Scientific Papers. Series B, Horticulture. Vol. LXVIII, No. 1, 2024
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

THE ORIGIN OF ROMANIAN BLUEBERRY CULTIVARS

Oana HERA

Research - Development Institute for Fruit Growing Pitești - Mărăcineni,
402 Mărului Street, Pitești, Argeș, Romania

Corresponding author email: oana.hera@yahoo.com

Abstract

Vaccinium corymbosum (L.) is a plant native to North America and belongs to the genus Vaccinium L., the Ericales order, family Ericaceae, Subfamily Vaccinioideae. Blueberries are classified under the family Ericaceae, subfamily Vacciniaceae, genus Vaccinium, and subgenus Cyanococcus. They are most commonly found growing in acidic and infertile soil conditions. Highbush blueberries have a higher total sugar content compared to other berries of the Vaccinium genus. The first blueberry crop in Romania was established in Bilcesti, Arges. Since 1980, blueberry breeding has been carried out at the Research Institute for Fruit Growing Pitesti - Maracineni, Romania, using different origins and parent plants. Controlled hybridization is used in the breeding process, where the parents or genitors are chosen based on the desired blueberry breeding objectives. The aim of this study is to expand the knowledge base on the pedigree and biometrical traits of Romanian blueberry cultivars.

Key words*: the first blueberry crop, controlled hybridization, pedigree, dendrogram of Vaccinium corymbosum.*

INTRODUCTION

Blueberries, belonging to the *Vaccinium* section *Cyanococcus*, are perennial shrubs that are extensively grown for their delicious fruits.
The *Vaccinium* genus comprises significant cultivated species like blueberry and cranberry (Kulkarni et al., 2020). Many *Vaccinium* species have been realized by blueberry breeders to develop cultivars adapted to diverse climates (Hanson et. al, 2007). Blueberries have a shallow root system, primarily located at depths less than 60 cm, especially on clay soils (Prodorutti et al., 2007). The species within this genus present different levels of ploidy (2*x*, 4*x* and 6*x*; $x = 12$), which results in evident morphological differences.
Vaccinium corymbosum (L.) (2 n=4×=48) is a tetraploid species and is the primary contributor to many highbush blueberry cultivars (Redpath L. et al., 2022). *Vaccinium* berries have gained tremendous attention in recent days due to the presence of a high amount of antioxidant properties (Sharma et al., 2022). Historical records indicate that the breeding of blueberries has deep roots in the incorporation of wild species and the crossing of different species (Brevis et al.,2008, Lyrene et al., 2012). The first blueberry breeder was Frederick V. Coville in Greenfield, America. He began observing the native highbush blueberries in the area in 1905 and started a blueberry breeding program using elite wild selections of *Vaccinium corymbosum* (L.). Coville, along with Elizabeth C. White, grew blueberries in plantations and released numerous crosses. Some of the breeding cultivars developed by F. V. Coville include 'Rubel' (wild selection in 1912), 'Pioneer' (1920), 'Katharine' (1920), 'Greenfield' (1926), 'Rancocas' (1926), 'Jersey' (1928), 'Concord' (1928), 'June' (1930), 'Stanley' (1930), 'Scammell' (1931), 'Redskin' (1932), 'Catawba' (1932), 'Wareham' (1936), 'Weymouth' (1936), and 'Dixi' (1936). After Coville died in 1937, researchers continued to release cultivars from his plantation and hybrids, including 'Angola' (1951), 'Atlantic' (1939), 'Berkeley' (1949), 'Bluecrop' (1952), 'Blueray' (1955), 'Burlington' (1939), 'Collins' (1959), 'Coville' (1949), 'Croatan' (1954), 'Earliblue' (1952), 'Ivanhoe' (1951), 'Murphy' (1950), 'Pemberton' (1939), and 'Wolcot' (1950). George Darrow continued his work through studies on the phylogeny of native species of *Vaccinium* (Darrow, 1952). In present, The National Clonal Germplasm Repository (NCGR) in Corvallis, Oregon, which is part of the United States Department of Agriculture (USDA) Agricultural Research Service (ARS), is dedicated to preserving the

