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Research Article

# Analysis of Inter-Primer Binding Site Retrotransposon Length Polymorphism in Selected Group of *Vaccinium corymbosum*

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The genetic variability in 13 selected genotypes of *Vaccinium corymbosum* using re-trotransposon-based iPBS markers system was characterized in this study. The four selected iPBS primers amplified 232 fragments, average 58 fragments per primer, approximately 53.16% of which were polymorphic. The polymorphism information content of iPBS marker was 0.238. The hierarchical cluster using unweighted pair group method with arithmetic mean analysis divided selected genotypes into two main groups and several subgroups. The values of the Jaccard coefficient of genetic relatedness were between 0.036–0.243. The similarity between two sample sets was 3.6%–24.3%. This study confirmed inter-PBS amplification technique as a reproducible and trustable marker system, but has proved to be an effective DNA fingerprinting technology to generate polymorphism in *Vaccinium corymbosum*.

**Keywords:** *Vaccinium corymbosum*, DNA polymorphisms, molecular marker

## 1 Introduction

Blueberries, scientifically known as *Vaccinium corymbosum*, are commonly referred to as highbush blueberries, and they belong to the family Ericaceae (Galletta, 2023) Blueberries, cranberries, and lingonberries – Google Scholar, n. d.). Blueberries are often considered as a 'superfruit' (Kalt et al., 2020), because they are rich in various nutrients and antioxidants, including vitamins (mainly vitamin C and vitamin K), minerals (such as manganese), dietary fiber, and various phytochemicals, including polyphenolic and anthocyanin compounds possessing high antioxidant capacity (Herrera-Balandrano et al., 2021). The antioxidant capacity found in this plant indeed offers a range of health benefits, including anti-inflammatory effects, combating oxidative stress, reducing the risk of chronic diseases, improving immune function, improving digestion, and promoting healthy aging (Rashidinejad, 2020).

Blueberry cultivars, also known as varieties, have been identified primarily based on their physical or morphological traits, including leaf shape, fruit size, and colour. However, this method has limitations, as these characteristics can be influenced by environmental conditions and agricultural practices (Aruna et al., 1993). Accurate identification of cultivars is important for practical breeding efforts aimed at developing new and improved blueberry varieties, and using a DNA marker system is a very common method to determine genetic diversity (Collard et al., 2005). Molecular markers based on DNA are ef-fective tools for genetic identification of blueberry cultivars and provide benefits such as significant polymorphism and independence from environmental factors (Spooner, 2005). Various genetic studies on *V. corymbosum* have utilized a range of molecular markers, including randomly amplified polymorphic DNA (RAPD) (Debnath, 2005; Levi & Rowland, 1997; Martinez et al., 2007; Matsumoto, 2019), microsatellite markers or simple sequence repeat (SSR)

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(Bassil et al., 2020; Bian et al., 2014; Liu et al., 2014; Rowland et al., 2014), and in-ter-simple sequence repeats (ISSR) (Garriga et al., 2013) primers. RAPD is a simple and cost-effective DNA marker widely used for rapid plant genotype identification. ISSR markers are considered more efficient and reliable than RAPD due to longer primer sequences and higher annealing temperatures, resulting in higher specifity (Tikendra et al., 2019). In another side, these markers belong to dominant markers, that mostly target non-coding regions of the genome (Wolfe & Liston, 1998). One of the functional markers located in or near candidate genes is iPBS. iPBS markers are described as identifying diverse LTR (long terminal repeats) sequences.

The identification is achieved by utilizing PBS (primer binding site), a conserved sequence located adjacent to the 5′LTR (Kalendar et al., 2010). These studies have played a significant role in tasks such as mapping, analyzing genetic diversity, and creating DNA fingerprints of blueberry cultivars.

In this study, we employed Inter-Primer Binding Site (iPBS) molecular markers to evaluate genetic diversity within a population of blueberries. This information could be valuable for conservation efforts and for identifying genetic resources for breeding programs. iPBS markers are specifically designed to target retrotransposons, which are repetitive DNA sequences (Kalendar et al., 2010). Compared to RAPD markers, iPBS markers are known for their higher specificity, reproducibility, and the ability to amplify specific genomic regions (Amom et al., 2020). iPBS markers have been valuable tools for a range of genetic and genomic investigations (Aydın et al., 2020; Başak et al., 2022; Guo et al., 2014; Özer et al., 2016; Žiarovská, 2022).

