/ha. Depending on the cultivar and weather, Hungarian apricots flower in March, April, and sometimes May. The most important traditional cultivars include 'Gönci Magyar kajszi', 'Ceglédi óriás', and 'Bergeron' [9], and the commercial cultivars include: 'Ceglédi arany', 'Ceglédi kedves', and 'Ceglédi piroska' from Cegléd; 'Pannónia' from Érd; 'Harmat' and 'Korai zamatos' from Budapest [9].

Numerous studies have been carried out for the determination of the genetic diversity of apricot. Traditional methods for identifying cultivars and rootstock in fruit trees are based on phenotypic traits [10]. However, the slow approach and environmental effects of a huge number of fruit trees and long generations are severe drawbacks of this differentiation method [11]. Because of the adaptation to various climate conditions, apricot can contain several clones that differ in minor genetics and phenotypic characteristics [12]. For this reason, the molecular marker is proper for cultivar identification. A variety of molecular markers has been applied to identify genetic diversity and the relationship among apricots. Simple Sequence Repeat (SSR) and RAPD are widely applied in the genetic variation analysis of apricots. AFLP and SRAP are also utilized. Nevertheless, inter-primer binding sequence (iPBS) application is still limited. A previous study by Baráneka et al. [11] on 22 iPBS primers for apricots showed a high coefficient of similarity, but the value was the lowest among the three marker systems studied.

The inter-primer binding sequence is a method based on isolation and displays the polymorphism of retrotransposon. The retrotransposon-based fingerprint system can clarify the insertion of hundreds to thousands of long nucleotides. Retrotransposon integration sites represent the joints between the conserved long terminal repeat ends and flanking, essentially random genomic DNA [12]. This iPBS marker can describe the polymorphic fingerprint for species in the plant kingdom, including angiosperms, gymnosperms, and some lower plants [11]. However, genetic diversity analysis with iPBS markers is applied to various species like yeast, grapevine, bamboo, and yacon. The iPBS primer is designed on the conserved parts of the primer binding site (PBS) sequences for tRNAs for reverse transcription during the replication cycle for the retrotransposons [13]. The iPBS marker method differs from earlier-utilized retrotransposon techniques because it can be applied to endogenous retroviruses and both *Gypsy* and *Copia* long terminal repeat retrotransposons and to other retrotransposon-based elements [12]. In this study, the iPBS marker method was used for assessing genetic diversities. Furthermore, the relationship between cultivars was determined with the examination of iPBS markers on genetic variance analysis in apricots.

2 Material and methods

2.1 Plant material and DNA extraction

Apricot accessions were collected from Research Stations in Cegléd NARIC Fruit Culture Research Institute in Hungary. All accessions were fresh, young leaf tissues. Each cultivar was collected with different accessions in different locations to ensure that the hypothesis of the DNA sample was not affected by environmental factors. The Veecot cultivar was the control. Table 1 lists the detailed information of each accession with the varieties name of six apricot cultivars. DNA was extracted from fresh, young leaf tissues according to NucleoSpin® Plant II protocols (Macherey-Nagel) with PVP (polyvinylpyrrolidone). The estimation of DNA concentration was conducted with gel electrophoresis in 2% agarose gel and stained with ethidium bromide.

2.2 iPBS-PCR amplification

Eleven iPBS retrotransposon primers designed by [14] that passed the test of application in apricot varieties were used in the present study (Table 2). Strong and clear amplified primers were selected for the evaluation of genetic diversity.

PCR amplification was performed in the Cycler equipment (BioRad). The 20 μL mixture contained 2 μL of template DNA, 0.4 μL of 25 mM $MgCl_2$, 2 μL of 1 X Dream Taq Green Buffer (Fermentas), 10.5 μL of sterile water, 0.1 μL of 1 X Dream Taq DNA Polymerase, 2 μL of 0.2 mM dNTP (Fermentas), and 3 μL of 1 μM primers. The profile for PCR thermal cycling was as follows: initial denaturation at 95 °C for 3 min; 30–35 cycles of 95 °C for 15 s; 53–57 °C (annealing temperature depending upon primers, Table 2) for 1 min; 68 °C for 1 min; and final extension at 72 °C for 5 min [14]. PCR products, after cycling, were separated in 2% (w/v) agarose gel in 0.5 × TBE buffer at 90 V, stained with EtBr (Ethidium Bromide) and photographed in a UV transilluminator (ChemImage Corporation, USA).

