

MORPHOLOGICAL DIVERSITY OF EUROPEAN CRANBERRY (*VACCINIUM OXYCOCCOS*) IN ŽUVINTAS RESERVE

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Clones of wild cranberry (*Vaccinium oxycoccos*) were selected and described in reserve Žuvintas in 1996 and 1999. The cuttings of these clones were collected, propagated and planted into acid peat (pH 4.0-5.0) beds in Kaunas Botanical Garden of VMU for further evaluation. Plants grown in the field collection distinguished themselves for larger and thicker leaves, bigger berries, which were predefined by better growth conditions in the field collection. The ripening period of investigated clones began in mid August through early September, whereas the ripening of berries came to an end in late September through early October. The period of late spring must be distinguished as critical for *V. oxycoccos* clones, while late spring frosts damages determined considerable decrease or complete destruction of berry yield of all clones in 2000 and 2004. The estimation of the yield and berry size indicated quite big differences among the Žuvintas clones *ex situ*. The conspicuous by good productivity and large berries the clones 99-Ž-11, 96-Ž-10, and 99-Ž-10 were selected in the collection and proposed for cultivar approval.

Key words: clone, collection, cranberry, morphological characteristic

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INTRODUCTION

Natural resources of European cranberry are found in raised bogs. This species grows on peat in poorly drained, subhygric or hygric sites that have a very high water level. The bog sites derive water from precipitation only and are nutrient-poor and low in productivity. The soil is very acid and pH ranges from 3.0 to 4.5. The existence of bogs vegetation was seriously threatened by the effects of land reclamation in Lithuania. Large areas of raised bogs suffered from eutrophication, which has adverse effect on existent bog

vegetation. The reduction of water level promoted the growth of shrubs (*Ledum palustre* L., *Calluna vulgaris* (L.) Hull, *Vaccinium uliginosum* L.) and reduced the vitality of European cranberry. The consumption of these valuable berries kept long-lasting traditions in Lithuania. This species was very appreciated in folk medicine, but land reclamation caused the critical decreasing of the natural resources of European cranberry in Lithuania (Budriūnienė 1998, Daubaras & Česonienė 2004).

The species *Vaccinium oxycoccos* L. (syn. *Oxycoccus palustris* Pers.) belongs to the family *Ericaceae* Juss. It is a dwarf, woody, evergreen shrub. Stems are woody, slender and rooting, occasionally up to 0.8-1.0 m with short flowering shoots. The leaves are leathery, dark shining green above, glaucous beneath, the margin of the blade is entire, frequently revolute. The white, pink or red flowers are grouped in racemes of 1-5. The fruit is an overwintering berry (Jaquemart 1997). This species distinguishes itself for high intraspecific diversity. Other researchers described cranberry clones growing in the same bog, however, differing mostly in berry size, colour and shape (Lekavičius & Butkus 1972, Cherkasov 1975) or shoot length and diameter predetermined by the ecological and phytocenological conditions (Stackevičienė & Labokas 2000). The research works in Estonia, Latvia, and Finland corroborated high level of phenotypic diversity of European cranberry as well. H. Vilbaste investigated diversity of this species in Estonian bogs and collected about 760 clones conspicuous by leaf and berry size, berry and flower colour and berry shape (Ruus & Vilbaste 1968). The cranberry collection with more than 190 clones was established in Latvia (Gronskij & Liepnice 1989). Investigations carried out in 1965-1970 in Lithuania showed that berry shape changed on a large scale. According to these investigations 17 forms of European cranberry were distinguished and described (Lekavičius & Butkus 1972).

The aim of this study was to compare morphological peculiarities, productivity, and seasonal development of European cranberry clones collected in reserve Žuvintas.