genetic resources of fruit crops. This genebank houses over 1800 accessions of Vaccinium L. from 34 different countries (Bassil et al., 2020). In Europe, Dr. Hermann from Germany made significant progress in blueberry breeding in 1929, establishing the first blueberry plantation in Germany and resulting in numerous cultivars. In Romania, blueberry breeding began in 1980 with Dr. Paulina Mladin at the Research Institute for Fruit Growing in Pitești - Mărăcineni. The breeding program continues to this day, focusing on meeting market requirements such as fruit size and extended ripening season. The program has resulted in a rich and diverse genetic material, with different stages of evaluation in the breeding process, including hybrids and comparative crops and microcultures.

That will serve to obtain new, competitive cultivars, as a new source for the continuation of the breeding program. The main objectives of breeders for highbush blueberry have been the selection of aromatic cultivars with the ability to be resistant to storage, fruiting period, disease and pest resistance and mechanized harvesting (Qu et al., 1998). The objectives of blueberry breeding are similar to those being realized in other breeding centres working with this crop (Pluta et al., 2012). As a working methodology, in particular, directed, controlled hybridization is used, using the rich collection from Pitesti and composed of 9 cultivars registered in the EURISCO European catalog: 'Azur' (1998), 'Safir' (1998), 'Augusta' (1999), 'Simultan' (2001), 'Delicia' (2001), 'Lax' (2002), 'Vital' (2009), 'Prod' (2009) 'Pastel' (2019).

The aim of this study is an expanded knowledge base of pedigree and biometrical traits for Romanian cultivars of blueberry.

Plant breeding success in the last century has been linked to a reduction in the genetic diversity present in the superior germplasm of plant species (Zoratti et al., 2015).

MATERIALS AND METODS

Plant material

The biological material was represented by 9 Romanian cultivars of blueberry (Figure 1): 'Azur' and 'Augusta cv.' originated from the cross 'Berkeley' × 'Bluecrop' cv.; 'Safir' cv. originated from the cross 'Pemberton' × 'Blueray' cv.; 'Simultan', 'Lax', 'Pastel' and 'Vital' cv. obtained from open pollination 'Spartan' cv.; 'Delicia' and 'Prod' obtained from open pollination 'Patriot' cv.

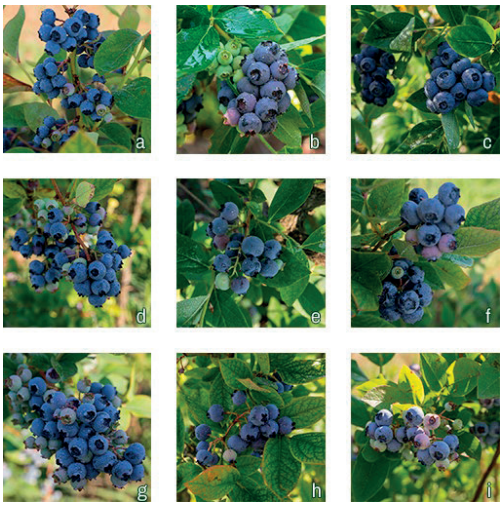

Figure 1. The biological material: a- 'Lax'; b-'Pastel'; c-'Safir'; d-'Vital'; e-'Prod'; f-'Delicia'; g-'Azur'; h-'Simultan'; i-'Augusta'

The experiment was set up at the Research Institute Growing Pitesti, within the Genetic and Breeding Department and tested in an experimental plot. The cultivars were planted at a distance of 3 m × 1 m on a mixture of soil and peat. The samples were harvested and immediately analyzed.

Determination of yield

By weighing total yield/plant (kg/plant).

