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Morphological and genetic diversity of European cranberry (*Vaccinium oxycoccos* L., Ericaceae) clones in Lithuanian reserves

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Abstract

The wild-harvested fruit of *Vaccinium oxycoccos* (European cranberry) is used medicinally in many European and North American countries; the plant, however, is seldom cultivated. In order to optimize the collection strategy and improve the horticulturally important characters of *V. oxycoccos* clones, comprehensive investigations of the species are necessary. In the present study we investigated the phenological, morphological and genetic diversity of 29 clones originating from two wild populations growing in two strictly protected Lithuanian reserves, Čepkeliai and Žuvintas. During an ex situ collection at Kaunas Botanical Garden, we observed great phenological variation between the collected *V. oxycoccos* clones. The following morphological traits most clearly distinguished our study clones: leaf size, berry shape, berry size and fruit colour at full maturity. The genetic variation of *V. oxycoccos* clones from the two populations was assessed using RAPD and SSR. RAPD analysis conducted with 9 primers resulted in 146 polymorphic loci for the total sample, and SSR analysis with 5 primers revealed 29 alleles for the total sample. A greater degree of polymorphism was demonstrated for the Čepkeliai population than for the Žuvintas population. The study allowed the selection of several clones having promising morphological traits for further testing in the field.

Keywords: clone, domestication, genetic resources, morphological character, peat bog, population, RAPD, SSR

Introduction

Of the 50000 or more plant species used by man for food and medicine, only a small fraction is cultivated, the remainder being harvested directly from their natural habitats [1]. However, due to human pressure, such as habitat loss, land conversion or over-harvesting, many of these plants, especially those of medicinal value, are considered to be at risk. The cultivation of such species could improve the status of natural populations by alleviating the pressure of over-harvesting, assure unrestricted access to that particular crop and allow the selection of more resistant and productive varieties [1].

One European example of such a plant species is *Vaccinium oxycoccos* L. (syn. *Oxycoccus quadripetalus* Gilib., *Oxycoccus palustris* Pers.; Ericaceae Juss.), which is widely wild-harvested throughout much of its natural range and cultivated only in Russia and Estonia [2]. This species, the European cranberry, is a dwarf, woody, evergreen clonal shrub with slender, rooting stems, occasionally up to 0.8–1.0 m tall, with short, usually erect flowering shoots. The leaves are leathery, dark, glossy green dorsally, glaucous ventrally and frequently revolute with an entire blade margin. Racemes of 1–5, white, pink or red, protandrous flowers are pollinated mostly by solitary or social bees [3] and high fruit production frequently occurs following autogamy [4]. The fruit is an over-wintering, edible berry (the cranberry). Although fruit-set in natural populations may be high, the plant mostly reproduces vegetatively, forming large clones some hundreds of years old [3]. This plant has three (or four, depending on taxonomic treatment) ploidy levels: mainly tetra-and hexaploid populations are found, but pentaploids are also reported from the Czech Republic and Sweden. Diploids are usually treated as a separate species, namely *V. microcarpum* (Turcz. ex Rupr.) Schmalh. [5,6].

Vaccinium oxycoccos has a circumboreal distribution. In Europe it usually grows on *Sphagnum* peat bogs and is present in the north-western part of the continent, from Ireland, the British Isles and Scandinavia, throughout Central and Eastern Europe, the Balkan countries, Bulgaria, and even extending as far east as Siberia (N. Asia) and Japan. It also occurs in Greenland and the northern part of North America [3]. The wild-harvested fruit of *V. oxycoccos* is considered a substitute for that of *V. macrocarpon* Aiton, widely cultivated in the US and GB [7], and is commercially used in Scandinavia, the Baltic States, Poland, Belarus, Ukraine, Russia, Alpine zone of Switzerland, France, and Italy [8,9], as well as the USA and Canada. The fruit

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is a source of phenolic compounds and athocyanins that have antibacterial, anticarcinogenic and antioxidant properties [10].

