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PRELIMINARY RESULTS REGARDING THE SELECTION OF NEW BLUEBERRY GENOTYPES (*VACCINUM CORYMBOSUM* L.)

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Abstract

Highbush blueberry (Vaccinium corymbosum L.) is a significant specie in terms of economical, nutritional, and medicinal point of view. Beside these attributes it is well known for its high anthocyanin content and antioxidant activity. Therefore, obtaining new valuable blueberry genotypes resilient to climatic changing conditions is a priority for breeders. The genotypes studied were obtained by a classical breeding method, respectively by free pollination, the seeds being prior cold stored and then sown in seedlings trays with acidic peat. Germination lasted even two years for some genotypes. The study presents the first phenotypic results for the obtained genotypes, highlighting differences and similarities regarding the foliar system and health status. Thirteen local (including 'Safir', 'Compact', 'Simultan') and international (Duke, Pink Lemonade, Berkeley, etc.) blueberry cultivars were used as parents. The results enclose twenty hybrids obtained from free pollination.

Key words: *highbush blueberry, high chill cultivars, genetic variability, blueberry breeding.*

INTRODUCTION

Blueberries (*Vaccinium* spp.) are one of the most economically significant and nutritionally valuable fruit crops worldwide (Edger et al., 2022). The demand for these antioxidant-rich berries has fuelled research endeavours to enhance cultivation practices, optimize yield, and improve fruit quality (Mladin et al., 2008; Patrick & Li, 2017; Hera et al.,2021; Edger et al., 2022; Babiker et al., 2023).

Core to these efforts is the field of phenotyping, a comprehensive approach that integrates genetic, physiological and environmental factors to characterize the measurable traits of blueberry plants throughout their development (Verde et al., 2013; Cândea-Crăciun et al., 2018; Manzanero et al., 2023). In recent years, the application of advanced phenotyping technologies has emerged as a transformative force in understanding the intricate genetic and physiological mechanisms governing blueberry growth and productivity. Phenotyping, broadly defined as assessing observable traits, offers a holistic perspective beyond conventional genetic studies. It involves the measurement and analysis of morphological, biochemical, and molecular characteristics, providing valuable insights into the complex interplay between genotype and environment (Lobos & Hancock, 2015; Asănică et al., 2017; Franeti et al., 2020).

Current article explores the recent strides in blueberry phenotyping, shedding light on the innovative methodologies and technologies driving progress in the field. By delving into the intricacies of blueberry phenotypic characterization, researchers aim to disclose the mysteries surrounding the dynamic responses of these plants to environmental stimuli, stressors, and genetic variations. Through the lens of phenotyping, we aim to elucidate the intricate interplay between genotype and phenotype in blueberries, offering insights that can guide breeding programs toward developing better cultivars with enhanced nutritional profiles, improved tolerance to different stressors, and increased adaptability to diverse growing environments. Integrating advanced phenotyping techniques marks a paradigm shift in blueberry research, unlocking new avenues for sustainable and resilient berry production in the face of a changing climate and evolving market demands.

MATERIALS AND METHODS

The biological material involved in the present work was represented by foreign cultivars ('Northland', 'Bluetta', 'Berkely', 'Coville', 'Draper', 'Duke', 'Nelson', 'Patriot', 'Spartan', 'Pink Lemonade') and Romanian ones ('Simultan', 'Compact', 'Lax' 'Safir') from the blueberry collection of the Faculty of Horticulture Bucharest. The blueberry collection is set up in containers where the soil-specific properties could be better satisfied. From the above blueberry cultivars fruits, as a result of free pollination, seeds were extracted, passed through the process of stratification in cold rooms, and later sown and grown into small pots. Some seeds germinated after two years. Two and three years after germination, one and three mature hybrids of each cultivar were obtained. In the phenotyping process, five leaves were collected for both cultivars and hybrids that were morphologically analyzed with the WinFolia system. WinFolia system included an Epson scanner and software for image analyses that accurately could measure the principal biometrical leaves parameters. It has been designed explicitly for analyzing leaves in terms of leaf morphology and, including color codes, to deliver the rate of the disease foliar percentage.