Determination of Average Weight

By weighing a sample of 50 fruits for each genotype (15 plants/genotype), the average weight of fruits was determined and the results were expressed in g/fruit.

Determination of shape index

The length and diameter of the fruit were determined by measuring the fruit using a digital caliper. The shape index (SI) of the fruit was calculated as the ratio of these two dimensions (Tudor et al. 2014, Jamieson, 2017).

Determination of fruit firmness
By measuring each fruit sample with a penetrometer Bareiss HPE II Fff nondestructive test, with a measuring surface of 0.25 cm².

Determination of total titratable acidity
TTA was determined by titrable method (Ermakov et al., 1987). The principle of the method is to neutralize a volume of aqueous fruit extract with a solution of NaOH 0.1N in the presence of phenolphthalein as an indicator.

Determination of Total Soluble Solid Content
Total soluble solids (TSS) were determined using a Kruss DR201-95 refractometer and the results were reported as °Brix at 20°C.

Statistical Analysis
All analyses were performed in triplicate and data were reported as mean standard deviation (SD). Excel 2021 (XLSTAT) was used for data statistical analysis. One-way analysis of variance (ANOVA) and two-way ANOVA and Duncan's multiple range tests were performed.
The genetic distance between varieties was determined using Minitab v. 18 software based on the Euclidean distance formula, which calculates the square root of the sum of squared differences: dik=$\sqrt{\sum_j (Xij - Xkj)^2}$.

RESULTS AND DISCUSSIONS

The analysis of the dendogram indicates four groups: group I formed by the cultivars: 'Lax' and 'Augusta'; group II formed by the cultivars: 'Pastel', 'Safir' and 'Simultan', group III formed by the cultivars: 'Azur' and group IV formed by the cultivars: 'Vital', 'Prod' and 'Delicia' (Figure 6).

The smallest genetic distance is between the cultivars 'Safir' and 'Simultan', and the largest genetic distance is between the cultivars 'Lax' and 'Vital'. The greatest degree of similarity is between the cultivars 'Safir' and 'Simultan', 'Prod' and 'Delicia', 'Lax' and 'Augusta', 'Azur' cv. clearly differs from all other cultivars.
The biggest yield of the cultivars tested was registered by 'Azur' cv. (3.93 kg/plant) and 'Delicia' cv. (3.58 kg/plant).
The lowest yield (shown in Table 1) was recorded by 'Vital' cv. (1.77 kg/plant).
For average fruit weight, there were no significant differences between cultivars, the highest fruit weight was recorded by 'Delicia' cv (3.1 g).
Regarding fruit firmness, statistically significant differences were found between cultivars, and it is a trait that directly affects the quality of blueberries. The average fruit firmness ranged from 12.63 N (for the 'Azur' cultivar) to 38.4 N (for the 'Simultan' cultivar).
According to previous studies, the content of organic acids in fruits can be influenced by culture and environmental conditions (Gündoğdu, 2019), genotypic differences and post-harvest handling procedures (Lee and Kader, 2000). In this study, the richest content of organic acids expressed as citric acid was 1.22% (for the 'Azur' cultivar), and the lowest content was 0.72% (for the 'Augusta' cultivar).
Along with other phytochemical compounds, the soluble solids content contributes to fruit flavor. TSS consisting of soluble carbohydrates and other non-carbohydrate compounds influences the refractive index of the aqueous extract obtained from a horticultural product (Diaconu, 2006).
In our study, for total soluble solids content ranged from 8.93 °Brix for 'Lax' cv. to 19.3 °Brix for 'Simultan' cv. (Table 1).

