

Evaluation of Morphological Peculiarities, Amount of Total Phenolics and Anthocyanins in Berries of European Cranberry (*Oxycoccus palustris*)

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Abstract

The clones with different size, shape and colouration of a berry were selected in the strictly protected areas Čepkeliai and Žuvintas. They were propagated and evaluated under *ex situ* conditions in the field collection of Kaunas Botanical Garden of Vytautas Magnus University. The assessing of the average berry weight corroborated high variability. Most of the clones investigated fall into the medium-sized group (average berry weight from 0.6 to 1.0g). The high variability of total amounts of phenolics and anthocyanins in the berries was detected. The clones of European cranberry on average accumulated 98.6 mg/100g of anthocyanins and 365 mg/100g of phenolics. Small and dark red berries accumulated the largest amounts of anthocyanins. The clones with the largest amounts of these compounds were selected for the permanent preservation *ex situ* in the collection at Kaunas Botanical Garden.

Key words: anthocyanins, clone, collection, cranberry, phenolics

Introduction

European cranberry (*Oxycoccus palustris* Pers.), species of the *Ericaceae* family, is distributed in sphagnum bogs in the boreal zone. This evergreen dwarf shrub grows in peat on poorly drained sites with a very high water table. This species is typical of the *Oxycocco-Sphagnetum* communities in oligotrophic habitats with the acid peat, pH 2.3-4.5 (Ozolinčius *et al.* 2004).

The research works in Lithuania as well as in Estonia, Latvia and Finland corroborated a high level of phenotypic diversity of European cranberry. H. Vilbaste investigated the 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 (Вильбасте, Вильбасте 1980). The cranberry collection with more than 190 clones was established in Latvia (Гронский, Лиепнице 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 (Лякавичюс, Буткус 1972).

European cranberry was widely spread in Lithuania till the sixth decade of the last century. The consumption of these valuable berries kept long-lasting traditions. This species was very appreciated in folk medicine but land melioration caused the critical decreasing of the natural resources of European cranberry in Lithuania (Daubaras and Česonienė 2004). The reduction in the ground water level diminished the vitality of *O. palustris*. The unique collection of genetic resources of this species was established at Kaunas Botanical Garden of Vytautas Magnus University with the purpose to preserve valuable clones selected in the strictly protected areas Čepkeliai, Žuvintas, Kamanos and in other bogs as well. The comprehensive evaluations of phenotypic and genetic diversity of European cranberry were conducted in 1997-2005 (Daubaras *et al.* 2004, Areškevičiūtė *et al.* 2006).

The latest assessment of European and American cranberry has revealed very valuable biochemical composition of the berries. Phenolic compounds in cranberries are a diverse group that includes anthocyanins, flavonoids, proanthocyanidins, phenolic

acids. These compounds have been identified as strong antioxidants, with the potential to prevent oxidative damage and protect against cardiovascular diseases and some cancers (Vinson *et al.* 2001, Wang and Stretch 2001, Vorsa *et al.* 2002; Xiaojun *et al.* 2002). The phenolic phytochemicals are secondary metabolites that distinguish for the function to protect plants against biological and environmental stresses, i.e. fungal or bacterial infections (Kähkönen *et al.* 1997). The red colour of cranberry fruit is due to the presence of anthocyanins. Of importance are anti-inflammatory, antioxidant and antiulcer activities of these compounds (Wang *et al.* 1999, Foo *et al.* 2000, Vinson *et al.* 2001).

The aim of this study was to assess morphological peculiarities of European cranberry berries and compare them by the total amounts of phenolics and anthocyanins. The result of evaluation could be the base for permanent preservation of the most valuable clones in the collection *ex situ* and promote the future breeding works.

Materials and methods

Eighteen clones of European cranberry were selected during expeditions in the strictly protected areas Čepkeliai and Žuvintas in 1998-1999. The definite geographical positions of their habitats were established by the GPS Magellan 315 receiver (GARMIN Corporation, USA). These clones with distinctive phenotypic peculiarities were propagated and planted into acid peat (pH 3.5-4.0) in the field collection of the Kaunas Botanical Garden of Vytautas Magnus University for further investigations.

The samples of berries were harvested after full maturity in 2003-2005 and evaluated. The average weight of a berry was calculated by assessing the average weight of 50 berries in three replications. Berry shape and coloration were determined according to the descriptor list for the genus *Oxycoccus* Hill (Budriūnienė 1997). All clones were divided into five groups according to the average mass of a berry by using this descriptor list: 1 – very small (<0.3g); 2 – small (0.3-0.5g); 3 – medium (0.6-1.0g); 4 – large – (1.1-1.5g); 5 – very large (1.5g).

