BIOLOGIJA. 2006. Nr. 1. P. 5–7

Genetic characterisation of wild cranberry (*Vaccinium oxycoccos*) from Čepkeliai reserve by the RAPD method

J. Areškevičiūtė[1],

A. Paulauskas[1],

L. Česonienė[2],

R. Daubaras[2]

[1] *Vytautas Magnus University, Department of Biology, Vileikos 8, 44404 Kaunas, Lithuania Judita_areskeviciute@fc.vdu.lt*

[2] *Kaunas Botanical Garden, Vytautas Magnus University, Z. E. Žilibero 6, 3018 Kaunas, Lithuania*

We investigated the genetic characteristics of morphologically different wild cranberries (*Vaccinium oxycoccos*) from Čepkeliai using random amplified polymorphic DNA (RAPD) analysis. Twenty random primers were tested on genomic DNA extracted from 13 wild cranberry sprout clones. Reproducible amplification patterns were obtained using nine primers, which gave from 5 to 20 bands. The RAPD data revealed a high genetic variability within a wild cranberry population.

Key words: *Vaccinium oxycoccos,* PCR, RAPD method, genetic variability

INTRODUCTION

Cranberries usually grow in uplands. There are two native kinds of cranberries – wild cranberry (*Vaccinium oxycoccos*) and small cranberry (*Vaccinium microcarpon)* in Lithuania. American cranberry (*Vaccinium macrocarpon)* was introduced from North America and is used only for cultivation [1]. The descriptions of cranberry growing in Lithuania rely on the morphology of berry leaves and sprouts. In our previous study of wild cranberry, a high degree of intraspecies morphologic variation was identified. It is not known why there is such a great morphologic variation within wild cranberry species [2].

The paucity of qualitative morphological descriptions contributes to cultivar misrepresentation [3, 4]. These features are influenced by environmental conditions and make them unreliable. During the last years, the molecular genetic method that utilizes the polymerase chain reaction (PCR) is gaining wide usage in ecological and behavioural research, taxonomy and genetics of plants and animals [5]. To evaluate the genetic variability of *V. oxycoccos* we have chosen the random amplified polymorphic DNR (RAPD) method.

The aim of our study was to investigate the genetic characteristics of morphologically different *V. oxycoccos* from the Čepkeliai reserve by the RAPD method.

MATERIALS AND METHODS

Sample collection. Thirteen clones with clearly parting vegetative indications (colour, size, shape of berry and productivity) were collected from the Čepkeliai bog during 1995–1999 and were chosen for DNR polymorphism analysis.

DNA Extraction and quantification. DNA from 13 clones was extracted using a purification kit (MBI Fermentas, Vilnius Lithuania). We modified this procedure by using a freshly prepared, warmed up to 37 °C precipitation solution. DNA concentrations were quantified with a spectrophotometer (Eppendorf, Hamburg, Germany) and samples were diluted to a 20 ηg · μl.

Amplification, scoring and analysis of RAPDs. DNR amplification, electrophoresis, ethidium bromide staining, band scoring and data analysis were conducted as described in [6, 7]. Selection of primers was done according to [4, 8] and nine ROTH primers. Primers were synthesized in MBI Fermentas (Vilnius, Lithuania). All chosen markers were tested for their suitability in genotyping *V. oxycoccos* species. DNA bands were visualized by UV transillumination (EASY Win32, Herolab, Germany). The size of DNA fragments was assessed by comparison with GeneRuler™ 10kbp DNA Lader Plus (MBI Fermentas, Vilnius, Lithuania).

The TREECON program [9] was used. Using the Nei and Li formula [10] genetic distances were eva-

luated and the dendrogram was drawn using the UPGMA (Unweighted Pair Group with Arithmetic Mean) method [11].

RESULTS

Based on the literature [4] we have chosen ten OPA primers. Screening of the same ten OPA primers as in [4] but from a different supplier revealed that only five were amplified. They were: OPA-01, OPA-04, OPA-05, OPA-09 and OPA-10. To obtain reliable results it was not enough. For this reason we decided to survey additional nine ROTH and one OPB primers for suitability in wild cranberry. From 20 different random oligonucleotide primers only nine were amplified (Table). Non-amplifiable primers were: OPA-2, OPA-7, OPA-8, OPA-11, OPA-17, ROTH-180-1, ROTH-180-2, ROTH-180-3, ROTH-180-5, ROTH-180-7, and ROTH-180-10. The DNA samples were amplified with reproducible primers which produced 113 bands.

