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# PLANT MORPHOLOGY AND RAPD MARKER CHARACTERIZATIONS OF *VACCINIUM OXYCOCCUS* LITHUANIAN POPULATIONS

# *VACCINIUM OXYCOCCUS* LIETUVAS AUGU POPULĀCIJU MORFOLOĢIJA UN RAKSTUROJUMS AR RAPD MARĶIERIEM

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## Abstract

Plant morphogenesis is a useful tool for physiological, biochemical, and molecular studies. The diversity in plant form is produced mainly because different parts of the plant grow at different rates. Furthermore, the growth of an individual structure is different in various dimensions. Such differential growth rates are very well determined by genetic factors.

Understanding the relationships among wild cranberry morphologic and genetic characteristics may provide insights for the better utilization of germplasm. The objectives of this research were to determine the relationships between the genotype and the development of morphologic features. Eleven morphologic characteristics (vegetative stage, flowering time, ripening start and ending, mass, volume and size of berry, berry form, leaf shape, steepness of upright) of 56 morphologically diverse genotypes from the 4 Lithuanian bogs were analyzed using random amplified polymorphic DNA (RAPD) classifications. To compare wild cranberry (*Vaccinium oxycoccus*) morphologic characteristic 213 RAPD bands were used. Comparing wild cranberry genetic and morphologic features there were found some significant variations.

## Kopsavilkums

Augu morfoģenēzes analīze ir noderīga metode fizioloģijas, bioķīmijas un molekulārajās studijās. Augu formu dažādība rodas galvenokārt tāpēc, ka dažādas auga daļas aug dažādos ātrumos. Turklāt, atsevišķas struktūras augšana var notikt vairākās dimensijās. Šīs augšanas atšķiribas tiek galvenokārt noteiktas ģenētiski.

Sakarības izzināšana starp savvaļas dzērveņu morfoloģisko un ģenētisko raksturojumu var ļaut labāk izmantot dzērveņu genofondu. Sī pētījuma mērķis bija noteikt sakarības starp genotipu un morfoloģisko pazīmju attīstību. Kopumā tika analizētas 56 morfoloģiski atšķirīgi genotipi no četriem Lietuvas purviem. Izmantojot klasifikāciju pēc RAPD marķieriem, tika pētītas vienpadsmit morfoloģiskās pazīmes (veģetatīvās fazes ilgums, ziedēšanas laiks, nogatavošanās sākums un beigas, ogas masa, tilpums un lielums, ogu un lapu forma, dzinumu stāvums). Savvaļas dzērveņu (*Vaccinium oxycoccus*) morfoloģisko atšķirību raksturošanai tika izmanotas 213 RAPD līnijas.

Tika atrastas dažas būtiskas atsķirības starp savvaļas dzērveņu ģenētisko un morfoloģisko raksturojumu.

**Key words**: genetic and morphological correlation; morphological characteristics; RAPD characterization

## Introduction.

The berries of wild cranberry *(Vaccinium oxycoccos* L.) are very valuable because they possess a large content of biologically active substances (flavonoids, acids, vitamins), that may help protect against heart disease, the ability of blood vessels to relax - in subjects with high blood cholesterol and atherosclerosis (Vorsa *et al*., 2002; Wang *et al*., 1996). Antioxidants found in this berries help to neutralize harmful free radicals that are thought to be linked to most chronic diseases including cancer, diabetes and heart disease (Budriuniene, 1998; Vedenskaja and Vorsa, 2004).

Multiple characteristics provide good support for the recognition of species and the determination of relationships among taxa. The strength of support for a lineage is based on the assumption that multiple characteristics have been acquired independently of each other. However, many suites of characteristics, such as the complex flowers of *Asclepiadaceae* and *Orchidaceae*, may be functionally integrated with each other and are probably not acquired independently. Others, such as the overall size of different organs, may have a common genetic and developmental basis in addition to functional integration (McLellan, 2005).

Genetic basis play important role in evolution of plant morphology (Davis, 2001; Conner, 2002; Ungerer *et al.,* 2002). Two possible mechanisms for these correlations are the association of more than one phenotypic characteristic with a single genotype, and linkage, when independent genes that determine different traits are inherited together because they are located near each other in the genome (McLellan, 2005).

The purpose of this paper is to examine correlations between eighteen different morphological traits among four populations of the *V. oxycoccus*. Also to compare the morphological and RAPD marker classifications of 56 morphologically diverse genotypes, and to determine the relationship of their RAPD and morphologic features.