The amount of total phenolics in the cranberry extracts was determined with the Folin-Ciocalteu reagent according to the method of Slinkard and Singleton (Slinkard and Singleton 1977) using gallic acid as a standard. Samples (1.0 ml, two replicates) were introduced into test cuvettes, and then 5.0 ml of Folin-Ciocalteu's reagent and 4.0 ml of Na₂CO₃ (7.5%) were added. The absorption of all samples was measured at 765 nm by using the Genesys-10 UV-VIS

spectrophotometer (Thermo Spectronic, Rochester, USA) after incubating at 20°C for 1.0 h. The results were expressed as milligrams of gallic acid equivalent (GAE) per 100 gram of fresh weight.

The pigments were extracted from 5g of fresh cranberry with 95% (v/v) grade ethanol acidified with 0.1 M HCl with a purpose to assay the total amount of anthocyanins (Rubinskiene *et al.* 2005). The berries were ground with quartz sand and the extraction was continued with 20 ml portions of solvent until the sample became colourless. The extract was diluted with acidified ethanol. The absorption was measured on a spectrophotometer Genesys-10 UV-VIS (Thermo Spectronic, Rochester, USA) at 535 nm. The amount of anthocyanins was expressed as prevailing cyanidin 3-galactoside and calculated in mg/100g using the extinction coefficient ($E_{1\text{ cm}}^{1\%}$) at 535 nm as 982 (Francis 1982).

The results were statistically analysed and the significance of differences was calculated using ANOVA for Excel vers. 3.1. The coefficients of correlation (*r*), determination (*R*²) and variation (*V*) were calculated using STAT for Excel ver.1.5 (Tarakanovas and Raudonius 2003).

Results

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 width and height of a berry varied from 1.00 to 1.35 cm and from 0.99 to 1.41 cm, respectively. The colour of berries was red or dark red at full ripeness. Only two clones (98-Č-19 and 98-Č-20) with pink berries were singled out while 99-Ž-10 with purple berries. The berries of clones 98-Č-06 and 99-Ž-09 were covered with a waxy coat.

Reliable differences in the average weight of a berry were ascertained since Fisher's criterion was $F_{05(\text{fact})}=42.04 > F_{05(\text{theor})}=1.7$ (Figure 1). The average berry weight of clones investigated was 0.94g ($LSD_{05}=0.052$). It has been found that the berries of twelve clones fall into the group of medium-sized (0.6-1.0g), the berries of five clones were large (1.1-1.5g). Only the clone from Čepkeliai (98-Č-17), which had small berries, was singled out. The average berry weight of clones 99-Ž-03, 99-Ž-11, 99-Ž-13, 98-Č-01, 98-Č-06 and 98-Č-19 varied slightly, variation coefficient $V < 10\%$. The largest variation of berry weight was detected in clone 99-Ž-03 ($V=19.3\%$).

The clones of European cranberry were compared according to the total amount of phenolic compounds. These clones accumulated from 197 (clone 98-Č-19)

Table 1. Morphological characteristics of *O. palustris* berries

| Clone | Length x width of a berry, cm | Prevailing shape of a berry | Colour of a berry |
|----------|-------------------------------|-----------------------------|-------------------|
| 99-Ž-03 | 1.11 x 1.12 | round | dark red |
| 99-Ž-04 | 1.19 x 1.21 | round | dark red |
| 99-Ž-07 | 1.38 x 1.29 | cylindrical | red |
| 99-Ž-09 | 1.02 x 1.04 | round | dark red |
| 99-Ž-10 | 1.00 x 1.28 | oblate | purple |
| 99-Ž-11 | 1.21 x 1.29 | oblate | red |
| 99-Ž-12 | 1.35 x 1.25 | oval | dark red |
| 99-Ž-13 | 1.29 x 1.21 | oval | dark red |
| 99-Ž-16 | 1.16 x 1.32 | oblate | dark red |
| 99-Ž-18 | 0.99 x 1.28 | oblate | dark red |
| 98-Č-01 | 1.17 x 1.22 | oblate | red |
| 98-Č-04 | 1.21 x 1.17 | round | dark red |
| 98-Č-06 | 1.17 x 1.24 | round | dark red |
| 98-Č-15 | 1.16 x 1.17 | cylindrical | dark red |
| 98-Č-15A | 1.11 x 1.35 | oblate | red |
| 98-Č-17 | 1.15 x 1.00 | oval | dark red |
| 98-Č-19 | 1.31 x 1.17 | oval | pink |
| 98-Č-20 | 1.41 x 1.34 | cylindrical | pink |