In nature, the plant grows on peat in poorly drained sites with a very high water level, and on very acidic soils (pH ranging from 3.0 to 4.5). In recent years, however, the peat bog vegetation has been seriously threatened by the effects of land reclamation. Large areas of raised bogs have also suffered from eutrophication, which has had an adverse effect on species composition. For example, in Lithuania, a drop in water level has promoted the growth of associated shrubs [*Ledum palustre* L., *Calluna vulgaris* (L.) Hull, *Vaccinium uliginosum* L.] and has reduced the vitality of *V. oxycoccos*, by causing a critical reduction in the natural resources of the species [11]. Despite this considerable habitat loss, cranberries are still harvested in the wild, which further erodes natural populations of the species. This, in turn, has stimulated interest in the further cultivation and breeding of this crop [12]. However, in order to promote future cranberry breeding and production, especially in countries having no such tradition, the morphological and horticulturally important characters of the plant require investigation [13,14]. Above all, there is a need for information about the genetic variability of the species, so as to optimize the sampling strategy, especially as the plant displays great intraspecific morphological diversity. For example, individual cranberry clones from the same population may differ considerably in terms of berry size, color and shape, as well as shoot length. This high degree of morphological variability has been reported, for instance, from Poland [15] and the Czech Republic [5]. Particular clones can also differ in their production of medicinally useful phytochemicals [10]. Investigations carried out in Lithuania during 1965–1970 confirmed that berry shape is a very variable character [11]. Since then, breeding and diversity of Lithuanian genotypes has become the subject of broad research and has provided a basis for *V. oxycoccos* studies. The application of molecular markers proved to be especially useful in assessing the diversity of the collected plant material [16].

It is important to select natural forms that display highest productivity, resistance to adverse environmental factors (diseases) and good fruit taste and size [17]. Owing to the complicated system of morphological descriptions used in separating individual cranberry clones, clone identification is prone to errors. Molecular markers, however, allow the direct assessment of genetic diversity as a means of determining objectively differences in genetic material.

The aim of this investigation, which is based on the results of earlier morphological studies [11,12], is to investigate phenological, morphological and genetic diversity, and horticultural value of Lithuanian *V. oxycoccos* clones collected from the wild at two nature reserves, namely, Žuvintas and Čepkeliai, where we have previously observed a high degree of morphological variation between populations.

Material and methods

Plant material and evaluation of phenological, morphological, and horticultural characters

Plant material for the study was collected from two strictly protected reserves, Žuvintas and Čepkeliai, during 1998–1999 (Fig. 1). The Žuvintas reserve is situated in the southern part of the central Lithuanian lowlands, (N54°29′ E23°40′). It comprises of a complex of Žuvintas and Amalvas wetlands, covering 6847 ha. The Žuvintas reserve is notable for its diverse plant communities. The Čepkeliai reserve is situated in Southern Lithuania, close to its border with Belarus (N54°00′ E24°30′). Raised bogs cover about 80% of the Čepkeliai wetlands.

Fig. 1 Locations of Žuvintas (1) and Čepkeliai (2) reserves in Lithuania.

During field work at both sites, we collected 29 distinctive clones differing clearly in vegetative characters, including berry size, shape, and color. Cuttings of selected clones (size 10–15 cm) were transferred into the field collection at Kaunas University Botanic Garden, Kaunas, Lithuania. The annual precipitation for Kaunas district is 500–750 mm, and the average temperature exceeds 6.7°C. The cuttings were planted in acid peat beds (pH 4.0–5.0) and cultivated under ex situ conditions for further investigations.

Phenological observations were conducted throughout the entire vegetative growth period during 2000–2010. On the same days, twice a week, we checked the collection, and the following phenological phases were recorded for each of the clones: commencement of shoot growth, commencement of flower bud development, commencement of flowering, end of flowering, commencement of fruit ripening, end of fruit ripening, and the end of the vegetative growth period.

Detailed evaluation of morphological diversity for these clones was carried out during the years 2004–2010. We measured or assessed the following characters for each clone: leaf size and shape, shape of leaf apex and base, recurving of leaf margin, color of fully opened flower, length of peduncle, berry size, berry shape and color, shape of berry in cross-section, extent of waxy layer of fruit, and color of flesh (mesocarp) of berry. Berries were weighed using an analytical balance (Ishida Co., Japan, model DJ-150E; sensitivity of 0.01 g) and the average weight calculated. For each clone, three replicates of 50 fruit were weighed. The yield production of each clone was calculated, again for triplicate samples, by weighing the total berries per 1 m^2. The average generative shoot length for each clone was calculated based on the measurement of 50 randomly selected shoots. The mean area of a leaf was determined by scanning triplicate samples of 30 randomly selected leaves from each clone with a CI-202 (CID Bio-Science, USA) portable laser leaf area meter.

Tab. 1 Primers and their sequences used for RAPD analysis of two populations of *Vaccinium oxycoccos* in Lithuania.