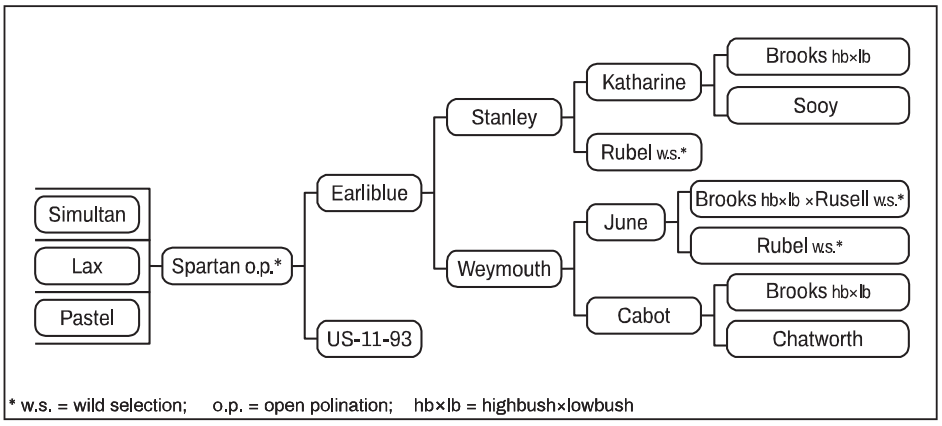

Figure 2. Pedigree of 'Simultan', 'Lax' and 'Pastel' cv. highbush blueberry

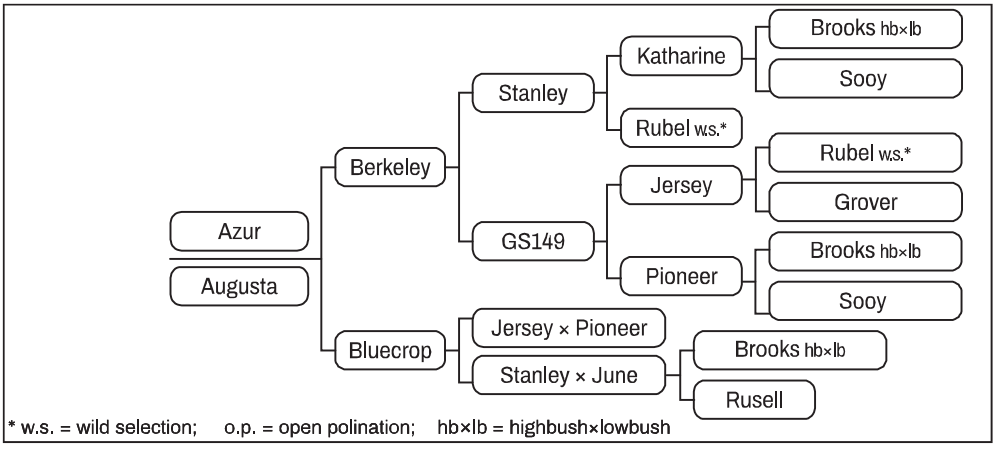

Figure 3. Pedigree of 'Azur' and 'Augusta' cv. highbush blueberry

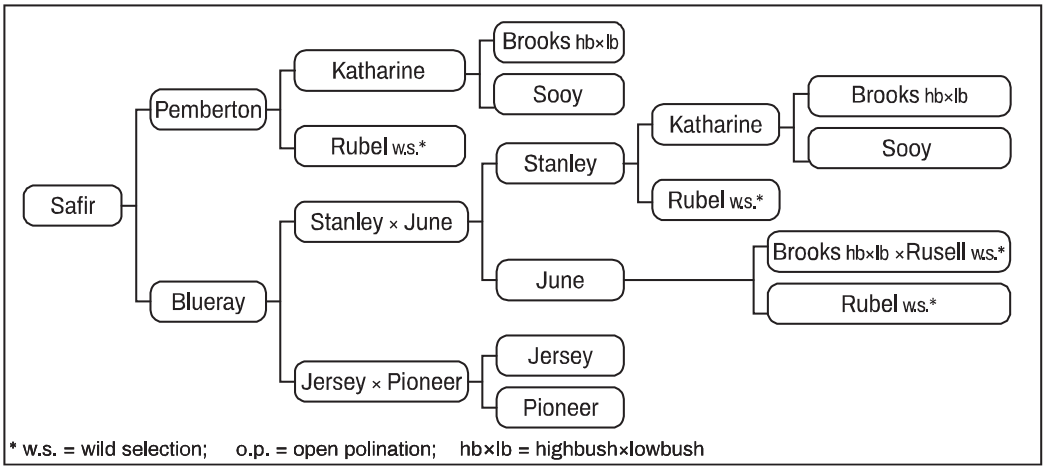

Figure 4. Pedigree of 'Safir' cv. highbush blueberry

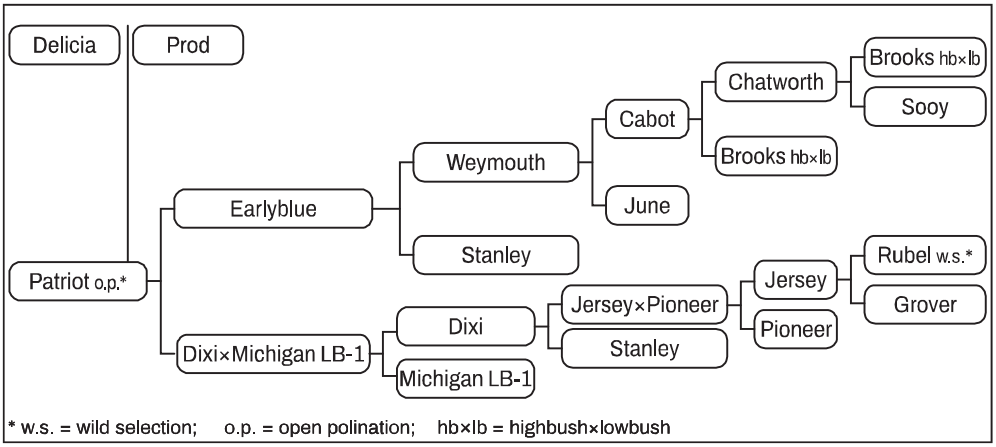