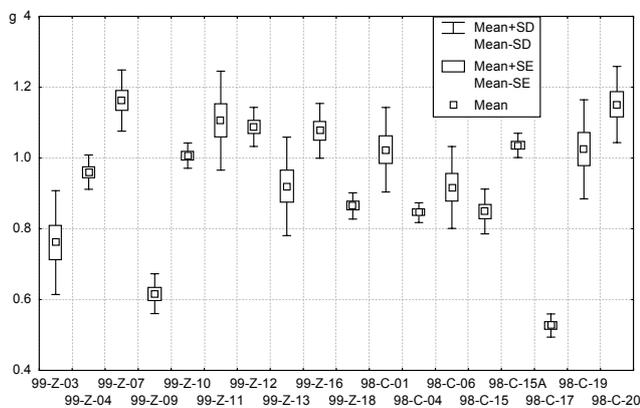


Figure 1. The average berry weight of *O. palustris* clones in 2003-2005, $LSD_{05}=0.052$

to 584 mg/100g (clone 98-Č-15A) of phenolic compounds (Figure 2). The total amount of phenolics on average attained 389 mg/100g in the clones from Čepkeliai and that in the clones from Žuvintas 347 mg/100g. The coefficient of correlation between the average berry weight and total phenolics amount was $r = -0.281$, i.e. very low.

In accordance with total amount of anthocyanins clone Č-98-17 from Čepkeliai was singled out

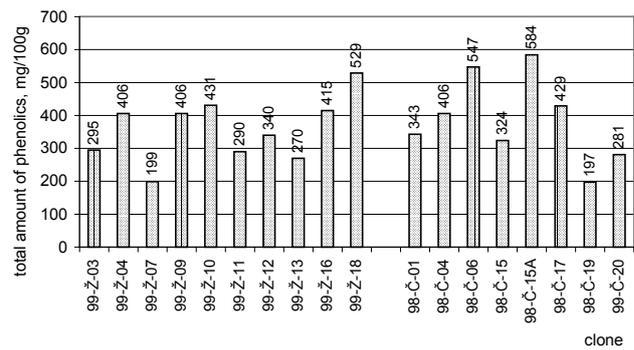


Figure 2. Total amount of phenolics in the berries of *O. palustris* clones, $LSD_{05}=12.99$

(206mg/100g) (Figure 3). The amount of anthocyanins attained from 56 to 137 mg/100g in Žuvintas clones and from 36 to 206 mg/100g in Čepkeliai clones. The clones of European cranberry on average accumulated 99 mg/100g of total amounts of anthocyanins.

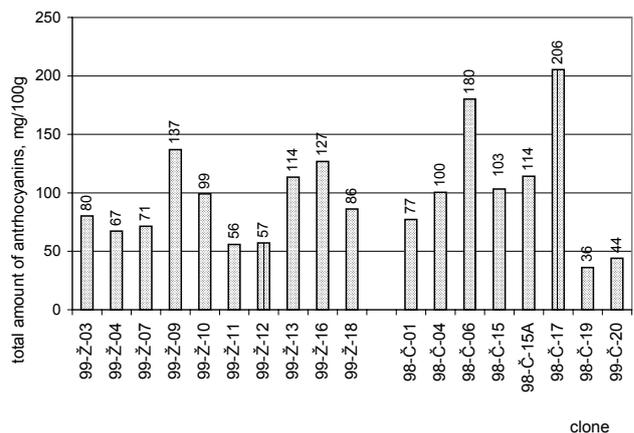


Figure 3. Total amount of anthocyanins in the berries of *O. palustris* clones, $LSD_{05}=14.54$

The results of biochemical analysis revealed that phenolics of clones of European cranberry on average included from 15.7% (clone 98-Č-20) to 47.9% (clone 98-Č-17) anthocyanins. The correlative analysis corroborated the negative relationship between the average berry weight and total amount of anthocyanins. The coefficient of correlation was $r = -0.657$ (at the probability level $\alpha=0.01$).

Discussion and conclusions

The collection of cranberry clones during expeditions *in situ* was based upon the selection of samples conspicuous in berry shape, colouration and size. The latest evaluation of European cranberry revealed high variability in berry shape and weight *ex situ* as

well. The berries of four clones 99-Ž-07, 99-Ž-11, 99-Ž-12, 99-Ž-16 from Žuvintas and one clone 98-Č-20 from Čepkeliai were distinguished for the largest average weight of a berry (1.16g, 1.11g, 1.09g, 1.08 and 1.15g, respectively). The berry weight of these clones could be equal to the average berry weight of American cranberry cultivars (Budriūnienė 1998).