Primer	Sequence of oligonucleotides (5'→3')	Length of obtained DNA fragments (bp)	Total number of bands per primer
Opa-01	5'-CAGGCCCTTC-3'	225–2200	22
Opa-04	5'-AATCGGGCTG-3'	225–1550	7
Opa-05	5'-AGGGGTCTTG-3'	225–2000	19
Opb-11	5'-GTAGACCCGT-3'	290–1900	13
Opa-10	5'-GTGATCGCAG-3'	100–2000	19
Opa-09	5'-GGGTAACGCC-3'	350–2750	14
Roth-06	5'-GCACGCCGGA-3'	100–1235	13
Roth-08	5'-CGCCCTCAGC-3'	300–1750	13
ROTH-09	5'-GCACGGTGGG-3'	200–2400	26
Total		**100–2750**	**146**

Genetic analysis

For DNA extraction, we used 100–130 mg of fresh, new cranberry shoots collected in spring. For RADP analysis, we used nine 10 nt-long primers of random sequence (Fermentas, Lithuania; Roth, Germany; Tab. 1). DNA was PCR-amplified using an automatic thermocycler (Mastercycler, Eppendorf, Germany) under the following conditions: initial denaturation for 4 min at 94°C, 44 cycles of denaturation for 1 min at 94°C, primers annealing for 1 min at 35°C, extension for 2 min at 72°C followed by a final extension for 5 min at 72°C. PCR reaction per primer was done not less than twice. The reaction products were fractionated by electrophoresis in 1.5% agarose gel and visualized using ethidium bromide stain and UV light. The length of DNA bands was estimated according to the gene ruler Gene RulerTM 100 bp DNA Ladder (Fermentas, Lithuania).

For SSR DNA amplification we used five 20–23 nt-long primers (Biomers, Germany; Tab. 2). SSR primers were chosen and the DNA amplification reaction performed according to Boches et al. [18].

PCR products were obtained by ABI 3130 xl Genetic analyzer (Applied Biosystems), length of fragments were set using standard of ROX-500 (Applied Biosystems) as an internal size standard. Allele sizes were visualized using GeneMapper v. 3.5 software (Applied Biosystems).

Data analysis

Data was analyzed using the statistical package STATISTICA 6 (Stat Soft., Inc.). Statistical differences were identified with ANOVA, followed by Fisher's LSD test at $P \leq 0.05$ and 0.01. Population genetic analysis, such as principal coordinates analysis (PCA) and analysis of molecular variance (AMOVA), analysis were performed using the GenAlEx 6 [19]. Calculation of the observed number of alleles, Nei's gene diversity H [20], Shannon's information index I, total gene diversity Ht, gene diversity within populations Hs, gene diversity between populations $Gst = (Ht - Hs / Ht)$, gene flow $Nm = 0.5\ (1 - Gst)/Gst$ and the generation of a Nei's genetic distance based dendrogram were achieved using POPGENE V 1.31 software.

Results

Phenological and morphological diversity

Vaccinium oxycoccos clones showed significant phenological plasticity. Although the duration of the vegetative growth period for the years 2004–2010 did not differ statistically between clones, in other years we observed great variation in the commencement of certain phenological phases (ranging from 10–19 days). For example, there were 3–9 day-long shifts between clones in the commencement of flower bud formation and 7–20 days-long shifts between clones in the commencement of berry ripening. The flowering phase, depending on the clone, started from mid May to the first ten days of June. Clones 99-Ž-02, 99-Ž-07, 98-Č-01, and 98-Č-09, in particular, would commence anthesis as late as the first ten days of June.

Tab. 2 Primers and their sequences used for microsatellite analysis of two populations of *Vaccinium oxycoccos* in Lithuania.

Primer	Sequence of oligonucleotides (5'→3')	Length of obtained DNA fragments (bp)	Total number of bands per primer
Ca169f	F:TAGTGGAGGGTTTTGCTTGG R:GTTTATCGAAGCGAAGGTCAAAGA	110–111	2
Ca421f	F:TCAAATTCAAAGCTCAAAATCAA R:GTTTAAGGATGATCCCGAAGCTCT	146–171	7
Ca483f	F:GTCTTCCTCAGGTTCGGTTG R:GAACGGCTCCGAAGACAG	298–319	5
Ca794f	F:CGGTTGTCCCACTTCATCTT R:GTTTGAATTTGGCTTCGGATTC	232–260	12
Vcc_j9	F:GCGAAGAACTTCCGTCAAAA R:GTGAGGGCACAAAGCTCTC	129–152	3
Total		**110–319**	**29**

The flowering period of different clones lasted 13–23 days. Berry ripening started from mid-August until early September, depending on clone, and ended in late September through to early October. The berry-ripening phase lasted 18–37 days. Clones 99-Ž-03, 99-Ž-07, 98-Č-05 and 98-Č-03 were characterized by the lengthy ripening period: 37, 35, 35 and 35 days, respectively. By contrast, clones 99-Ž-02 and 98-Č-02 had the shortest ripening period, lasting 18 and 19 days, respectively (Fig. 2). The ripening time depended both on prevailing weather conditions, as well as on the inherent biological characters of the clone. In some clones, frost damage during spring 2004 resulted in considerable reduction or complete destruction of berry yield.