MATERIAL AND METHODS

Plant material for the evaluations was collected in the strictly protected reserve Žuvintas in 1996 and 1999, where great morphological variation had been noticed tentatively. Nineteen clones with clearly distinctive vegetative properties, berry size, shape, and colour were selected in

this reserve. The *GPS Magelan 315* receiver was used to establish geographical positions of their habitats. The clones were planted in the field collection to avoid the influence of various ecological factors in natural habitats. The collected cuttings of these clones were planted into acid peat (pH 4.0-5.0) beds in the field collection of Kaunas Botanical Garden for further investigations under *ex situ* conditions.

A detailed evaluation of morphological diversity of these clones was carried out in 1999-2008. For the morphological characterization different stems, leaves and berry properties per clone were used: leaf size and shape, shape of leaf apex and base, leaf margin bending, colour of a fully opened flower, length of peduncle, berry size, shape, colour, cross-section shape, and waxy layer intensity, as well as berry flesh colour and taste. The average weight of a berry was calculated by weighing 50 berries in three replications. A 1-5-point scale was used for berry weight estimation where 1 stands for very small (<0.3 g), 2 – small (0.3-0.5 g), 3 – medium (0.6-1.0 g), 4 – large (1.1-1.5 g), and 5 – very large berries (>1.5 g). The yield production of the clones was estimated. The average generative shoot length was ascertained by measure of 50 shoots. The mean area of a leaf was determined by scanning 30 leaves with a scanner (HP Scan Jet 3600) in three replications and applying two noise removal filters. The image threshold was set with user-chosen values and the amount of dark pixels representing the actual area of leaves were counted.

Phenological observations were accomplished during entire vegetation period. The collection of *V. oxycoccos* was observed and assessed twice a week on the same days of the week. The following main phases of seasonal development were recorded: the beginning of shoot growth; the beginning of budding; the beginning of flowering; the end of flowering; the beginning of ripening; the end of ripening; the end of vegetation.

Mathematical - statistical assessment on the data has been fulfilled using program packet SELEKCIJA, which operates as applied to the basic program in the *Excel* packet. Specific differences were identified with LSD (the least significant difference) (Tarakanovas & Raudonius 2003).

RESULTS

It was observed that in the field collection some of cranberry morphological properties varied seldom and within a very narrow range. Therefore, it was difficult to identify the differences. These properties were shape of a leaf apex and base, leaf margin bending, and length of peduncle. The other group of properties included the ones, which were difficult to estimate, because they are related to plant seasonal development phases. For instance, it was difficult to estimate flower properties of clones as well as the colour of fully ripe berries and intensity of a waxy layer. Herewith differences in these properties become much more evident while cultivating plants in the same place, under the same environmental conditions. If compared to the natural bogs, plants grown in the field collection distinguished themselves for larger and thicker leaves, bigger berries, which were predefined by better growth conditions in the field collection.

The determination of berry shape of European cranberry revealed high variability. The most common were clones with round or oblate berries (Table 1). The average length and width of a berry varied from 0.99 to 1.38 cm and from 1.01 to 1.36 cm, respectively. The clones 96-Ž-03 and 99-Ž-07 were conspicuous by their unique egg-shaped and cylindrical berries. The colour of berries was red or dark red at full maturation. Only berries of clone 99-Ž-10 were distinguish for pink colour. The berries of clones 99-Ž-16, 99-Ž-09, and 96-Ž-11 were covered with a waxy coat.

Statistically reliable differences in the average weight of a berry were ascertained since Fisher's criterion was $F_{01(\text{fact})} = 31.69 > F_{01(\text{theor})} = 1.54$. The

clones 99-Ž-11, 96-Ž-13, 96-Ž-10, 99-Ž-14 were distinguish for the largest berries. It has been found that the berries of twelve clones fall into the group of medium-sized (0.6-1.0g), the berries of seven clones were large (1.1-1.5g). The average berry weight of Žuvintas clones varied slightly (the variation coefficient $V < 10\%$). The largest variation of berry weight was detected in clones 99-Ž-08 ($V = 15.1\%$), 99-Ž-10 ($V = 13.7\%$), and 99-Ž-11 ($V = 13.2\%$). The average berry weight of clones investigated was 0.92 g (Table 2).