## 2 Material and Methods

### 2.1 Plant Material

The plant material for this study consists of twelve accessions of *V. corymbosum* and one accession of *V. myrtillus* sourced from local stores. These accessions originated from seven different locations (not specifically described) around the world, which were specified by distributor. The geographic locations include Slovakia, Argentina, Spain, Italy, South Africa, Chile, Peru, and Poland (Table 1). The study included sample no. 13, another species *Vaccinium myrtillus* from Slovakia, serving as a control for species differentiation using iPBS profiles.

**Table 1** Genotypes codes and locations

| Code | Location | Code | Location |
|---|---|---|---|
| **1** | Argentina | **8** | Chile |
| **2** | Spain | **9** | South Africa |
| **3** | Peru | **10** | Poland |
| **4** | Italy | **11** | Slovakia |
| **5** | Argentina | **12** | Peru |
| **6** | Poland | **13** | Slovakia |
| **7** | Poland | | |

### 2.2 DNA Extraction

DNA was extracted from the fruit using the EliGene Plant DNA Isolation Kit following the manufacturer's protocol. Extracted DNA was diluted and stored at -20 °C. The quality and quantity of the isolated genomic DNA was determined spectrophotometrically using a NanoPhotometerTM (IMPLEN), and the quality of DNA was also assessed by ITS-PCR.

### 2.3 PCR and iPBS Performing

After screening twenty-one primers, four highly polymorphic iPBS primers were selected for use in the polymerase chain reaction (PCR). The chosen primers were obtained from the study conducted by Kalendar et al. (2010). The PCR amplification was carried out in the total reaction volume of 10 µL, consisting of 2 µL of genomic DNA (10–24 ng), 800 nM of both forward and reverse primers, 5 µL of 2x EliZymeTM HS Robust Mix, and 2.2 µL of PCR-grade water. The PCR protocol consisted of an initial denaturation at 95 °C for 3 min, followed by 35 cycles at 95 °C for 30 s, an annealing temperature of 55 °C for 30 s, and polymerization at 72 °C for 2 min. A final extension step was performed at 72 °C for 5 min. The PCR products were separated on 2% agarose gel by electrophoresis. The products were stained with the intercalating dye GelRed (Biotium) and visualized under the UV light using a BioDocAnalyzer Box 2 (Biometra).

### 2.4 Data Processing and Statistical Analysis

The scoring of PCR products was conducted using a binary system (0 for absence, 1 for presence) via GelAnalyzer (2023) software (GelAnalyzer, n.d.). The results were analysed using the free software DendroUPGMA (2023) (DendroUPGMA: Dendrogram construction using the UPGMA algorithm, n.d.) to prepare a UPGMA dendrogram, and the genotypes were grouped into clusters based on the Jaccard coefficient. The Marker Efficiency Calculator (iMEC) (Amiryousefi et al., 2018) web-based tool was used to compute the polymorphic information content, marker index, and resolving power.

## 3 Results and Discussion

Numerous studies have confirmed that the iPBS marker system offers reproducibility and efficiency when applied to genomic analyses in plants. As a result, this marker system is widely used in various plant species, including Fagaceae (Coutinho et al., 2018), *Vitis vinifera* (Žiarovská, 2022), Peruvian rosewood (Baloch et al., 2022), *Carthamus tinctorius* (Ali et al., 2019), *Solanum andigenum* (Demirel et al., 2018), *Chenopodium quinoa* (Barut et al., 2020), Laurus nobilis (Karık et al., 2019) and in many other studies. This study is the first report to investigate genetic diversity in *Vaccinium corymbosum* using iPBS markers. The results of our analysis revealed valuable insights into the genetic variation within this species. In our iPBS marker analysis, these four primers generated the total of 232 scorable bands. The number of alleles observed in the primers ranged from 47 (iPBS 1,846) to 67 (iPBS 1,867), with an average of 58 alleles per primer, and 124 (53%) of these bands were polymorphic. The average number of polymorphic bands per primer was 31. Among the primers, primer iPBS 1,846 displayed the highest level of polymorphism at 67.27%, while primer iPBS 1,882 exhibited the lowest, with only 36.51% polymorphism. The average of polymorphic information content (PIC) was 0.238, ranging from 0.203 (iPBS 1,867) to 0.258 (iPBS 1,882). The low value of PIC suggests a narrow range of polymorphism at the level of retrotransposons in the blueberry genome. The resolving power of the primers range from 14,308 (iPBS 1,846) to 22,154 (iPBS 1,882) (Table 2).