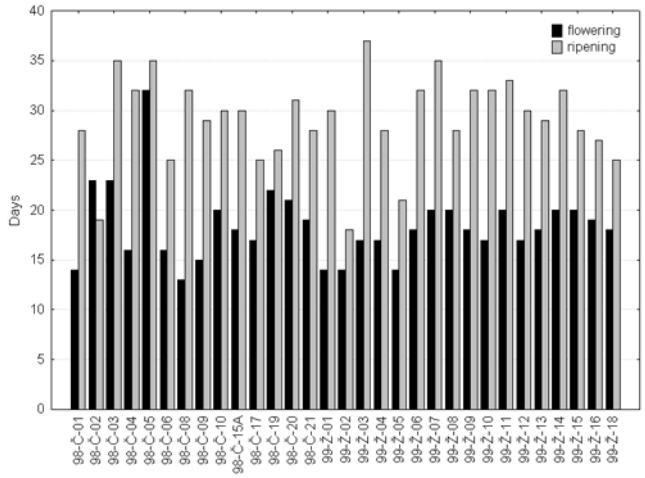

Fig. 2 Flowering and ripening duration for various *Vaccinium oxycoccos* clones, during 2000–2010.

We observed that variation in some morphological characters relative to collecting conditions was so low that it was difficult to distinguish between them. This was especially true for leaf characters, such as the shape of leaf apex and base, recurving of the leaf margin, and peduncle length. Some characters were strongly related to the phenological phases of the plant. For example, it was difficult to define flower properties, as well as the color of ripe berries and the extent of the waxy layer. However, these differences were much more evident in plants grown under collection condition, as compared with those from natural populations. For example, collection plants had larger and thicker leaves and larger berries owing to better growing conditions. The average berry length and width varied from 9.9–13.8 mm and from 10.1–13.6 mm, respectively. The usual color of berries at full maturation was red or dark red, except for clone 99-Ž-10, which produced pink fruits. The berries of clones 99-Ž-16, 99-Ž-09, and 99-Ž-11 were coated with a waxy layer. Most of the clones studied produced oblate berries (42%), whereas 25.8% were characterized by ovate fruit.

Most of the investigated samples had ovate leaves. The average leaf area, depending on clone, varied from 24 to 47 mm^2 (Tab. 3). Analysis of leaf size showed that most clones (84%) develop moderately sized leaves, 7% have small leaves, and 9% large leaves. Clones 99-Ž-05 and 99-Ž-06 had the smallest leaves (24 mm^2 and 28 mm^2, respectively) and clones 99-Ž-14 and 99-Ž-11 the largest (44 mm^2 and 41 mm^2, respectively). The average length of a generative shoot varied from 32.0–53.2 mm.

The average berry weight was 0.91 g (Tab. 3). The greatest variation in average berry weight was recorded for clones

Tab. 3 Summary statistics for selected morphological characters of the studied *Vaccinium oxycoccos* clones from the Lithuanian nature reserves.

Character	*M*	*SD*	M_{min}–M_{max}	*F*
Berry weight (g)	0.91	0.16	0.51–1.99	9.67**
Average yield (kg)	0.46	0.25	0.08–1.10	1.66*
Leaf area (mm^2)	37	4	24–47	20.09**
Generative shoot length (mm)	41.9	5.5	32.0–53.2	7.6*

F – Fisher's criterion; *M* – mean; M_{min}-M_{max} – range of the mean values; *SD* – standard deviation. * $P < 0.05$ **; $P < 0.01$.

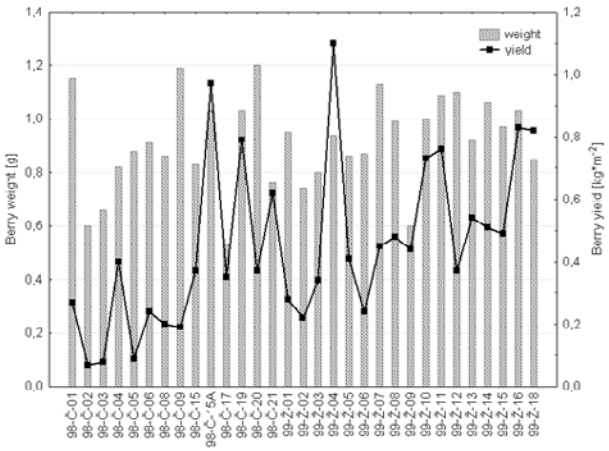

Fig. 3 Variation in average berry weight ($LSD_{0.01}$ = 0.196) and average berry yield ($LSD_{0.05}$ = 0.235), between *Vaccinium oxycoccos* clones collected from Žuvintas and Čepkeliai populations (Lithuania); data pooled over the years 2000–2010.