Each primer generated from 5 to 20 individual bands per clone and provided a distinct and reproducible pattern of the amplified PCR fragments (Fig. 1). The number of fragments and the degree of intraspecies polymorphisms varied among the primers (Table).

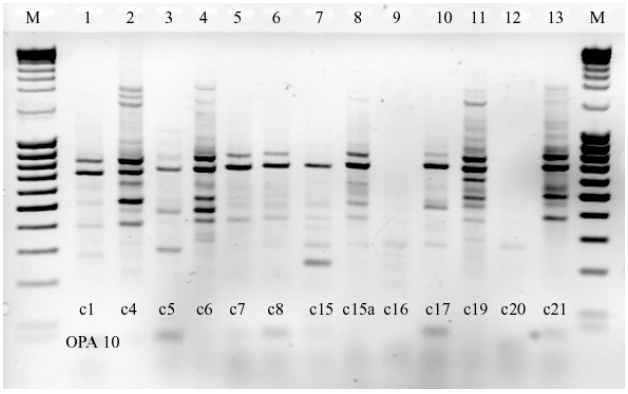

Fig. 1. Reproducibility of RAPD fingerprinting. DNA fingerprints from different samples of *V. oxycoccos* were obtained by PCR with primer OPA-10. 1–13 runners – different clones of *V. oxycoccos.* M – marker

Genetic distances among wild cranberries collected from the Čepkeliai reserve were analysed and the genetic dendrogram was drawn (Fig. 2).

DISCUSSION

The method presented here allows the estimation and comparison of population genetic structure at the genotypic level. This research has been challenged by several investigations [4, 8]. Novy & Vorsa [4] used the silver-stained random amplified polymorphic DNR (ssRAPD) method with cranberry cultivars. In this investigation, ten OPA and two U.B.C. primers (synthesized by Operon Technologies Almeda Calif) were amplified. Stewart & Excoffier [8] made research with American cranberries (*V. macrocarpon*) and used the RAPD method. In this research seven primers were amplified: OPA-4, OPA-7, OPA-9, OPA-13, OPA-18, OPB-4 and OPB-18 (Operon Technologies, Almeda, CA, USA). Two primers, OPA-4 and OPA-9, were amplified by Novy & Vorsa [4] and Stewart & Excoffier [8] and our results.

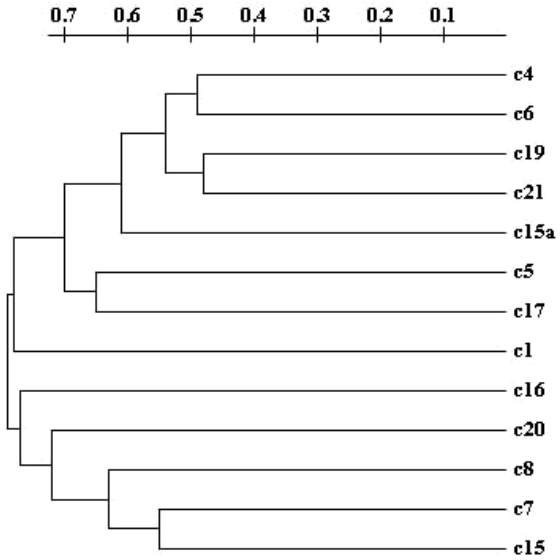

Fig. 2. Dendrogram based on Nei genetic distance [10] constructed for thirteen analysed clones of *V. oxtcoccos*

Table. **Primer names and sequences amplified by RAPD-PCR for individuals of *V. oxycoccos,* the number of polymorphic bands per primer, and the range of molecular weight in base pairs (bp) amplified by PCR for individuals of *V. oxycoccos***

Primer	Sequence (5' to 3')	Number of bands	Range of molecular weight (bp)
OPA-01	5'-CAGGCCCTT-3'	12	275–1300
OPA-04	5'-AATCGGGCT-3'	5	225–1550
OPA-05	5'-AGGGGTCTT-3'	14	225–2000
OPA-09	5'-GGGTAACGC-3'	13	350–2750
OPA-10	5'-GTGATCGCA-3'	18	80–2000
OPB-11	5'-GTAGACCCG-3'	9	290–1700
180-6	5'- GCACGCCGGA-3'	13	80–1235
180-8	5'- CGCCCTCAGC-3'	9	300–1750
180-9	5'- GCACGGTGGG-3'	20	200–1750

The partial discrepancy among our results and those by Novy & Vorsa [4] and Stewart & Excoffier [8] could be due to the use of primers from different suppliers (Operon Technologies vs. Fermentas) or of different species.