The above-mentioned clones produced large berries *in situ* as well as *ex situ*. The weight of a berry is one of the most important peculiarities in the breeding. Recent DNA studies have revealed a high level of genetic diversity of European cranberry (Areškevičiūtė *et al.* 2006).

Cranberries and specific biochemical components of their berries are being associated with human health attributes, such as maintenance of urinary tract health and antioxidant status (Vorsa *et al.* 2002). The berries of European cranberry are one of the best sources of phenolic compounds as compared with other berry plants, such as strawberry, black currant, raspberry *etc.* (Moyer *et al.* 2002). The results of this study corroborated significant variability for total phenolics as well as for anthocyanins amounts. The berries of clones 98-Č-15A, 98-Č-06, 98-Č-17 and 99-Ž-18 had the largest amounts of phenolics: 584, 547, 429 and 529 mg/100g, respectively.

Anthocyanins form a part of phenolics, however there was no linear dependence between the total amounts of anthocyanins and phenolics established. A regression analysis revealed that the response of total amounts of phenolics to total amounts of anthocyanins could be best expressed by the second-order polynomial equation $y = -0.0119x^2 + 4.3453x + 77.443$ at the coefficient of determination $R^2 = 0.4477$ (Figure 4). Therefore the amount of anthocyanins was not the main factor, which determines the

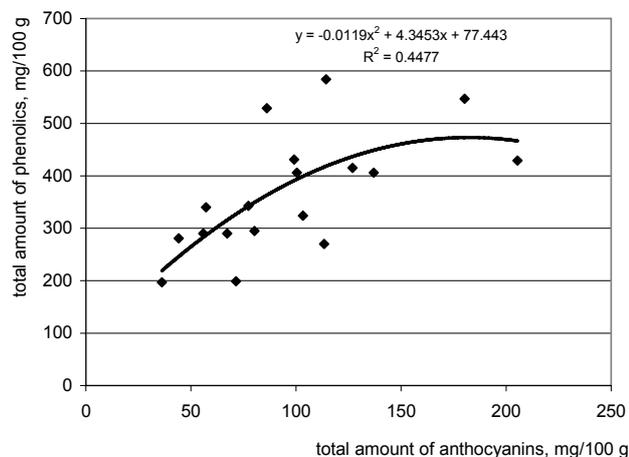


Figure 4. Relation between the total amount of phenolics and the total amount of anthocyanins in the berries

total amount of polyphenols in the berries of European cranberry.

The level of total anthocyanins amount was negatively correlated with the berry weight. The clones with small and dark red berries (98-Č-17, 98-Č-06, 99-Ž-09, 99-Ž-13) accumulated the largest amounts of anthocyanins (Table 1, Figures 1 and 3). This data motivate the selection of clones with putative large amount of anthocyanins *in situ* and the preservation in the collection *ex situ* with the purpose to use them in further breeding works.

The natural resources of this valuable berry plant were significantly reduced over the last 40 years. This research promotes the selection of clones with valuable properties *in situ* and preservation in the field collection of European cranberry germplasm at Kaunas Botanical Garden.

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ИССЛЕДОВАНИЕ МОРФОЛОГИЧЕСКИХ ПРИЗНАКОВ, ОБЩЕГО КОЛИЧЕСТВА ФЕНОЛЬНЫХ СОЕДИНЕНИЙ И АНТОЦИАНИНОВ В ЯГОДАХ КЛЮКВЫ БОЛОТНОЙ (*OXYCOCCUS PALUSTRIS*)

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Резюме

В заповедниках Чапкяляй и Жувинтас собраны образцы клонов клюквы болотной, которые выделялись разнообразием величины формы и окраски ягод. Клоны размножены и посажены в коллекцию Каунасского ботанического сада университета Витаутаса Великого, где и проводились данные исследования. Установлена высокая изменчивость среднего веса одной ягоды. Большинству клонов были характерны ягоды среднего веса (0,6-1,0г). Исследованные клоны отличались по количеству фенольных соединений и антоцианинов. В ягодах клюквы болотной в среднем установлено 99 мг/100г антоцианинов и 365 мг/100г фенольных соединений. Мелкие и темно окрашенные ягоды накапливали больше антоцианинов. По количеству этих соединений отобраны клоны клюквы болотной для хранения в коллекции Каунасского ботанического сада.

Ключевые слова: антоцианины, клон, коллекция, клюква, фенольные соединения