98-Č-09 and 98-Č-05, with variation coefficients of 29.1% and 20.2%, respectively. Variation in berry weight of the remaining clones was small (the variation coefficient $V < 10\%$) or moderate ($10\% < V < 20\%$). Clones 98-Č-01, 98-Č-09, 98-Č-20, 99-Ž-07, 99-Ž-11, and 99-Ž-12 produced the largest berries, weighing, on average, 1.03–1.19 g (Fig. 3).

We found considerable differences in fruit yield between the studied clones. The most productive clone was 99-Ž-04 (average yield of 1.76 kg/m^2), whereas the average yield of other clones varied from 0.07 kg/m^2 (99-Č-02) to 0.97 kg/m^2 (98-Č-15A). In some years, berry yield of certain clones could reach as much as 2.0 kg/m^2.

Genetic analyses

Evaluation of RAPD patterns resulted in 146 fragments. The fragment sizes of different bands recorded for all the samples ranged from 175 to 2750 bp (Tab. 1, Fig. 4), reflecting a rich allelic diversity among the populations, with 99.32 % of loci being polymorphic. The number of bands per primer ranged from 7 (for OPA-4) to 26 (for ROTH-09). More unique fragments were detected for Čepkeliai population as compared with the Žuvintas population. Furthermore, the level of polymorphism was greater in the former (Tab. 4).

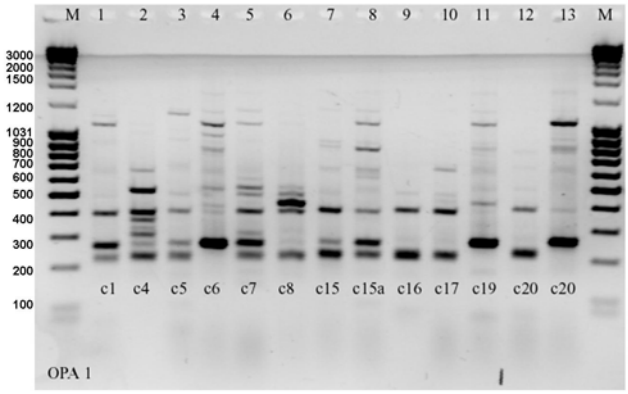

Fig. 4 RAPD patterns obtained using the primer OPA-01 for the investigated *Vaccinium oxycoccos* clones, Čepkeliai reserve, M-Gene RulerTM 100 bp DNA Ladder.

Tab. 4 Genetic polymorphism of *Vaccinium oxycoccos* clones collected from Žuvintas and Čepkeliai populations as assessed by RAPD.

	Čepkeliai	Žuvintas
Fragment number	109	79
Private fragment number	66	36
% polymorphism level	74	54

Five primers (Ca169f, Ca421f, Ca483f, Ca794f, Vcc_j9) were chosen for SSR analysis, and following the reaction, 29 fragments were obtained ranging in size from 110–319 bp. The greatest number of fragments (12) was obtained using Ca794f primer, whereas the lowest (2) was obtained using primers Ca169f and Vcc_j9 (3; Tab. 2). Primer Ca169f was monomorphic for the Žuvintas population.

In order to estimate genetic variation between populations, Shannon's information index (*I*), Nei's gene diversity (*H*), the observed number of alleles per locus (*Na*), and the number and percentage of polymorphic loci were calculated. Summary Nei's genetic diversity for both populations was 0.17, and ranged from 0.1 (Čepkeliai population) to 0.2 (Žuvintas population), whereas the Shannon's information index for Žuvintas and Čepkeliai populations was 0.29. The genetic variation among the studied populations and calculated using AMOVA was 29% and 3%, respectively for the RAPD and the SSR methods. The genetic variation within *V. oxycoccos* populations was 97%, compared with 81% for *V. oxycoccos* clones, as estimated by the SSR method.

PCA analysis showed that the studied populations contained individuals with location-specific alleles. Each represented a different haplotype, thus resulting in two distinct groups within the distribution range (Fig. 5).

Discussion

Our study shows that some morphological characters of the European cranberry, *V. oxycoccos*, can be used for distinguishing clones. Of the 13 leaf, flower and fruit traits surveyed in the present paper, leaf size, berry shape and berry size are the most useful, the other characters demonstrating insufficient variation to be of any value in clone identification. Similar results were reported for clones and cultivars of the American cranberry, *V. macrocarpon* [21]. These characters, especially those related to fruit, can also be used to select plants for further experimental cultivation.