## Material and methods.

Morphologic features analysis. Fifty six cranberry cuttings with clearly differing vegetative indications (color, size, shape of berry and productivity) (Daubaras et all., 2004) were collected from 4 populations in Lithuania bogs (Čepkeliai (54$^{o}$00’-54$^{o}$03’N, 24o25’-24$^{o}$35’E), Žuvintas (54$^{o}$23’- 54$^{o}$30’N, 23$^{o}$25’-23$^{o}$40’E), Kamanos (56$^{o}$15’-56$^{o}$20’N, 21$^{o}$35’-22$^{o}$45’E) and Aukštatija(55 $^{o}$40’ - 55$^{o}$00’N, 25 $^{o}$80’ – 26 $^{o}$25’)) during 1995–1999. The cuttings were arranged in the Kaunas Botanical Garden collection into special peat (pH 4.0-5.0) beds. These clones were selected for further evaluation *ex situ*. Eleven morphological properties of shoots, leaves and berries for the morphological characterization of the clones were used along the descriptor list for cranberries (Budriūnienė, 1998). Depending on the size berries were classified in to 5 groups: very small (<0.3 g); small (0.3 - 0.5 g); medium (0.6-1.0g); large (1.1-1.5 g); very large (>1.5 g). Flowering shoots were measured at the end of growth. The main phenological phases of the investigated clones were recorded also. During four years (2001 - 2004) clones were observed and evaluated according to the phenological parameters (stages) as: vegetative stage, beginning of blossoming, beginning of berry ripening, and end of berry ripening. The length of generative shoots, volume of the berries, leaf length, leaf width, and berry weight was also measured. These morphological data were used for statistical analysis.

DNA analysis. DNA extraction and RAPD analysis was done as it is described previously (Areškevičiūtė et all, 2006). Nine (OPA-1, OPA-4, OPA-5, OPA-9, OPA-10, OPB-11, ROTH-6, ROTH-8, ROTH-9) 10 base pair length primers of random sequence (Fermentas, Lietuva; Roth, Germany) were used. DNA amplification was performed in a 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.

Calculation of the observed number of alleles, Nei's (Peer et all., 1994; Nei, 1973) gene diversity (H), Shannon's Information Index (I), total gene diversity (Ht), gene diversity within populations (Hs), gene diversity among populations (Gst = (Ht–Hs) / Ht), gene flow (Nm = 0.5 (1–Gst) / Gst) and generation of a Nei's genetic distance based dendrogram were carried out with POPGENE V 1.31 software (Yeh and Yang 1999). Principal coordinates analysis were performed with the GenAlEx 6 program (Peakala and Smouse 2006).

Statistical Analyses. The test for the significance of each 213 RAPD product band was done using the Pearson Chi-Square ($p \geq 0.05$) (Kish, 1987).To find out relatedness between RAPD fragments and phenological stages we searched for correlations. Stepwise multiple correlations (two-tailed significance level 0.05) were used to determine which combinations of genetic variables were associated with morphologic traits were carried out with SPSS 13.0 for Widows software.

## Results and Discussion

In our study, RAPD markers proved to be a powerful method for the detection of spatial genetic variation. Based on the literature (Nei and Li, 1979).we have chosen nine 6 OPA and 3 ROTH primers. With nine primers, we obtained 213 fragments and could differentiate the 56 *V. oxycoccus* clones, reflecting a rich allelic diversity among the populations.

The size of the amplified fragments ranged from 80 to 2750 bp, and all loci were polymorphic. The number of bands per primer ranged from 13 (RAPD OPB-11) to 30 (RAPD ROTH-180-09). The polymorphism level for populations was as follows: Čepkeliai 52.11%, Žuvintas 37.09 %, Aukštaitija 43.19% and in Kamanos population 36.62 %.

To estimate genetic variation between populations, the values of Shannon's Information Index (I), Nei'sgene diversity (H) and the observed number of allelesper locus (Na), the number and percentage of polymorphic loci were calculated (Table 1). For the total sample, Shannon's Information Index was 0.2 and Nei's gene diversity 0.10. The observed number of alleles per locus ranged from 0.1 in Čepkeliai population to 1.4 in Kamanos and Žuvintas population. The estimated total proportion of diversity among populations ($G_{ST}$) and gene flow (Nm) were 0.15 and 2.9 respectively.