The estimation of the berry yield *ex situ* indicated quite big differences among the Žuvintas clones. The most productive was the clone 96-Ž-11 with an average yield 1.75 kg/m². The average yield of other clones varied from 0.3 kg/m² (the clone 99-Ž-11) to 1.3 kg/m² (the clone 99-Ž-04).

Leaf shape was evaluated as well. Majority of the investigated samples had egg-shaped leaves. The analysis of leaf size showed that most clones (84%) were characteristic of medium leaves, 7% had small leaves, 9% - large ones. The clones with the smallest leaves were 99-Ž-05 (0.26 cm²), 99-Ž-06 (0.28 cm²), and 96-Ž-11 (0.29 cm²). The clones 96-Ž-14, 99-Ž-11, and 96-Ž-13 were distinguish for the largest leaves, respectively 0.44 cm², 0.42 cm², and 0.41 cm². Average length of generative shoot varied from 3.25 cm to 5.43 cm (Table 2).

V. oxycoccos clones were characteristic of significant phenological plasticity. In different years the beginning of phenological phases possessed the wide amplitude (from 10 to 19 days) of variation. A comparison of the duration of the vegetation period in 1999-2008 revealed no reliable differences between the clones. It was estimated out that differences between the clones in the phase of flower bud formation varied from 3 to 9 days and in the phase of the beginning of berry ripening – from 7 to 20 days. There is a risk of late frosts during the cranberry flowering period in late May through mid June, which could cause the loss of flowers and proportionately the berry yield. The flowering period persisted from 12 to 23 days (Fig. 1). The berry ripening of

Table 1. Morphological characteristics of *Vaccinium oxycoccos* berries

Clone	Berry length, cm	Berry width, cm	Prevailing shape of a berry	Berry colour
96-Ž-02	1.14	1.28	oblate	dark red
96-Ž-03	1.29	1.01	egg-shaped	dark red
96-Ž-10	1.18	1.36	round	dark red
96-Ž-11	1.35	1.32	oblate	dark red
96-Ž-12	1.15	1.04	oblate	dark red
96-Ž-13	1.29	1.30	round	red
96-Ž-14	1.15	1.10	round	red
99-Ž-01	1.20	1.25	round	red
99-Ž-02	1.18	1.22	round	red
99-Ž-03	1.11	1.12	round	dark red
99-Ž-04	1.19	1.21	round	dark red
99-Ž-07	1.38	1.29	cylindrical	red
99-Ž-09	1.02	1.04	round	dark red
99-Ž-10	1.00	1.28	oblate	pink
99-Ž-11	1.21	1.29	oblate	red
99-Ž-12	1.35	1.25	ovale	dark red
99-Ž-13	1.29	1.21	ovale	dark red
99-Ž-16	1.16	1.32	oblate	dark red
99-Ž-18	0.99	1.28	oblate	dark red
LSD_{01}	0.049	0.064		
$S_x\%$	1.50	2,131		