Apart from morphological traits, phenological characters, such as the length of the vegetative growth period, the time of flowering and fruit maturity are also useful in identifying suitability as crop plants. In assessing the cultivation possibilities of *V. oxycoccos* clones, late spring is a critical period since, especially in Lithuania, but also in other Central European and Baltic countries, there is a risk of late frosts in mid-May, when cranberry usually flowers. Therefore, early-flowering individuals would be prone to flower loss and consequently, a reduction in berry yield. Indeed, in this respect, our study showed great phenological variation among *V. oxycoccos* clones. Four distinct clones (99-Ž-02, 99-Ž-07, 98-Č-01, 98-Č-09) were characterized by a postponed, late flowering phase, and would therefore seem much better suited for cultivation under Central European conditions. We also noted differences in the onset of berry ripening and the duration of the ripening period, which need also to be considered when selecting genotypes for further evaluation.

Hitherto, the identification of cranberry clones has been based mainly on morphological traits. This approach, however, has considerable limitations, since variation in morphological characters often reflects environmental factors [22,23]. Therefore, morphological identification should ideally be supplemented by other methods of assessing diversity. The data on genetic diversity of the organisms investigated here, together with information on variation in morphological traits, nevertheless, regardless of environmental conditions, provides a good means of assessing the quality of plant material to be collected in future.

A rapid means of genotype identification for germplasm characterization [24] is very important in the case of vegetatively propagated plant species such as the cranberry. As shown in our study, the summary Nei's genetic diversity value for both populations was 0.17, whereas Shannon's information index scored 0.29 for both sites, indicating that the investigated populations reproduce mainly by vegetative means (similar low results were also obtained by Bartish et al. [25], for some species of *Chaenomeles* Lindl.). Alternatively, this may be caused by frequent autogamous seed production [24], the two explanations being not mutually exclusive.

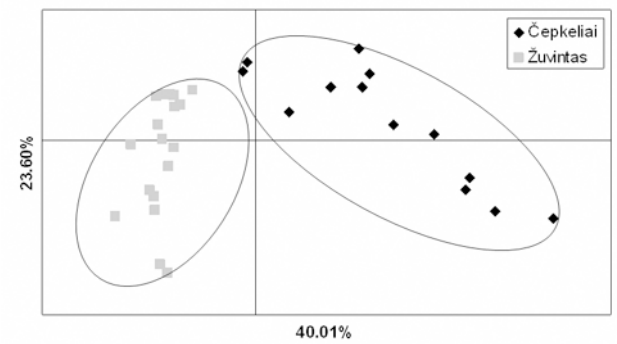

Fig. 5 PCA of the genetic distances assessed using RAPD for *Vaccinium oxycoccos* populations from Žuvintas and Čepkeliai reserves.

Of the numerous molecular markers available, the random amplified polymorphic DNA (RAPD) technique has become the subject of much debate. Nevertheless, limitations to the reproducibility of RAPD markers have been largely overcome by improvements in laboratory techniques and band scoring procedures [26] and this method has been successfully used to investigate the degree of cloning in many plant species [27,28]. Indeed, comparison of RAPD and AFLP molecular markers has confirmed the reproducibility of RAPD markers [29].

In our study, RAPD markers proved to be a powerful method for the detection of spatial genetic variation, allowing the selection of particularly valuable genotypes. For example, it has been demonstrated for *V. stamineum* L., that plants with the greatest genetic diversity within and between populations are better adapted to cope with different environmental conditions [27].

Analyses of the genetic structure of *V. macrocarpon* and certain other plants showed that, for many species, the greatest genetic variation may be detected within populations [30,31], in contrast to the results obtained for *Oryza rufipogon* Griff. [32]. Average molecular variance between populations of *Vaccinium species* was 87.7%, whereas the value obtained from within populations of the same species was 27.7% [30]. With regard to the genus *Vaccinium*, greatest intrapopulation variation was detected for American cranberry, *V. macrocarpon*: more than 91% [30], followed by *V. uliginosum* L. – 90.3% [27] and *V. myrtillus* L. – 86.19% [29,33]. By contrast, our study of *V. oxycoccos* populations exhibited relatively low (71%) intrapopulation genetic variation, based on RAPD. This also contrasts with our SSR results, which showed 97% genetic variability between Žuvintas and Čepkeliai populations, whereas in other vegetatively propagated clonal species it ranged from 71–86% [34–36].

Greater genetic variation was found in the Čepkeliai population than in the Žuvintas population. This may be due to greater penetration of the latter site and the intensive picking of berries resulting in a reduction in propagation by seed and the promotion of clonal growth.

In conclusion, *V. oxycoccos* seems a promising crop for cultivation under Central European conditions. This study shows that some useful morphological characters such as leaf size, berry size and berry shape can be used to assess potentially interesting genotypes. The considerable genetic diversity found within the studied populations indicates that the selected clones from Čepkeliai and Žuvintas reserves are well suited to the prevailing environmental conditions and may prove a useful source of plant material for future study.

