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Short Communication

ASSESSMENT OF IPBS PRIMERS POTENTIAL TO BE USED IN GENETIC DIVERSITY STUDIES OF WILD CLOUDBERRY (*RUBUS CHAMAEMORUS* L.) POPULATIONS

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*Inter-primer binding site (iPBS) molecular retrotransposon-based markers are a universal and simple tool to estimate genetic diversity. The goal of this study was to select highly informative iPBS primers to be used in studies of intraspecific genetic diversity of cloudberry (*Rubus chamaemorus *L.), a common plant species of the boreal zone, associated with raised bog habitats and transitional bogs. Initially, 75 iPBS primers were screened and three of the most informative primers (indicated by numbers 2298, 2277, and 2229) were selected based on the highest number of polymorphic bands and PIC (polymorphic information content) values. Electrophoretic analysis revealed 34 bands amplified with primer 2229, 25 bands with primer 2298, and 22 bands with primer 2277. The percentage of polymorphic loci ranged from 90.9 to 97.1% and PIC values ranged from 0.4885 to 0.4965, indicating potential of the selected primers to be applied in population genetic studies of* R. chamaemorus*.*

Keywords: *retrotransposon-based markers, primer screening, population genetic diversity, Latvia, Belarus.*

Cloudberry (*Rubus chamaemorus* L.) is a perennial dioecious herb from the family *Rosaceae* with subarctic and boreal circumpolar distribution, found mainly in the Northern Hemisphere (Nilsen, 2005). It is most common in the tundra zone and boggy areas. *R. chamaemorus* populations in Europe mainly occur in Fennoscandia, the Baltic countries and Scotland, and its continuous distribution range stretches through Russia to Mongolia and Japan (Thiem, 2003). *R. chamaemorus*, being a glacial relict, has also some small, isolated locations in Central European countries, like Poland, Germany, the Czech Republic (Taylor, 1971; Thiem, 2003; Leišová-Svobodová *et al.,* 2018) and Belarus, where it is considered as critically endangered species and/or has a high protection level. For example, in Belarus species occurs only in northern part of its territory (Kachanovsky, 2015). The distribution range of *R. chamaemorus* in Latvia is close to the southern limit of the species, but it is common throughout the territory and is associated with raised bog, and transitional bog forest habitats, and with nutrient-poor and acidic peatlands (Taylor, 1971; Auniņš, 2013). In total, there are about 500 sites of the species in Latvia (Laiviņš *et al*., 2009). The species mainly reproduces asexually and spreads by rhizomes (Taylor, 1971). Nevertheless, reproduction by seeds is rare, and less effective, but is considered as a crucial mechanism for genetic exchange (Rantala, 1978).

R. chamaemorus is an octoploid plant with 2C = 8x = 56, and DNA content 2.46 ± 0.05 pg/2C (Thiem and Swilinska, 2003). During the last two decades, only a few studies on *R. chamaemorus* have been aimed at determination of intraspecific genetic diversity and differentiation of its natural populations. A low level of allozyme diversity among Finland populations of *R. chamaemorus* was found by Korpelainen (1994), in the first attempt to study its molecular diversity. Ehrich *et al*. (2008) studied amplified frag-

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ment length polymorphism (AFLP) in a broad phylogeographical study of *R. chamaemorus* that involved sampling sites from Scotland, Norway, Siberia, Alaska, and Canada. The authors found great discriminative power of AFLP markers and their capacity to represent genetic relationships among *R. chamaemorus* populations growing in several distinct areas of northern central Siberia and Beringia. Unfortunately, ALFP markers were not shown to be sensitive enough to discriminate geographically related *R. chamaemorus* populations. Recently, Leišová-Svobodová *et al*. (2018) used microsatellites (SSR) to examine *R. chamaemorus* genetic diversity parameters of wild cloudberry populations from Norway and the Czech Republic, and found them to be genetically different.