Figure 5. Pedigree of 'Delicia' and 'Prod' cv. highbush blueberry

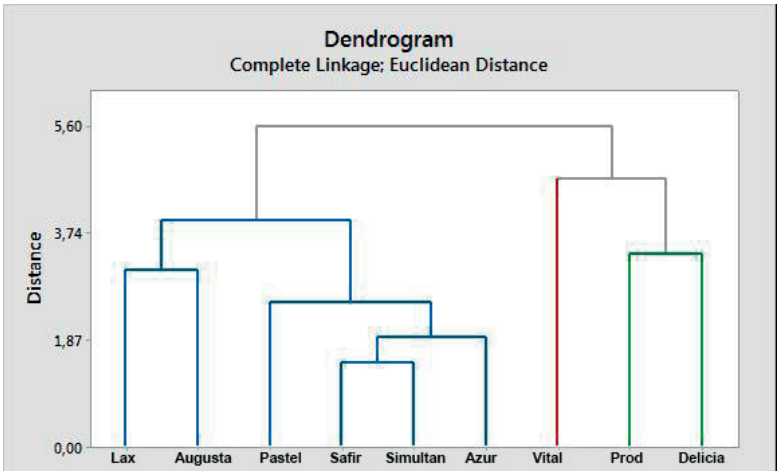

Figure 6. Dendrogram of 9 blueberry cultivars generated by Minitab v.18, cluster analysis of the Euclidean Distance based on biometrical traits

Table 1. The average values of yield, berry weight, size index, firmness, acidity and °Brix at 9 blueberry cultivars