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Authors' contributions

The following declarations about authors' contributions to the research have been made: collected plant material and performed morphometric analyses: LČ; designed the study: RD; performed genetic analyses: JŽ; analysed the data: AP; wrote the manuscript: MZ.

References

1. Scoones I, Melnyk M, Pretty JN. The hidden harvest: wild foods and agricultural systems: a literature review and annotated bibliography. London: Sustainable Agriculture Programme, International Institute for Environment and Development; 1992.
2. Stackevičienė E, Labokas J. Fruiting peculiarities of wild cranberry (*Oxycoccus palustris* L.) in Čepkeliai bog. Hort Veg Grow. 2000;19(3):255–264.
3. Jacquemart AL. *Vaccinium oxycoccos* L. (*Oxycoccus palustris* Pers.) and *Vaccinium microcarpum* (Turcz. ex Rupr.) Schmalh. (*Oxycoccus microcarpus* Turcz. ex Rupr.). J Ecol. 1997;85(3):381. http://dx.doi.org/10.2307/2960511
4. Fröborg H. Pollination and seed production in five boreal species of *Vaccnium* and *Andromeda* (Ericaceae). Can J Bot. 1996;74(9):1363–1368. http://dx.doi.org/10.1139/b96-165
5. Suda J, Lysák MA. A taxonomic study of the *Vaccinium* sect. *Oxycoccus* (Hill) W. D. J. Kock (Ericaceae) in the Czech Republic and adjacent territories. Folia Geobot. 2001;36(3):303–320. http://dx.doi.org/10.1007/BF02803183
6. Suda J. Sympatric occurrences of various cytotypes of *Vaccinium* sect. *Oxycoccus* (Ericaceae). Nord J Bot. 2002;22(5):593–601. http://dx.doi.org/10.1111/j.1756-1051.2002.tb01914.x
7. Mabberley DJ. Mabberley's plant-book: a portable dictionary of plants, their classification and uses. 3rd ed. Cambridge: Cambridge University Press; 2008.
8. Kardell L. Occurrence and berry production of *Rubus chamaemorus* L., *Vaccinium oxycoccus* L., *V. microcarpum* Turcz. & *V. vitis-idaea* on Swedish peats. Scand J For. Res. 1986;1:125–140.
9. Łuczaj Ł, Szymański WM. Wild vascular plants gathered for consumption in the Polish countryside: a review. J Ethnobiol Ethnomed. 2007;3(1):17. http://dx.doi.org/10.1186/1746-4269-3-17
10. Cesonienė L, Daubaras R, Jasutienė I, Venclovienė J, Miliauskienė I. Evaluation of the biochemical components and chromatic properties of the juice of *Vaccinium macrocarpon* Aiton and *Vaccinium oxycoccos* L. Plant Foods Hum Nutr. 2011;66(3):238–244. http://dx.doi.org/10.1007/s11130-011-0241-5
11. Daubaras R, Česonienė L. Phenotypic properties of clones of wild cranberry (*Oxycoccus palustris* Pers.) and their stability. Balt For. 2004;10:87–90.
12. Daubaras R, Česonienė L, Labokas J. Phenotypic diversity of wild cranberry (*Vaccinium oxycoccos*) in Lithuania. Acta Hortic. 2004;663:617–620.
13. Makeev VA, Cherkassov AF, Makeeva GY. Results and future outlook for *Oxycoccus palustris* selection. Gomel: Forest institute NASB; 2000. p. 178–180.
14. Ravanko O. The taxonomic value of morphological characteristics in *Oxycoccus* (subgenus of *Vaccinium*, Ericaceae) species in Finland. Ann Bot Fenn. 1990;27:235–239.
15. Gugnacka-Fiedor W. Zmienność morfologiczna taksonów rodzaju *Oxycoccus* Hill. Stud Soc Sci Torun Sect Bot. 1987;11:1–57.
16. Areškevičiūtė J, Paulauskas A, Česonienė L, Daubaras R. Genetic characteristic of wild cranberry collected from Čepkeliai reserve using RAPD method. Biologija. 2006;1:5–7.
17. Vorsa N. Wisconsin Cranberry School 1994 proceedings. In: Roper TR, editor. Breeding the American cranberry. Madison WI: Wisconsin State Cranberry Growers Association; 1994. p. 1–4.
18. Boches PS, Bassil NV, Rowland LJ. Microsatellite markers for *Vaccinium* from EST and genomic libraries. Mol Ecol Notes. 2005;5(3):657–660. http://dx.doi.org/10.1111/j.1471-8286.2005.01025.x
19. Peakall R, Smouse PE. Genalex 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol Ecol Notes. 2006;6(1):288–295. http://dx.doi.org/10.1111/j.1471-8286.2005.01155.x
20. Nei M, Li WH. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc Natl Acad Sci USA. 1979;76(10):5269–5273. http://dx.doi.org/10.1073/pnas.76.10.5269
21. Novy RG, Vorsa N. Identification of intracultivar genetic heterogeneity

