Identification of DNA Fingerprint Pattern Variability of Known Retrotrasposons in *Plantago lanceolata* L.

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Abstract
Plantago lanceolata L. belongs to important medical plants and is numerously represented in the plant communities of meadows, fields, pastures and permanent grasslands. Here, analysis of the presence of selected transponable elements sequences in the genome of *Plantago lanceolata*, L were performed. Retrotransposon are an inevitable part of plant genomes are one of the DNA markers that possess good amplification profiles useful for variability analysis. Known sequences of retrotransposons Tst-1, Cassandra, BARE-1, FaRe-1 and HACRE1 were used for a total of 10 specific primer designation and IRAP analysis as well as selected iPBS markers. The presence of the known retrotransposon sequences were confirmed by PCR analysis with different polymorphic potential. A total of 22 different iPBS markers were used from which 15 were confirmed to amplify polymorphic fingerprint patterns. Comparing obtained fingerprint profiles, iPBS markers resulted in more abundant profiles with higher potential to detect intraspecific differences for *Plantago lanceolata* L.

Keywords: *Plantago lanceolata*, iPBS, retrotransposons

1. Introduction

The plantain is one of the medicinal plants actively used in pharmacy. It contains many medicinal substances which include glycosides, flavonoids, enzymes, pectins, mucilage, tannins, bitter substances, ascorbic acid and saponins. Plantain further contains minerals such as silicic acid and salts of calcium and potassium (Mika, 1991). The most important active substances from a medicinal point of view include glycosides, flavonoids and mucilage.

Up to now, different DNA based markers were utilized in studies of *Plantago lanceolata* L., such as AFLP (Amplified Fragment Length Polymorphism) or MSAP (Methylation-Sensitive Amplification Polymorphism) [1]. To our knowledge, no retrotransposon based markers were used in the studies of platain. Retrotransposons are multiple fragments of DNA that are capable of moving in the genome. Their genetic information encodes proteins essential for enzymatic retrotransposon intracellular and extracellular domains [2]. Retrotransposons are divided into two basic types, namely retrotransposons with long-terminal repeats (LTR) and retrotransposons without LTR. The size of LTR can range from 100 bp to 5 kbp. For classification of LTR retrotransposons, they were categorized according to length, gene order, and sequence similarity [3].

In plants, different retrotransposons are very abundant, what provide them as very good markers for analysis of genetic variability. The Cassandra retrotransposon has been identified in more than 50 species of the plant kingdom. It was firstly isolated from the *Prunus domestica*.

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Cassandra retrotransposons have a size of 565 to 860 bp with different lengths of LTRs [4]. Retrotransposons of the BARE group were firstly isolated from barley [5]. Among the most common retrotransposons of this group is the BARE-1 retrotransposon [6], that actively generates new insertion variation and thus determines the structure of the genome.

DNA markers derived from retrotransposon sequences are reported often as to be very close to the ideal markers, because of retrotransposon characteristics, as they possess the high number of copies in genomes and are situated on the various locations with the well accessibility to analyse length polymorphism between species or inside of species. Retrotransposons are often chosen in the phylogenetic relationships studies and genetic variability analyses and the most frequently used retrotransposon based molecular marker methods are IRAP (Inter-Retrotransposon Amplified Polymorphism), REMAP (REtrotransposon-Microsatellite Amplified Polymorphism) [7,8] and iPBS (Inter Primer-Binding Sites Polymorphism) [9].

The aim of the study was identification of five known retrotransposon sequences (Tst-1, Cassandra, Bare-1, FaRe-1 and Hacre-1) in the genome of *Plantago lanceolata*, L. by IRAP technique and verification of iPBS technique to generate abundant fingerprints in this specie.

2. Materials and methods

Plant material

Young healthy leaves of *Plantago lanceolata*, L. were collected *in situ* in the area of Zobor, Nitra. Immediatelly after the collection they were treated with etanol for the purpose of the surface desinfection. When transporting them to the laboratory, they were stored under the -20°C until the further processing. Genomic DNA extraction was performed by GeneJET Plant Genomic DNA kit (ThermoScientific) according the manufacturer instructions. Quantity and quality of extracted DNA was checked by NanoPhotometer P-Class (Implen).

Retrotransposon marker techniques and PCR protocols

Primers used in the study were as folows:

Tst-1 IRAP – 5′ ATG ACT AAA TCT GCC TAC TCA TTC AAC A 3′

Cassandra IRAP – 5′TGT AAC ACC CCG TAC TTT TC 3′

Bare-1 IRAP – 5′ TTT TTG TTT CCC ATG GGA CGT TCC CCA ACA 3′

FaRe-1 IRAP – 5′ TGG TTT CAC GCT TTG GCA GAG GA 3′

Hacre-1 IRAP – 5′ GTT TGA GAC GCG AAT GGG CGC G 3′

A total of 22 iPBS primers were screened for their ability to amplify fingerprints in the genom of plantain – 1838, 1854, 2270, 1899, 2279, 2274, 2039, 2036, 2042, 2027, 2019, 2032, 1826, 1830, 1833, 1846, 1879, 1881, 1886, 1892, 2380, 2374 [9].