Shannon's index estimates of intraspecific genetic diversity within *V. oxycoccus* were higher than in other plant species (Jogaitė et all, 2006). Shannon's information index has general applications in ecology and is relatively insensitive to the skewing effects caused by the inability to detect heterozygous loci (Dawson, 1995).

Table 1. Values of genetic diversity indicated in Lithuanian populations of *V. oxycoccus*.

| Population | Shanon's Information Index (I) | Nei's gene diversity (H) | Observed mean number of alleles (Na) | The number of polymorphic loci | The percentage of polymorphic loci %(P) |
|---|---|---|---|---|---|
| Čepkeliai | 0.225 | 0.142 | 1.5211 | 111 | 52.11 |
| Žuvintas | 0.120 | 0.071 | 1.3709 | 79 | 37.09 |
| Kamanos | 0.086 | 0.045 | 1.3662 | 78 | 36.62 |
| Aukštaitija | 0.189 | 0.120 | 1.4319 | 92 | 43.19 |
| Total | 0.195 | 0.102 | 1.9953 | 213 | 99.53 |

Owing to this life history trait on genetic diversity, a low genetic diversity within but a high diversity among populations is expected (Kreher, 2000). Plants with highest genetic diversity within and among populations can better adapt to different environmental conditions.

Analyzing molecular variance of *Vaccinium* and other plant species were observed that biggest part of molecular variance were within populations (Stewart and Excoffier, 1996; Jordano and Godoy 2000; Jürgens et all., 2007). Average molecular variance within *Vaccinium* species populations were 87.7% and within populations were 27.7 %. Highest molecular variance was detected within american cranberry (*V. macrocarpon*) populations (more than 91 %) (Stewart and Excoffier, 1996). In *V. ulingosum* – 90.3 %(Kreher et all., 2000) in *V. myrtillus* – 86.19 %(Albert et all., 2004, Garkava-Gustavson et all., 2005).Thus, our study revealed a comparably low DNA polymorphism level in *Vaccinium oxycoccus* populations (Table 1). RAPDs indicate that Lithuanian *V. oxycoccus* appears to maintain a quite low level of the genotypic variance among populations (25 %). and within (75 %) Lithuanian *V. oxycoccus* populations was found compared to American *V. macrocarpon* and the other *Vaccinium* species.

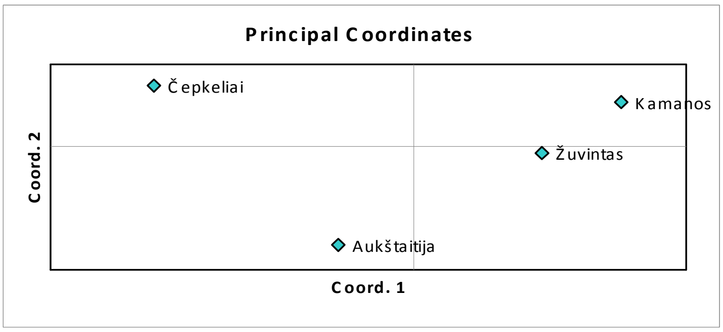

Figure 1. Distribution of *V. oxycoccus* populations in principal coordinates analysis.

To estimate the relationship between *V. oxycoccus* populations, Nei's genetic distance between pairs of populations was calculated. To generate graphs principal coordinate analysis (PCA) was used. Graphs show genetic distances among samples and populations. The PCA analyses have revealed that Čepkeliai populations of *V.oxycoccus* had homogenous genotype and all samples are in one lineage. Kamanos, Žuvintas and Aukštaitija populations are found in one site and have mixed genotypes. (Figure 2). The resulting different and mixed lineages can confirm the prediction that these three Lithuanian *V. oxycoccus* populations were derived from one population before glaciation and the Čepkeliai population differed from them. This prediction also confirms and PCA analysis (Figure 1). Postglacial decolonization one more factor that could have influenced these differences is because the populations Čepkeliai, Žuvintas, Kamanos and Aukštatija (Webb and Bartelein, 1992). According to Lithuanian deglaciation periods these four populations genetically separated into different lineages this can confirm glaciation stages in Lithuania (Figure 1). The second factor explaining our results is wide river Nemunas separating Čepkeliai from other evaluated bogs.