in cranberry using silver-stained RAPDs. Hort Sci. 1995;30(3):600–604.
22. Kroon H, Stuefer JF, Dong M, During HJ. On plastic and non-plastic variation in clonal plant morphology and its ecological significance. Folia Geobot. 1994;29(2):123–138. http://dx.doi.org/10.1007/BF02803790
23. Ellison AM, Buckley HL, Miller TE, Gotelli NJ. Morphological variation in *Sarracenia purpurea* (Sarraceniaceae): geographic, environmental, and taxonomic correlates. Am J Bot. 2004;91(11):1930–1935. http://dx.doi.org/10.3732/ajb.91.11.1930
24. Debnath SC. An assessment of the genetic diversity within a collection of wild cranberry (*Vaccinium macrocarpon* Ait.) clones with RAPD-PCR. Genet Resour Crop Evol. 2006;54(3):509–517. http://dx.doi.org/10.1007/s10722-006-0007-3
25. Bartish IV, Garkava LP, Rumpunen K, Nybom H. Phylogenetic relationships and differentiation among and within populations of *Chaenomeles* Lindl. (Rosaceae) estimated with RAPDs and isozymes. Theor Appl Genet. 2000;101(4):554–563. http://dx.doi.org/10.1007/s001220051515
26. Nybom H, Bartish IV. Effects of life history traits and sampling strategies on genetic diversity estimates obtained with RAPD markers in plants. Perspect Plant Ecol Evol Syst. 2000;3(2):93–114. http://dx.doi.org/10.1078/1433-8319-00006
27. Kreher SA, Foré SA, Collins BS. Genetic variation within and among patches of the clonal species, *Vaccinium stamineum* L. Mol Ecol. 2000;9(9):1247–1252. http://dx.doi.org/0.1046/j.1365-294x.2000.01002.x
28. Persson HA, Gustavsson BA. The extent of clonality and genetic diversity in lingonberry (*Vaccinium vitis-idaea* L.) revealed by RAPDs and leaf-shape analysis. Mol Ecol. 2001;10(6):1385–1397. http://dx.doi.org/10.1046/j.1365-294X.2001.01280.x
29. Albert T, Raspé O, Jacquemart AL. Clonal diversity and genetic structure in *Vaccinium myrtillus* populations from different habitats. Belg J Bot. 2004;137(2):155–162.
30. Stewart CN, Excoffier L. Assessing population genetic structure and variability with RAPD data: application to *Vaccinium macrocarpon* (American Cranberry). J Evol Biol. 1996;9(2):153–171. http://dx.doi.org/10.1046/j.1420-9101.1996.9020153.x
31. Jordano P, Godoy JA. RAPD variation and population genetic structure in *Prunus mahaleb* (Rosaceae), an animal-dispersed tree. Mol Ecol. 2000;9(9):1293–1305. http://dx.doi.org/10.1046/j.1365-294x.2000.01009.x
32. Ge S. RAPD variation within and between natural populations of the wild rice *Oryza rufipogon* from China and Brazil. Heredity. 1999;82(6):638. http://dx.doi.org/10.1046/j.1365-2540.1999.00516.x
33. Garkava-Gustavsson L, Persson HA, Nybom H, Rumpunen K, Gustavsson BA, Bartish IV. RAPD-based analysis of genetic diversity and selection of lingonberry (*Vaccinium vitis-idaea* L.) material for ex situ conservation. Genet Resour Crop Evol. 2005;52(6):723–735. http://dx.doi.org/10.1007/s10722-003-6123-4
34. Godt MJW, Hamrick JL. Population genetic analysis of *Elliottia racemosa* (Ericaceae), a rare Georgia shrub. Mol Ecol. 1999;8(1):75–82. http://dx.doi.org/10.1046/j.1365-294X.1999.00539.x
35. Bockelmann AC, Reusch TBH, Bijlsma R, Bakker JP. Habitat differentiation vs. isolation-by-distance: the genetic population structure of *Elymus athericus* in European salt marshes. Mol Ecol. 2003;12(2):505–515. http://dx.doi.org/10.1046/j.1365-294X.2003.01706.x
36. Tsyusko OV, Smith MH, Sharitz RR, Glenn TC. Genetic and clonal diversity of two cattail species, *Typha latifolia* and *T. angustifolia* (Typhaceae), from Ukraine. Am J Bot. 2005;92(7):1161–1169. http://dx.doi.org/10.3732/ajb.92.7.1161