Table 1. Name of apricot cultivar for DNA extraction

Number	Cultivar Name
1	Gönci magyar kajszi C.381
2	Nagygyümölcsű magyar kajszi C.383
3	MK135 C.384
4	Magyar kajszi C.256
5	Magyar kajszi alapfajta (magyar kajszi C.235)
6	Veecot (Control)

Table 2. List of iPBS primers, their annealing temperature and sequence [14]

Designation of primers	Annealing temperature (°C)	Sequencing of primers
2085	53	ATGCCGATACCA
2222		ACTTGGATGCCGATACCA
2226		CGGTGACCTTTGATACCA
2230		TCTAGGCGTCTGATACCA
2251		GAACAGGCGATGATACCA
2401		AGTTAAGCTTTGATACCA
2272	55	GGCTCAGATGCCA
2232		AGAGAGGCTCGGATACCA
2237		CCCCTACCTGGCGTGCCA
2220	57	ACCTGGCTCATGATGCCA
2221		ACCTAGCTCACGATGCCA

2.3 Data analysis

The amplified bands were scored as 1 (present) or 0 (absent). The clear, repetitive, and well-separated bands were selected for scoring. The total band, polymorphic band, and the percentage of the polymorphic band were numbered and calculated.

For the evaluation of marker effectiveness, the polymorphic information content (PIC) was calculated according to [15] $IC = 1 - (f^2 + (1 - f))^2$, where f is the frequency of the marker. The resolving power was calculated by using a formula according to [16] $p = \sum Ib$, where Ib is the informative band calculated from formula $Ib = 1 - (2 * |0.5 - p|)$, where p is the frequency of varieties containing band I. The average of the informative band (AvIb) was calculated by averaging the informative bands in each marker.

3 Results and discussion

Fig. 1 shows the molecular characterization of the six cultivars with eleven iPBS retrotransposon primers. The amplification was successful with ten iPBS retrotransposon primers: 2085, 2222, 2230, 2251, 2401, 2272, 2232, 2237, 2220, and 2221. Only the 2226 marker did not work in the case of apricot samples.

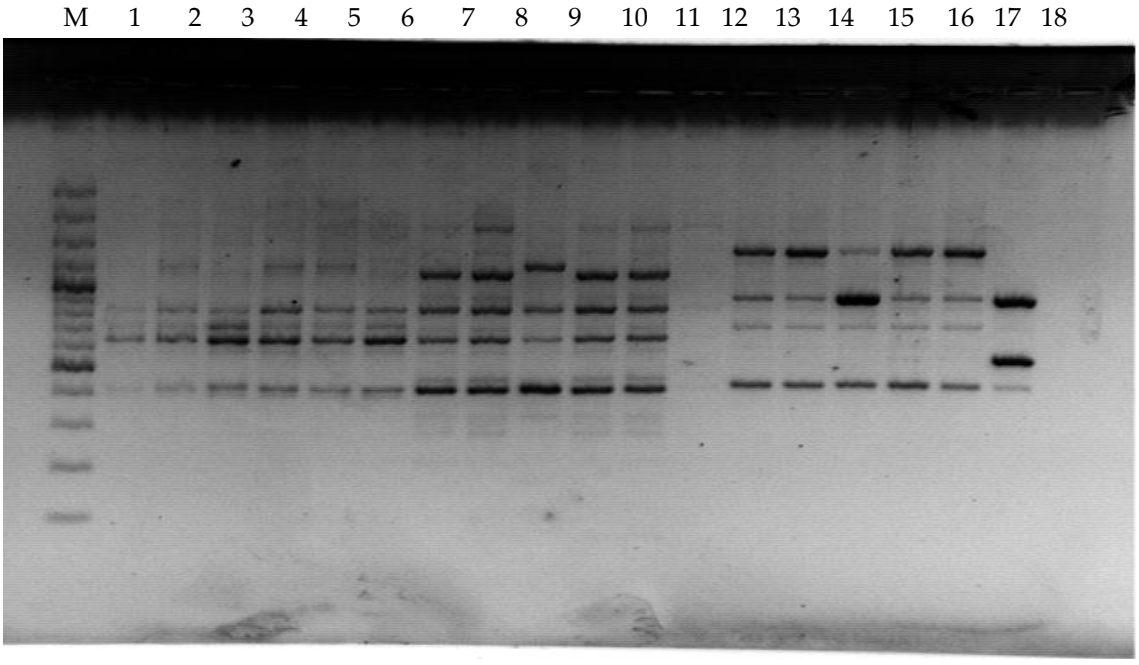

Fig. 1. Amplification profile of different iPBS markers in six apricots genotypes

Notes: 1. iPBS 2230: Gönci magyar kajszi C.381; 2. iPBS 2230: Naggyümölcsü magyar kajszi C.383; 3. iPBS 2230: MK135 C.384; 4. iPBS 2230: Magyar kajszi C.256; 5. iPBS 2230: Magyar kajszi alapfajta; 6. iPBS 2230: Veecot; 7. iPBS 2251: Gönci magyar kajszi C.381; 8. iPBS 2251: Naggyümölcsü magyar kajszi C.383; 9. iPBS 2251: MK135 C.384; 10. iPBS 2230: Magyar kajszi C.256; 11. iPBS 2251: Magyar kajszi alapfajta; 12. iPBS 2251: Veecot; 13. iPBS 2401: Gönci magyar kajszi C.381; 14. iPBS 2401: Naggyümölcsü magyar kajszi C.383; 15. iPBS 2401: MK135 C.384; 16. iPBS 2401: Magyar kajszi C.256; 17. iPBS 2401: Magyar kajszi alapfajta; 18. iPBS 2401: Veecot M: 100 bp Ladder + (plus) Molecular weight marker.