Inter-primer binding site (iPBS) molecular retrotransposon-based markers are considered to be an efficient tool for genetic polymorphism detection due active moving and recombination of retrotransposons (Kalendar *et al*., 2010). Application of iPBS revealed possibility to detect genetic differences also among individuals within a population. This ability would be highly useful in genetic studies of *R. chamaemorus* due to reduced intraspecific genetic diversity of the species, depending on the high rate of vegetative reproduction. The goal of the current study was focused on the screening and selection of iPBS primers most suitable and informative to reveal genetic diversity of *R. chamaemorus* specimens collected from geographically related populations.

Fresh leaves of *R. chamaemorus* were collected from eight Latvian and Belarus geographically related coenopopulations of naturally raised bog habitats in the North-Eastern part of Latvia and Northern Belarus (Table 1).

Leaf samples were stored in room temperature in silica gel until DNA extraction. Total genomic DNA was extracted from drained leaf samples using a cetyltrimethylammonium bromide (CTAB) protocol (Doyle and Doyle, 1987) with some modifications. Extracted DNA quality and concentration were checked by electrophoresis in 1% agarose gel.

PCR for iPBS analysis was performed following Kalendar *et al*. (2010). PCR reactions were performed in 0.2 ml tubes containing a 25 µl reaction mixture with 1x HotStart PCR buffer, 1 mM primer, 0.2 mM each dNTP, 1.25 U HotStart DNA polymerase, and 20–25 ng DNA. DNA amplification was performed in a GeneAmp PCRSystem 9700. The PCR programme included the following steps: initial denaturation at 95 °C for 3 min, 35 cycles (95 °C for 20 s, 50–60 °C for 60 s, 68 °C for 60 sec), and the final synthesis for 10 min at 72 °C.

The PCR products were electrophoresed at 100 V for 6 hours on 1.7% agarose gel with 1x TAE electrophoresis buffer, and were stained with ethidium bromide (EtBr).

Initially, 75 iPBS primers were tested and the most perspective 9 primers were chosen for further screening using 24 *R. chamaemorus* DNA samples representing eight different

Table 1. Characteristics of Rubus chamaemorus coenopopulations

Country	Locality	Latitude N	Longitude E
LV	Asenieku bog	56°15 58.6	26°29 49.9
LV	Zalezera bog	56°28 20.0	24°33 32.7
LV	Baltais bog	57°31 24.6	27°9 23.6
LV	Nitaure	57°6 36.8	25°8 52.1
BY	Zhada	55°25 44.1	27°58 49.0
BY	Lonno	55°38 01.4	27°9 23.6
BY	Elna	57°31 24.6	27°9 23.6
BY	Velikij moch	55°37 59.4	27°27 19.5

Table 2. Description of iPBS primers

Primer ID	Sequence	Tm (°C)a
2095	GCTCGGATACCA	44.8
2276	ACCTCTGATACCA	42.7
2277*	GGCGATGATACCA	46.2
2393	TACGGTACGCCA	47.1
2229*	CGACCTGTTCTGATACCA	53.5
2231	ACTTGGATGCTGATACCA	52.9
2249	AACCGACCTCTGATACCA	54.7
2251	GAACAGGCGATGATACCA	54.3
2295	AGAACGGCTCTGATACCA	55.0
2298*	AGAAGAGCTCTGATACCA	51.6

* Primers selected for further analysis

a Oligonucleotide concentration og 1 µM (Kalendar *et al*., 2010)