After optimization of PCR parameters of IRAP and iPBS, following protocols were used with 80 ng × microL^{-1} and with final concentrations of primers 1200 nmol × microL^{-1} for IRAP and 900 nmol × microL^{-1} for iPBS:

Tst-1 IRAP – 94°C - 2 min; 40 cycles of: 94°C 60 s; 55 °C 60 s; 72°C 180 s; final 72°C 10 min;

Cassandra IRAP – 95°C - 3 min; 35 cycles of: 95°C 30 s; 55°C 40 s; 72°C 120 s; final 72°C 5 min;

Bare-1 IRAP – 95°C - 3 min; 40 cycles of: 95°C 45 s; 58°C 60 s; 72°C 120 s; final 72°C 10 min;

FaRe-1 IRAP – 95°C - 3 min; 45 cycles of: 95°C 45 s; 60°C 60 s; 72°C 120 s; final 72°C 10 min;

Hacre-1 IRAP – 95°C - 3 min; 35 cycles of: 95°C 30 s; 62°C 40 s; 72°C 120 s; final 72°C 10 min;

iPBS - 95°C – 3 min; 45 cycles of: 95°C 30 s; 55°C 40 s; 72°C 120 s; final 72°C 5 min.

Data analysis

PCR products were separated in 2% (w/v) agarose gels in 1 × TBE buffer. Gels were stained by GelRed™ and digitally photographed. All the accessions were growth and sampled through the two seasons to ensure the stability of the markers and all the PCR amplifications were repeated at least twice to establish reproducibility of polymorphic fragments and scored independently by KODAK EDAS software.

3. Results and discussion

In this study, five different retrotransposons were screened for their presence in the genome of plantain by IRAP technique.

In the analyses for the presence of BARE-1 retrotransposon sequences, amplification of the

fragments was successful and a total of nine amplicons were obtained (figure 1).

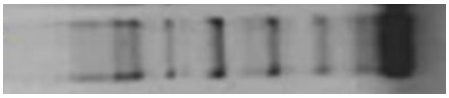

Figure 1. Amplification profile of IRAP fingerprint for Bare-1 retrotransposon in *Plantago lanceolata*, L.

In the analyses for the presence of Cassandra retrotransposon sequences, amplification of the fragments was successful and a total of eleven amplicons were obtained (figure 2).

Figure 2. Amplification profile of IRAP fingerprint for Cassandra retrotransposon in *Plantago lanceolata*, L.

In the analyses for the presence of Tst-1 retrotransposon sequences, amplification of the fragments was successful, but here, only four amplicons were obtained (figure 3).

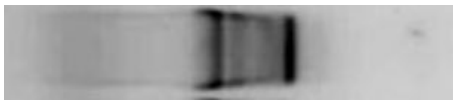

Figure 3. Amplification profile of IRAP fingerprint for Tst-1 retrotransposon in *Plantago lanceolata*, L.

Analyses of Hacre-1 retrotransposon by IRAP technique returned no successful amplification in any of tested annealing temperatures in the optimization reactions of PCR.

In the analyses for the presence of FaRe-1 retrotransposon sequences, amplification of the fragments was successful with nine amplified fragments visible in agarose gel (figure 4).

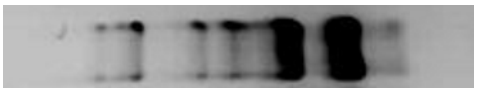

Figure 4. Amplification profile of IRAP fingerprint for FaRe-1 retrotransposon in *Plantago lanceolata*, L.

Retrotransposons are abundant, dispersed, ubiquitous, persistent, active, and are in high copy numbers in the plant genomes, what resulted in many retrotransposon-based markers that have been developed [10]. This technique has been reported to evaluate the insertional polymorphism and genetic diversity in several crop species such as rice [11], banana [12,13], masson pine [14], alfalfa [15], cowpea [16], sunflower [17], cotton [18], wheat [19], mung bean [20], bean [21] or sweet orange [22].

On the other hand, IRAP markers are specific ones with different levels of possible transferability. Retrotransposons tend to transfer between closely related as well as distantly unrelated species by mode of vertical and horizontal transmission by crossing the species' fencing [23].

In the iPBS analyses, six of screened primers returned no amplification profile (table 1).