A total of 86 amplified bands were scored, and thus 8.6 amplicons were amplified with each primer. The total band of each primer varied from 6 (2230, 2237) to 10 (2085, 2251, 2272, 2232, 2221). The number of polymorphic bands ranged from 0 to 4, and the mean number of polymorphic bands per genotype was 2.6. The percentage of polymorphic bands varied from 0 (2230, 2272) to 40% (2232), with an average of 18.6%. A contrasting result was reported by Baráneka et al. [11] with 157 polymorphic bands amplified, with an average of amplified amplicons of 7.5. These differences could be attributed to the different iPBS marker contributions and different numbers of genotypes used in the studies. However, according to Baráneka et al. [11], polymorphism among the analyzed clones is rare, which is similar to this study. These authors also mentioned that the reason for low polymorphism is due to the lower number of evaluated products generated by iPBS compared with other markers like SSAP or AFLP.

Table 3. Polymorphic information content (PIC), average of informative band (AvIb) and resolving power (Rp) of iPBS markers

Primers	Total band	Polymorphic band	Percentage Polymorphic band (PPB%)	Polymorphic information content (PIC)	Average of informative band (AvIb)	Resolving power (Rp)
2085	10	2	20.0	0.06	0.07	0.67
2222	8	1	12.5	0.03	0.04	0.33
2230	6	0	0.0	0.00	0.00	0.00
2251	10	3	30.0	0.17	0.17	1.67
2401	7	2	28.6	0.07	0.10	0.67
2272	10	0	0.0	0.00	0.00	0.00
2232	10	4	40.0	0.11	0.13	1.33
2237	6	1	16.7	0.05	0.06	0.33
2220	9	2	22.2	0.06	0.11	1.00
2221	10	1	10.0	0.03	0.03	0.33
Average	8.6	1.6	18.6	0.06	0.07	0.63

For further evaluation of the performance of iPBS markers and assessment of the genetic diversities among the genotypes, the polymorphic information content, an average of the informative band (AvIb), and resolving power (Rp) were calculated. The PIC values were used to estimate the ability of discrimination and to measure the efficiency of polymorphic genotypes in discriminating genetic diversity among the cultivars **[**14]. The highest PIC value (0.17) is from iPBS primer 2251, and the zero PIC value is from iPBS primers 2230 and 2272. The average PIC value is 0.06, indicating insufficient information between these genotypes and those of other studies about genetic diversity analysis with iPBS markers in grape (0.445) [13] or rice (0.35) [17]. The resolving power calculated through the total of informative bands in one marker reflects the number of polymorphic bands in the pattern and the value of the polymorphic band. In durum wheat studies, the resolving power was found to be more suitable to describe the discriminatory ability of primers in a diversity study than PIC [18]. The highest Rp value is 1.67 with an average of informative bands of 0.17 (2251), and the lowest Rp is zero with an average of informative bands of zero (2230, 2272). In contrast to other studies on genetic diversity in grapes [13], the AvIb and Rp values show a low level of polymorphism. Both Rp and PIC show low polymorphism in this study. No studies in apricot genetic diversity calculate the polymorphic information content, an average of informative bands and resolving power because of the low polymorphism of this marker on apricots. iPBS markers cannot be utilized for the rapid genetic diversity analysis studies of Hungarian apricot cultivars.

4 Conclusion

'Gönci Magyar kajszi,' and 'Ceglédi óriás,' are two classic Hungarian apricot cultivars. In this study, iPBS markers were used for assessing genetic diversity and determining the relationship between apricot cultivars. The results show that only MK135 C.384 and Veecot were discriminated. Gönci magyar kajszi C.381, Naggyümölcsü magyar kajszi C.383, Magyar kajszi C.256, and Magyar kajszi alapfajta (magyar kajszi C.235) were not discriminated. Furthermore, the 18-mer iPBS primer was more efficient than the 12-mer iPBS primer. However, owing to a large number of 18-mer iPBS primers (8) compared with 12-mer iPBS primers (2) used in this study, the effectiveness of the 12-mer iPBS primers in the apricot genetic analysis could not be concluded. In the future, different molecular markers would be applied to Magyar kajszi apricot genotype analysis, and for that reason, it was expected to obtain more information on genetic diversity and relationships in plant phylogenetic analysis as well as plant breeding.

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