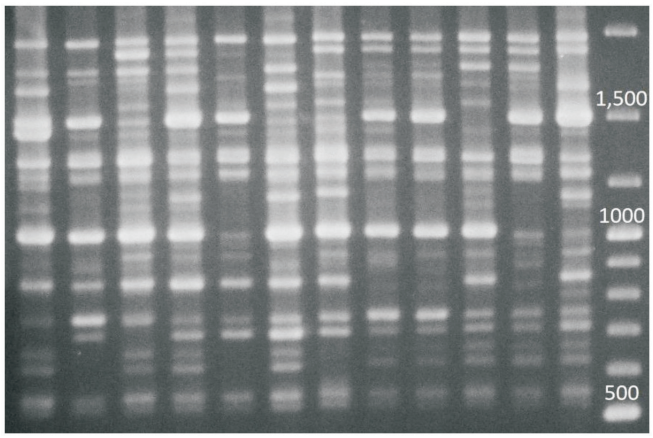


Fig. 1. Rubus chamaemorus samples amplified with iPBS primer 2229.

coenopopulations (Table 2). Primer performance was evaluated as described by Kalendar *et al*. (2010). After separation of the amplification products on agarose gel (Fig. 1), presence (1) or absence (0) of each band was scored manually and data collected were used to create a binary matrix. The efficiency of iPBS primers was evaluated by calculation of polymorphic loci percentage and polymorphic information content (PIC) as mentioned, for example, by Roldán-Ruiz *et al*. (2000) and Soengas *et al*. (2006).

In total, 81 bands were scored, ranging from 200 bp (2298) to 2000 bp (2229). The number of bands per most informa-

Table 3. Percentage of polymorphic loci and PIC for iPBS primers

*P*rimer ID	Polymorphic loci %	PIC	Number of polymorphic bands	Total number of bands
2229	96	0.4899	33	34
2298	90.9	0.4965	24	25
2277	97.06	0.4885	20	22

tive primers varied from 22 to 34, and number of polymorphic bands varied from 20 to 33. Three primers (2298, 2277, and 2229) expressing highest polymorphic patterns (based on polymorphic loci percentage and PIC values) were chosen as the most informative and suitable to conduct *R. chamaemorus* genetic diversity studies (Table 3). For the selected iPBS primers, the polymorphism information content (PIC) was 0.4885–0.4965, which was very close to the theoretical maximum for dominant markers (0.5). This represents high level of genetic diversity among analysed samples.

Based on the most polymorphic iPBS primers (2229, 2277, and 2298) found in the current study, we are planning to carry out comprehensive analysis of the genetic structure of existing *R. chamaemorus* populations, with the goal to reveal unique and genetically variable populations in Latvia and Belarus for genetic diversity conservation and breeding.

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IPBS PRAIMERU IZMANTOŠANAS POTENCIĀLA NOTEIKŠANA LĀCENES (*RUBUS CHAMAEMORUS* L.) SAVVAĻAS POPULĀCIJU ĢENĒTISKIEM PĒTĪJUMIEM

iPBS (*inter-Primer Binding Site* — starppraimeru sasaistes apgabals), uz retrotranspozoniem balstīts molekulārais marķieris, ir universāls un vienkāršs instruments lai novērtētu populāciju ģenētisko daudzveidību. Šī pētījuma mērķis bija atlasīt maksimāli informatīvus iPBS primerus, kurus izmantot lācenes (*Rubus chamaemorus* L), kas ir bieži sastopama boreālās zonas augsto purvu biotopu augu suga, ģenētiskās daudzveidības noteikšanai. Sākotnēji tika pārbaudīti 75 iPBS praimeri no kuriem, pamatojoties uz lielāko polimorfo lokusu skaitu un PIC vērtībām, tika atlasīti trīs informatīvākie (apzīmēti ar numuriem 2298, 2277 un 2229). Ar gēla elekroforēzi tika parādīts, ka praimeris 2229 izdala 34 lokusus, praimeris 2298 — 25 lokusus un 2277 — 22 lokusus. Atkarībā no praimera 90,9 līdz 97,1% lokusu bija polimorfi, savukārt PIC (*Polymorphic Information Content* — polimorfās informācijas saturs) vērtības svārstījās no 0.4885 līdz 0.4965, kas norāda uz izvēlēto praimeru augstu potenciālu *R. chamaemorus* populāciju ģenētiskajā izpētē.