LSD_{01} - the least significant difference, $P < 0.01$

$S_x\%$ - the relative error of an average

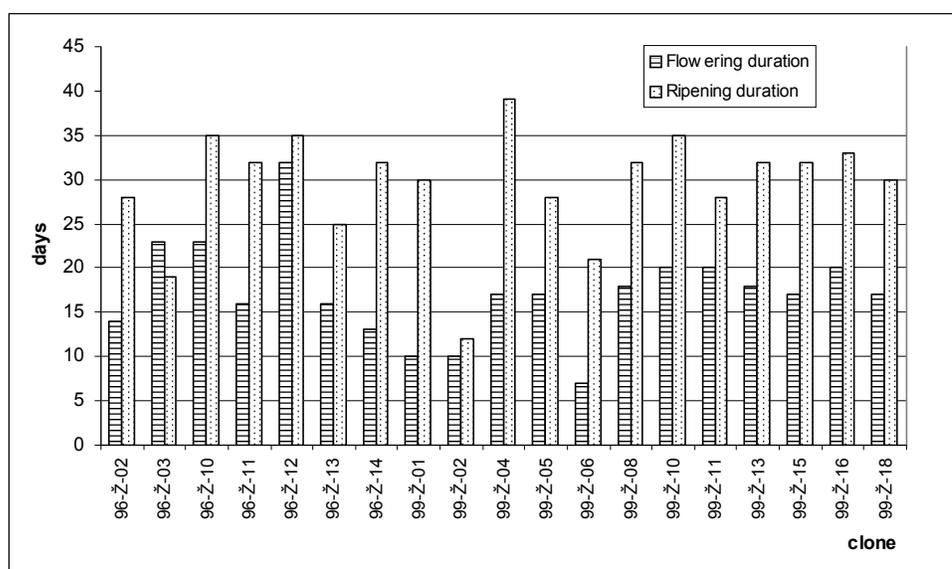


Fig. 1. Duration of *Vaccinium oxycoccos* clones flowering and ripening periods

Table 2. Summary statistics of *V. oxycoccos* clones characteristics

Characteristic	M	SD	M _{min} -M _{max}	F
Berry weight, g	0.92	0.17	0.67-1.09	31.69**
Leaf area, cm ²	0.39	0.05	0.26-0.44	20.09**
Generative shoot length, cm	4.23	0.57	3.25-5.43	7.6*

M- mean, SD - standard deviation, M_{min}-M_{max} - range of mean values

F – Fisher's criterion, ** P<0.01, * P<0.05

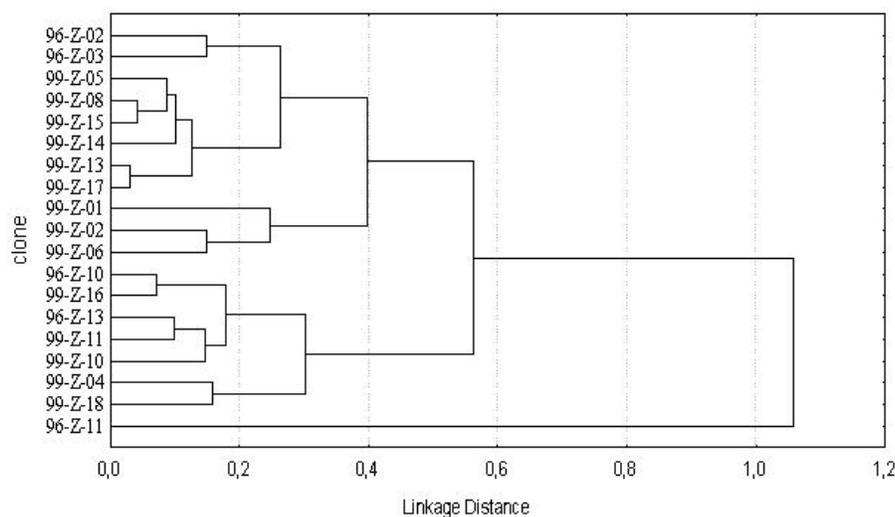


Fig. 2. UPGMA dendrogram of 19 *Vaccinium oxycoccos* clones according to an average berry weight and an average yield

Žuvintas clones continued from 19 to 35 days. The clones 99-Ž-04, 96-Ž-12 and 99-Ž-10 were characteristic of the lengthy ripening, respectively 39, 35, and 36 days. The clone 99-Ž-02 were distinguish for the shortest flowering and ripening periods. The ripening time of berries depended on prevailing meteorological conditions as well as on the clone peculiarities. The beginning of ripening was observed from mid August through early September, whereas the ripening of berries came to an end in late September through early October. While assessing adaptation possibilities of *V. oxycoccos* clones the period of spring must be distinguished as critical: damage determined after late frost in spring resulted in a considerable decrease or complete destruction of berry yield of all clones in 2000 and 2004.