Table 1. Ability of amplification of iPBS fingerprints in the genome of *Plantago lanceolata* L. for markers used in this study.

iPBS marker	amplification profile
1838	+
1854	+
2270	+
1899	+
2279	+
2274	+
2039	+
2036	-
2042	+
2027	+
2019	+
2032	-
1826	+
1830	+
1833	+
1846	-
1879	+
1881	-
1886	+
1892	-
2384	+
2374	-

Different numbers of amplified fragments were obtained by individual iPBS primers from three up to fifteen (figure 5) with variable reproducibility in repeating amplifications (figure 6).

The method of iPBS was succesfully used for analysis in many different plant species such *Linum ussitatisimum* [24], *Saussurea esthonica* [25], *Liparis loeselii* [26] or *Prunus armeniaca* [27] where was prooved as reliable DNA marker system. iPBS technique is used for both –

isolation of long terminal repeats retrotransposons as well as an efficient and a general marker system. Kalendar et al. [9] has reported it as suitable for the universal use for both retroviruses and LTR retrotransposons. The method is applicable to any organism with retrotransposons containing primer binding sites that are complementar to the tRNA.

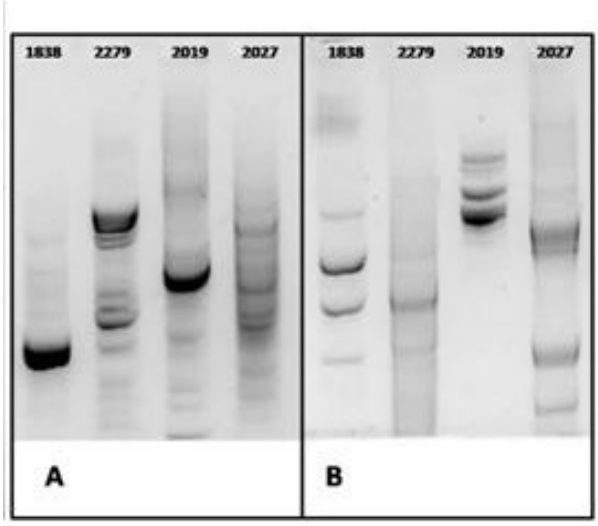

Figure 5. Amplification profile of selected iPBS markers in repetitive analysis of *Plantago lanceolata*, L.

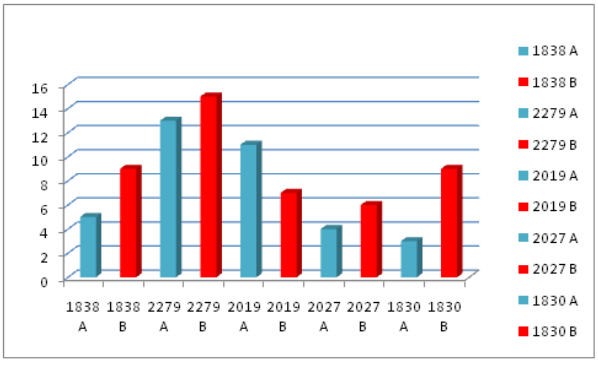

Figure 6. Numbers of obtained fingerprint profiles of selected iPBS markers in repetitive analysis of *Plantago lanceolata*, L.

Ones the specific iPBS primers are selected from the universal set for the particular organism, the method becames an effective and reproducible one. This represent its main pros when comparing it to the unspecific length polymorphism techniques sucha s RAPD (Randomly Aplified Polymorphic DNA) or ISSR (Inter Simple Sequence Repeats polymorphism) [28].

Along with the iPBS, another retrotransposon based DNA markers such as IRAP, REMAP, RBIP or SSAP are stil used widely for the analysis of genetic relationships [29-31].

In Plantago spp, ISSR markers were previously succefuly used to estimate the genetic structure and population differentiation in *Plantago brutia* [32]. Five ISSR primers were utilized to analyze five *Plantago* species collected from different regions of Saudi Arabia [33]. The ability of ISSR markers for detection of genetic diversity of *P. almogravensis* and *P. algarbiensis* populations and analysis of polymorphic information of ISSR were proved as affective in the analysis [34].

Here, both of the techniques, IRAP and iPBS were confirmed as transferable DNA markers for variability analysis of *Plantago lanceolata*, L.

4. Conclusions

For the purposes of molecular analysis of *Plantago lanceolata*, L., two retrotransposon marker techniques were evaluated in this study – IRAP and iPBS. Based on the type of the used technique, different results were obtained in their ability to amplified length polymorphism amplicons by PCR. The most stable technique that have the potential for the purpose of *Plantago lanceolata*, L. population studies were iPBS and IRAP technique for Cassandra retrotransposon.

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