Cutivar	Yield*	Berry Weight (g)	Size index	Firmess (N)	TTA (%)	TSS (° Brix)
Lax	3±0,2cd	2,57±0,76a	0,87±0,06a	20,73±7,1cd	0,75±0,13a	8,93±1,03b
Pastel	2,78±0,3c	2,99±0,71a	0,76±0,09bc	22,9±3,06bcd	1,25±0,5a	10,73±1,1b
Safir	2,67±0,48c	2,7±0,2a	0,71±0,09c	24±0,95bcd	0,91±0,15a	11,17±1,24b
Vital	1,77±0,25d	2,77±0,81a	0,72±0,02c	35,74±6,68ab	1,22±0,48a	9,58±0,65b
Prod	2,55±0,54c	2,47±0,51a	0,72±0,01c	28,93±10,89abc	0,7±0,08a	13,37±3,1b
Delicia	3,58±0,56ab	3,1±0,56	0,7±0,04c	28,63±3,7abc	0,93±0,07a	11,33±0,95b
Azur	3,93±0,12a	3,08±0,51a	0,88±0,04a	12,63±0,15d	1,04±0,67a	10,43±0,65b
Simultan	2,63±0,55c	2,4±0,52a	0,85±0,06ab	38,4±0a	1,19±0,4a	19,3±8,75a
Augusta	2,63±0,55c	2,03±0,32a	0,75±0,07bc	28,73±13,97abc	0,72±0,13a	10,87±1,5b

*Means with the same letter are not significantly different at 5% level

CONCLUSIONS

Dr. Coville's significant contribution to understanding the cultural requirements of blueberries is immeasurable. However, we can assess the value of the blueberries from the bushes he breeded. The cultivars created by Dr. Coville contributed to the breeding of the 9 Romanian blueberry cultivars. The Rubel cv., common ancestor, is a selection from the spontaneous flora in 1912.

The different cultivars he introduced and the seedlings he cultivated, which were later released, are illustrated in Figure 2 to Figure 5. This paper shows the broad origin of the nine Romanian blueberry cultivars.

According to the results presented in our study, the smallest genetic distance is between the cultivars 'Safir' and 'Simultan', and the largest genetic distance is between the cultivars 'Lax' and 'Vital'. The greatest degree of similarity is between the cultivars 'Safir' and 'Simultan', 'Prod' and 'Delicia', 'Lax' and 'Augusta', 'Azur' cv. clearly differs from all other cultivars.

The biggest yield of the cultivars tested was registered by 'Azur' cv. (3.93 kg/plant) and 'Delicia' cv. (3.58 kg/plant).

It is proposed to create a new *Vaccinium corymbosum* (L.) from these cultivars. Our results will strengthen the breeding program.

ACKNOWLEDGEMENTS

This research work was carried out with the support of the Research Institute for Fruit Growing Pitesti, and also was financed from Project ADER 6.4.1.: Innovative preservation/storage and processing technologies applicable to new fruit varieties offered in the farms established through Submeasure 4.1a - "Investments in fruit farms" for the purpose of efficient exploitation on the market.

REFERENCES

Bassil, N., Bidani, A., Nyberg, A., Hummer, K., & Rowland, L. J. (2020). Microsatellite markers confirm identity of blueberry (Vaccinium spp.) plants in the USDA-ARS National Clonal Germplasm Repository collection. *Genetic Resources and Crop Evolution*, *67*, 393-409.

Brevis, P. A., Bassil, N. V., Ballington, J. R., & Hancock, J. F. (2008). Impact of wide hybridization on highbush blueberry breeding. *Journal of the American Society for Horticultural Science*, *133*(3), 427-437.

Darrow, G. M. (1952). Breeding of small fruits in the United States. *The Scientific Monthly*, *75*(5), 288-297.

Diaconu C., 2006. Biological and cultural value of strawberry (*Fragaria x ananassa*) germplasm and its utility in genetic breeding programs. *Doctoral thesis, ASAS Bucureşti*.