Microsoft Excel 2016 and IBM SPSS v. 28.0.1.1 software were used for the statistical analyses of the data with a significance level of $p = 0.05$ were used.

RESULTS AND DISCUSSIONS

The first data included the visual analysis of the hybrids compared to the genitors. After scanning with the WinFolia program, images were obtained with the five leaves for each cultivar and hybrid. In Table 1, the mother genitor and the corresponding hybrids were presented. They are valuable for future applications for plant/cultivar/hybrid recognition.

In the second phase, morphological parameters were analyzed for hybrids and cultivars. WinFolia software delivers results on leaf area, perimeter, vertical length, width, ratio (W/L), form coefficient, blade length, lobe angles, and petiole length and area (Tables 2 and 3).

When analysing the leaf area, hybrids proved to have a large variability, the values being between 5.488 (PLS 7-3) and 23.218 cm^2 (PLS 52-1), the same characteristics being observed in the other parameters.

For the petiole length, there were no significant differences between variants. For the petiole area, PLS 2-1 had the highest value (0.016 cm^2), followed by PLS 18-3, PLS 25-23, PLS 22-6, PLS 52-13, PLS 7-4, PLS 2-2, PLS 59-14, PLS 45-1, PLS 20-1, PLS 18-1, PLS 33-11 (no significant differences between them). The group of PLS 7-3, PLS 18-2, PLS 29-15, PLS 52-3, PLS 49-22, PLS 21-2, PLS 52-1, PLS 21-3 had lower values.

Table 1. Cultivars and the corresponding hybrids analyzed with the WinFolia program

Variety	Genotype	Genotype	Genotype
Northland	PLS 52-1	PLS 52-3	PLS 52-13
Bluetta	PLS 45-1		
Simultan	PLS 7-4	PLS 7-3	
Spartan	PLS 2-2	PLS 2-1	
Berkeley	PLS 21-2	PLS 21-3	
Compact	PLS 33-11		
Pink Lemonade	PLS 59-14		

For the healthy status of the plants, Winfolia software was used with image analysis based on color. At the hybrids, all the variants presented similar values (Table 2), except PLS 21-3, which had a lower value (71.37%). In general, hybrids had very good, healthy foliage. Based on the morphological parameters, hybrids were analyzed in clusters (Figure 1). According to the obtained dendrogram, five common groups were obtained. First group included PLS 52-3, PLS 18-1, PLS 29-15, PLS 2-2, second group PLS 25-23, PLS 22-6, PLS 33-11, PLS 52-1, third group PLS 21-3, PLS 18-3, PLS 45-1, PLS 7-4, fourth group PLS 52-13, PLS 59-14, PLS 21-2, and the fifth group PLS 2-1, PLS 18-2, PLS 49-22, PLS 7-3. The first and second groups shared characters through the hybrid PLS 29-15 with PLS 33-11. Groups three and four shared characters through the hybrid PLS 59-14 with PLS 18-2.

Based on the morphological parameters, cultivars were analyzed in clusters (Figure 2). According to the obtained dendrogram, four common groups were obtained. The first group included Coville PLS 19, Patriot PLS 22, and Nelson PLS 29; the second group Simultan PLS 7, Draper PLS 25, and Berkeley PLS 21; the third group Lax PLS 13, and Safir PLS 18; the fourth group Bluetta PLS 45, Northland PLS 52, and Duke PLS 20.