After comparison of our data with the results reported in [4, 8] we can infer that more experiments for a deeper comparison of genetic variation of wild cranberries should be done. We have to test other RAPD primers (used in ssRAPD [4] study) that were not investigated by us. We could also suggest investigators who use the ssRAPD method for cranberry genotypic analysis to test markers that were used only in our research and then to compare the data.

Thus, DNA typing methods such as RAPD profiling, which do not display allele frequencies but provide many polymorphic markers, may be used in a comprehensive population analysis. According to our results, the genetic variability is high and coincides with morphological variation. However, wild cranberry is a polyploid complex comprising diploids, tetraploids and hexaploids [12]. Therefore, a deeper research is needed. Nevertheless, the results presented in this study provide useful information about the genetic variability of Lithuanian cranberries collected from the Čepkeliai reserve.

ACKNOWLEDGMENT

This work was partially supported by Lithuanian Ministry of Education and Science programme and by Norwegian Centre for International Cooperation in Higher Education (grant CCP 03/02: ENLINO Master program network).

Received 1 December 2005
Accepted 30 January 2006

References

1. Butkus V, Jasionis I, Urbonas V, & Červokas V. Mažieji miško turtai. 1987; Vilnius.
2. Daubaras R, Česonienė L. Baltic Forestry. 2004.
3. Novy RG, Kobak C, Goffreda J, Vorsa N. Theor Appl Genet 1994; 88 1004–10.
4. Novy RG, Vorsa N. Hort science1995; 30(3): 600–4.
5. Hadrys H, Balic M, Schierwater B. Molecular Ecology 1992; 1: 55–63.
6. Žvingila D, Verbylaitė R, Abraitis R, Kuusienė S, Ozoličius R. Baltic Forestry. 2002; 18(2): 2–7.
7. Miceikienė I, Paulauskas A, Grigaliūnaitė I, Malevičiūtė J, Tubelytė-Kirdienė V. Genetikos praktikumas. IV DNR polimorfizmo tyrimo metodai. 2002 m.
8. Stewart CN Jr, Exocoffier L. Evolutionary Biology 1996; 9: 153–71.
9. Van de Peer Y, De Wachter R. Appl Biosci 1994; 10: 569–70.
10. Nei M, Li W. Proc Natl Acad Sci USA 76. 1979; 5269–73.
11. Yeh FC, Boyle TJB. Belgian Journal of Botany 1997; 129: 157.
12. Ravanko O. Ann Bot Fennici 1990; 27: 235–9. Helsinki. ISSN 0003–3847.

J. Areškevičiūtė, A. Paulauskas, L. Česonienė, R. Daubaras

ČEPKELIŲ REZERVATO PAPRASTOSIOS SPANGUOLĖS (*VACCINIUM OXYCOCCOS*) GENETINĖ CHARAKTERISTIKA PAGAL AAPD ANALIZĘ

Santrauka

Atsitiktinai padaugintos polimorfinės DNR metodu (APPD) buvo tirta morfologiškai skirtingų paprastosios spanguolės (*Vaccinium oxycoccos*) klonų, surinktų iš Čepkelių rezervato, genetinė įvairovė. Šiam tikslui buvo išskirta DNR iš 13 skirtingų paprastosios spanguolės klonų. Pasirinkta ir patikrinta 20 skirtingų pradmenų, iš kurių 9 pradmenys davė rezultatus. Buvo aptikta nuo 5 iki 20 fragmentų. AAPD duomenys atskleidė didelę, morfologiškai besiskiriančių *V. oxycoccos* klonų iš Čepkelių rezervato genetinę įvairovę.