DISCUSSION

Natural resources of European cranberry can found in Scandinavia, Baltic States, Poland, Byelorussia, Ukraine, Russia, and Alpine zone of Switzerland, France, Italy (Kardell 1986, Pliszka, 1997). Cranberry productivity in natural habitats is determined by different factors, i.e. bog type, ecological and phytocenological conditions (Labokas & Stackevičienė 2000). Nevertheless, natural resources of this valuable berry plant were significantly reduced in Lithuania. For this reason the collecting of European cranberry genetic resources is necessary. The cranberry collection in Kaunas Botanical Garden of VMU demonstrates wide range of morphological diversity. Evaluation of Žuvintas clones revealed statistically reliable differences in respect of berry

and leaf size, productivity, and seasonal development. Ravanko (1990) maintains that European cranberry is a polyploidy complex comprising diploids, tetraploids and hexaploids, whereas leaf dimensions could be used for distinguishing diploids from polyploids.

The similarity of different clones of *V. oxycoccus* were defined on the basis of hierarchical cluster analysis according to an average berry weight and an average yield. For division of *V. oxycoccus* into clusters, Unweighted pair-group average method was taken (Fig. 2). Altogether, two main clusters of different size were recognized. Seven clones 99-Ž-18, 99-Ž-04, 99-Ž-10, 99-Ž-11, 96-Ž-13, 99-Ž-16, and 96-Ž-10 with the large berries and good yield were included into the first cluster. The second large cluster was comprised of two subclusters at the Euclidean distance 0.408. The clones 99-Ž-06, 99-Ž-02, and 99-Ž-01 with medium berries and small yield formed the first small subcluster. The clones 99-Ž-17, 99-Ž-13, 99-Ž-14, 99-Ž-15, 99-Ž-08, 99-Ž-05, 96-Ž-03, and 96-Ž-02 with medium berries and varied yield were amalgamated into the second subcluster. The clone 96-Ž-11 which has been distinguished by large berries (1.1 g) and high yield were separated from all other on the Euclidean distance 0.106.

Significant differences in the yield and the berry size of clones in the *ex situ* collection indicate a good possibility for European cranberry breeding. This is also confirmed by the Russian investigators, who have recently released new cultivars of *V. oxycoccus*. The selection of economic valuable clones of European cranberry were carried out in Kostroma Forest Research Station. The long-term researches were resulted in selection of six *V. oxycoccus* clones. These clones were registered by the State Commission of Russian Federation for Testing and Protection of Selection Achievements as the first Russian cultivars 'Alaya Zapovednaya', 'Krasa Severa', 'Sazonovskaja', 'Severyanka', 'Sominckaja', and 'Khotavetskaya' (Cherkasov et al. 1998, Makeev et al. 2000). Six cultivars suitable for garden cultivation have been selected from the wild clones in Estonia as well: 'Kuresoo',

'Nigula', 'Soontagana', 'Maima', 'Virussaare', and 'Tartu'. In distinguishing the different cultivars special characteristics including berry size and shape have been used (Ruus & Vilbaste 1968).

The research accomplished promotes the selection of clones with valuable properties *in situ* and preservation in the field collection of European cranberry germplasm at Kaunas Botanical Garden. Integrated evaluation of morphological and economical peculiarities of Žuvintas clones based the selection of the valuable clones 96-Ž-11, 96-Ž-10, and 99-Ž-10. These clones were entitled, respectively 'Amalva', 'Vita', and 'Žuvinta'. The proposal to approve the cultivars were put forward. The origination of these cultivars will promote cultivation of *V. oxycoccus* in Lithuania.

Conclusions:

1. The clones of *V. oxycoccus* collected in Žuvintas reserve demonstrate wide range of morphological diversity and productivity.
2. The flowering period of Žuvintas clones continues from 12 to 23 days. The ripening period lasts from 19 to 35 days.
3. The accomplished evaluation of morphological and economical peculiarities of *V. oxycoccus* clones based the selection of the valuable clones 96-Ž-11, 96-Ž-10, and 99-Ž-10, which are prospective for cultivation.

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