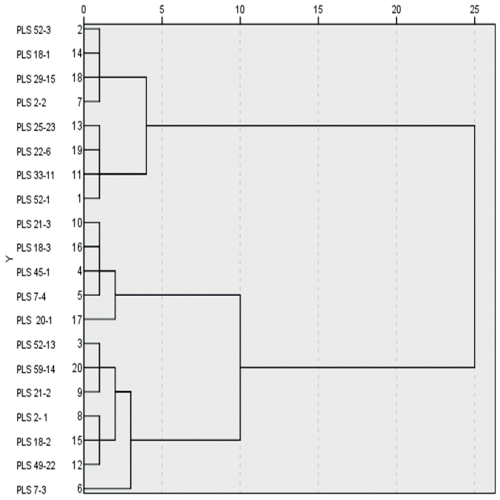

Figure 1. Hybrids grouped in clusters based on the morphological parameters

When the cultivars were analyzed (Table 3), the same variability was observed for the leaf area, where values ranged between 96.169 (Bluetta PLS 45) and 194.795 cm^2 (Pink Lemonade PLS 9) (results expressed for the five leaves). There was a slight variability for petiole length; the values ranged from 0.232 (Bluetta PLS 45) to 0.520 cm (Pink Lemonade PLS 9).

The petiole area recorded the highest value for Pink Lemonades PLS 9 (0.091 cm^2). The lowest value for the Bluetta PLS 45 cultivar (0.026 cm2), followed upwards by Draper PLS 25, Northland PLS 52, Compact PLS 33, Berkeley PLS 21, Duke PLS 20, Simultan PLS 7, Safir PLS 18, Patriot PLS 22, Lax PLS 13, Coville PLS 19, Nelson PLS 29, and Spartan PLS 2.

Table 2. Biometric data variability for the studied hybrids

Hybrid code	Leaf area	Perimeter	Vertical length	Width	Healthy status (%)	Petiole Length	Petiole Area
PLS 7-3	5.488 h	96.147 h	41.182 i	19.812 f	713.740 b	.0586 a	.0050 b
PLS 2-2	10.007 g	123.052 g	47.989 gh	28.990 e	955.300 a	.0594 a	.0055 b
PLS 59-14	10.375 g	127.278 g	51.274 gh	29.024 e	969.600 a	.0631 a	.0057 b
PLS 52-13	10.715 g	129.527 fg	52.392 fgh	29.464 e	971.180 a	.0647 a	.0061 b
PLS 21-2	11.286 fg	133.093 efg	53.476 fgh	29.837 e	971.660 a	.0675 a	.0064 b
PLS 18-2	11.313 fg	135.459 efg	54.322 fgh	30.108 de	980.140 a	.0688 a	.0066 b
PLS 29-15	11.971 efg	137.358 defg	55.135 efg	30.243 de	980.900 a	.0710 a	.0072 b
PLS 21-3	12.270 defg	137.591 defg	55.812 efg	30.582 de	981.080 a	.0774 a	.0073 b
PLS 45-1	12.850 defg	141.390 cdefg	57.268 defg	32.343 cde	982.840 a	.0800 a	.0074 ab
PLS 18-1	12.912 defg	146.856 cdef	59.267 def	32.546 cde	983.280 a	.0808 a	.0079 ab
PLS 52-3	13.245 defg	147.865 cdef	61.773 cde	33.122 cde	983.620 a	.0867 a	.0086 ab
PLS 2- 1	13.592 defg	148.485 cdef	61.807 cde	33.765 cde	984.060 a	.0887 a	.0090 ab
PLS 18-3	13.818 defg	149.144 cdef	63.161 cd	34.273 cde	984.540 a	.0921 a	.0094 ab
PLS 25-23	15.067 cdef	152.090 cde	63.500 cd	34.612 cde	984.920 a	.0926 a	.0098 ab
PLS 49-22	15.184 cdef	156.122 bcd	64.448 cd	35.594 cd	986.240 a	.0940 a	.0103 ab
PLS 33-11	15.721 bcde	157.712 bc	64.482 cd	36.881 c	987.060 a	.1026 a	.0111 ab
PLS 7-4	16.110 bcd	159.650 bc	68.275 b	37.287 bc	989.940 a	.1108 a	.0112 ab
PLS 22-6	18.207 bc	171.630 ab	72.678 ab	37.694 bc	991.000 a	.1142 a	.0123 ab
PLS 20-1	18.961 b	182.756 a	74.710 ab	42.096 ab	1.000.000 a	.1201 a	.0132 ab
PLS 52-1	23.218 a	185.819 a	79.011 a	45.821 a	1.000.000 a	.1418 a	.0161 a