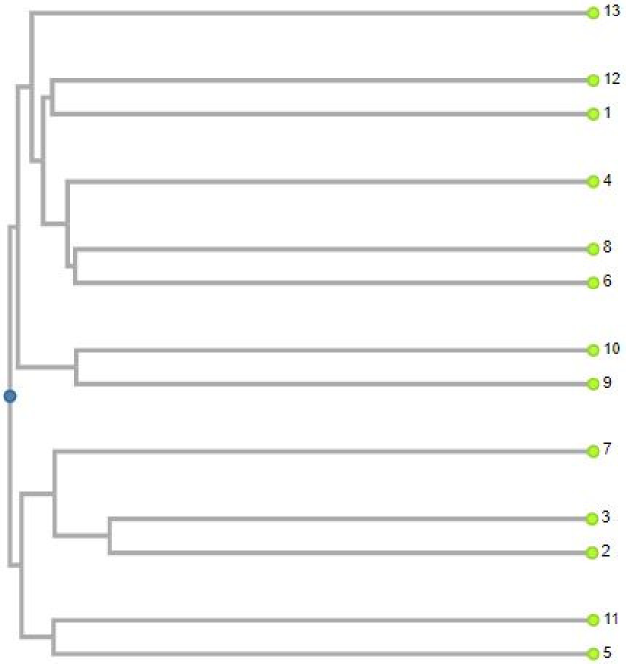

**Figure 1** Dendrogram obtained from Unweighted Pair-Group Method with Arithmetic mean (UPGMA) cluster analysis using the Jaccard coefficient
numbers of genotypes correspond to table 1 data

The similarity value computed with the Jaccard coefficient, calculated based on iPBS primers, ranged between 0.036 and 0.243. The highest similarity coefficient, which was 0.243, was observed between genotypes 2 and 3, that indicates that these two genotypes are most genetically similar among the selected genotypes. The lowest similarity coefficient of 0.036 was observed when assessing the genetic similarity between genotypes 11 and 13, indicating significant genetic variability between these two genotypes. UPGMA dendrogram according to the Jaccard coefficient determined two main clusters divided to the subclusters. The first main cluster included 8 genotypes (13, 12, 1, 4, 8, 6, 10, and 9), while the second main cluster had five genotypes (7, 3, 2, 11, and 5) (Fig. 1). The iPBS marker system was successful in confirming species distinction, particularly in the case, where sample number 13 (species *Vaccinium myrtillus*) was identified as different. This suggests that the iPBS marker technique is effective for distinguishing between different species, including within-species differences between genotypes.

In prior investigations into the genetic diversity of blueberries, one study employed SSR markers, which exhibited a range of PIC values ranging from 0.23 to 0.94, with an average of 0.67 (Bassil et al., 2018). In an ISSR molecular marker analysis, six ISSR primers were used, resulting in the generation of a total of 87 bands,

**Table 2** Summary of iPBS Marker Characteristics

| Marker | Total bands | | | Polymorphic bands | | PIC | RP |
|---|---|---|---|---|---|---|---|
| | num. | min | max | num. | ratio (%) | | |
| **iPBS 1,846** | 55 | 6 | 12 | 37 | 67.27 | 0,213 | 14,308 |
| **iPBS 1,867** | 67 | 0 | 17 | 43 | 64.18 | 0,203 | 17,692 |
| **iPBS 1,882** | 63 | 7 | 17 | 23 | 36.51 | 0,258 | 22,154 |
| **iPBS 2,270** | 47 | 1 | 13 | 21 | 44.68 | 0,239 | 14,923 |
| **Total** | 232 | - | - | 124 | 53.45 | - | - |
| **Average** | 58 | - | - | 31 | - | 0,228 | 17,269 |

of which 70 (80.4%) displayed polymorphism (Garriga et al., 2013).

## 4 Conclusions

This study aimed to assess the genetic variation present in the iPBS fingerprints generated from selected genotypes of *Vaccinium corymbosum*. The analysis confirmed that the iPBS technique is easily applicable, highly reproducible, and effective in assessing genetic diversity. The study observed a relatively low level of polymorphism, when iPBS primers were employed. The low level of polymorphism was reflected in the low PIC values. Despite this limitation, the dendrogram analysis revealed complete separation among all the examined *Vaccinium corymbosum* genotypes and showed complete separation for the species *Vaccinium myrtillus*.

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