Ermakov, A. I., Arasimovich, V. V., & Yarosh, N. P. (1987). Methods of biochemical research of plants. *Leningrad: Agropromizdat*, 429c.

Gündoğdu, M., 2019. Effect of rootstocks on phytochemical properties of apricot fruit. *Turkish Journal of Agriculture and Forestry, 43*(1): 1-10.

Hanson, E. J., Berkheimer, S. F., & Hancock, J. F. (2007). Seasonal changes in the cold hardiness of the flower buds of highbush blueberry with varying species ancestry. *Journal of the American Pomological Society*, *61*(1), 14.

Kulkarni, K. P., Vorsa, N., Natarajan, P., Elavarthi, S., Iorizzo, M., Reddy, U. K., & Melmaiee, K. (2020). Admixture analysis using genotyping-by-sequencing reveals genetic relatedness and parental lineage distribution in highbush blueberry genotypes and cross derivatives. *International Journal of Molecular Sciences*, *22*(1), 163.

Lyrene, P. M., & Olmstead, J. W. (2012). The use of inter-sectional hybrids in blueberry breeding. *International journal of fruit science*, *12*(1-3), 269-275.

Pluta, S., & Zurawicz, E. (2012, June). The high-bush blueberry (Vaccinium corymbosum L.) breeding programme in Poland. In *X International Symposium on Vaccinium and Other Superfruits 1017* (pp. 177-180).

Prodorutti, D., Pertot, I., Giongo, L., & Gessler, C. (2007). Highbush blueberry: Cultivation, protection, breeding and biotechnology. *The European journal of plant science and biotechnology*, *1*(1), 44-56.

Sharma, U., Igamberdiev, A. U., & Debnath, S. C. (2022, August). DNA methylation and epigenetic variation in Vaccinium plants. In *XXXI International Horticultural Congress (IHC2022): International Symposium on Advances in Berry Crops 1381* (pp. 1-14).

Ștefănescu, E., B., Szabo, K., Mocan, A., Crișan, G., (2019). Phenolic compounds from five *Ericaceae* species leaves and their related bioavailability and health benefis, *Molecules journal*.

Sturzeanu M., Militaru M., Butac M., Călinescu M., Iancu A., Petre Gh., Iurea E., Guzu G., (2022). *Eficientizarea ameliorării genetice a soiurilor de pomi şi arbuşti fructiferi.* Proiect ADER 7.2.2/2019. ISBN: 978-606-764-074-

Redpath, L. E., Aryal, R., Lynch, N., Spencer, J. A., Hulse-Kemp, A. M., Ballington, J. R., ... & Ashrafi, H. (2022). *Nuclear DNA contents and ploidy levels of North American Vaccinium species and interspecific hybrids*. *Scientia Horticulturae*, *297*, 110955.

Qu L., Hancock J. F., Whallon J. H.,1998. *Evolution in an autopolyploid group displaying predominantly*

bivalent pairing at meiosis: genomic similarity of diploid Vaccinium darrowi and autotetraploid V. corymbosum (Ericaceae). American Journal of Botany, 85(5), 698-703.

Tudor V, Asanica A, Neagu T. First results of some day-neutral strawberry cultivars behavior in the Bucharest area conditions. Uni. Agron. Sci. and Veterinary Medicine of Bucharest 58 (2014): 101-106.

Zong, Y., Kang, H., Fang, Q., Chen, X., Zhou, M., Ni, J., & Guo, W. (2019). Phylogenetic relationship and genetic background of blueberry (Vaccinium spp.) based on retrotransposon-based SSAP molecular markers. *Scientia Horticulturae*, *247*, 116-122.

Zoratti, L., Palmieri, L., Jaakola, L., & Häggman, H. (2015). Genetic diversity and population structure of an important wild berry crop. *AoB Plants*, *7*, plv117.

https://eurisco.ipkgatersleben.de/apex/eurisco_ws/r/eurisco/home).