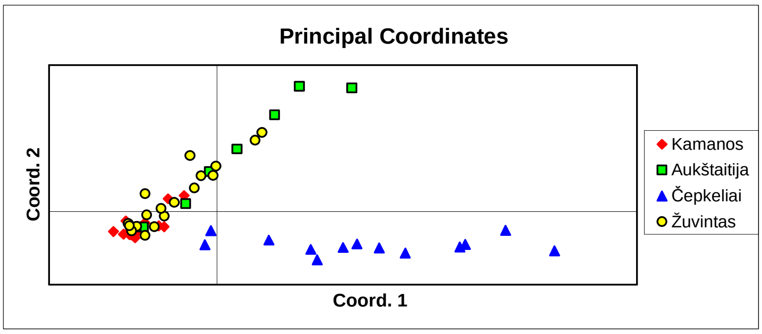

Figure 2. Distribution of *V. oxycoccus* populations by clones in principal coordinates analysis. Different population clones are marked by different marker.

Using statistic analysis 119 out of 213 RAPD band products were identified as significantly contributing to the genetic and morphologic classification (OPA-1,125, 275, 400, 450, 500, 550, 625, 650, 700, 750, 850, 1131, 1200, 12800, 1300, 1750 bp. OPA-4 225, 550, 600, 810, 1040, 1150, 1550 bp. OPA-5 280, 350, 375, 400, 440, 500, 550, 590, 600, 700, 750, 800, 900, 1031, 1040, 1230, 2000 bp. OPA-9 320, 350, 390, 400, 465, 550, 630, 650, 680, 710, 750, 850, 1234, 1500, 2900 bp. OPA-10 290, 325, 375, 400, 450, 500, 550, 690, 700, 750, 800, 850, 900, 690, 1031, 1150, 1200, 1234, 1550, 2000 bp. OPB-11 300, 400, 650, 700, 750, 850, 1031, 1131, 1200, 1700, 1900 bp. ROTH-6 175, 420, 460, 500, 520, 600, 700, 820, 1031, 1150 bp. ROTH-8 440, 600, 950 bp. ROTH-9 110, 190, 200, 250, 300, 310, 320, 350, 420, 440, 450, 500, 580, 650, 680, 700, 850, 900, 1031, 1350, 1750, 2400 bp.). Some significant molecular fragments and morphologic data correlations were also found (Table 2).

Table 2. Calculated significant fragments and phenological parameters correlations per primer in Lithuanian populations of *V. oxycoccus*.

| Morphologic characteristics | roth 9 | opa 1 | opa 4 | opa 5 | opa 9 | opa 10 | opb 11 | roth 6 | roth 8 | total |
|---|---|---|---|---|---|---|---|---|---|---|
| Vegetative stage | 4* | 5 | 1 | 4 | 3 | 3 | 6 | 3 | 1 | 31 |
| beginning of blossom | 3 | 4 | -** | 1 | 2 | 3 | 5 | 2 | 1 | 21 |
| beginning of berries ripening | 1 | 1 | - | - | 1 | 2 | - | - | - | 6 |
| end of berries ripening | 1 | - | 1 | 1 | 1 | - | 1 | - | 1 | 6 |
| length of generative uprights | 2 | 2 | - | - | 1 | - | 1 | - | - | 6 |
| volume of berry | 3 | - | - | 1 | - | - | 1 | - | - | 5 |
| leaf length | 2 | 1 | 1 | 2 | 1 | 1 | 1 | 1 | 1 | 11 |
| leaf width | 4 | 3 | 1 | 3 | - | 3 | 3 | - | - | 17 |
| berry mass | 5 | - | 1 | 2 | 1 | 4 | - | 1 | - | 14 |

* Numbers inside table shows how many correlations were find with significant fragments and morphologic feature per primer.

** Trace shows that there were no correlation.

According to correlations between phenological parameters and significant fragments per primer, we can see that biggest correlations were with vegetative stage and beginning of flowering. Low correlations were with the beginning of berry ripening, the end of berry ripening, length of generative shoots, and the capacity of the berries.

## Conclusions

According to correlations between morphologic features and primers fragments we can see that the biggest correlations were with vegetative stage and the beginning of flowering. Low correlations were between the beginning of berry ripening, the end of berry ripening, length of generative shoots, and the volume of the berries.

The resulting different and mixed lineages can confirm the prediction that these three Lithuanian *V. oxycoccus* (Žuvintas, Kamanos and Aukštaitija) populations belonged to one population before glaciations and the Čepkeliai population differed from them. This could be because of the influence of the river Nemunas and of postglacial decolonization

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