*a-h are corresponding to the post-hoc Duncan test analysis

Table 3. Biometric data variability for the cultivar used as parents in the study

Cultivar code	Leaf area	Perimeter	Vert Length	Healthy	Petiole Length	Petiole Area
Bluetta PLS 45	96.169 f	118.749 e	48.328 f	407.560 b	.2319 c	.0259 c
Draper PLS 25	111.356 ef	123.246 e	48.768 f	523.700 b	.2583 bc	.0304 c
Northland PLS 52	111.789 ef	126.968 e	53.408 ef	745.260 a	.3077 abc	.0316 c
Compact PLS 33	118.053 def	128.674 de	53.577 ef	823.740 a	.3088 abc	.0374 c
Berkeley PLS 21	122.679 def	130.574 de	54.932 def	856.260 a	.3527 abc	.0454 bc
Duke PLS 20	126.651 cdef	132.434 de	57.641 cde	867.360 a	.3579 abc	.0484 bc
Simultan PLS 7	139.347 bcde	146.197 cd	58.623 cde	891.340 a	.3766 abc	.0501 bc
Safir PLS 18	151.869 bcd	146.236 cd	61.536 bcd	905.520 a	.4016 abc	.0509 bc
Patriot PLS 22	159.347 abc	153.176 bc	63.432 bc	942.640 a	.4186 abc	.0559 abc
Lax PLS 13	162.810 ab	154.533 abc	63.669 bc	963.360 a	.4477 abc	.0583 abc
Coville PLS 19	168.732 ab	158.255 abc	63.906 bc	978.180 a	.4537 abc	.0605 abc
Nelson PLS 29	193.175 a	161.860 abc	68.682 ab	982.940 a	.4667 ab	.0747 ab
Spartan PLS 2	193.446 a	168.218 ab	73.355 a	984.740 a	.4799 ab	.0753 ab
Pink Lemonade PLS 9	194.795 a	172.250 a	73.389 a	989.060 a	.5203 a	.0911 a

*a-f are corresponding to the post-hoc Duncan test analysis

The leaf perimeter recorded the lowest value of 118.749 cm for the Bluetta PLS 45 cultivar, increasing significantly to 172.25 for the Pink Lemonade PLS 9. Vertical length has no significant values in terms of variability (data expressed for five leaves).

The WinFolia program used to analyze leaf health highlighted the Bluetta PLS 45 cultivar with the lowest value, having specific signs of disease, and the Pink Lemonade PLS 9 cultivar with the highest value (Table 3). In general, cultivars had good leaf health.

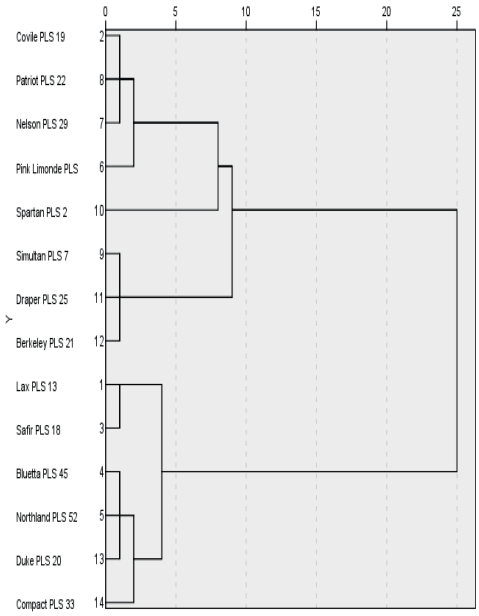

Figure 2. Cultivars grouped clustered by the morphological parameters

Based on the morphological parameters, cultivars were analyzed in clusters (Figure 2). According to the obtained dendrogram, four common groups were obtained. The first group included Coville PLS 19, Patriot PLS 22, and Nelson PLS 29; the second group Simultan PLS 7, Draper PLS 25, and Berkeley PLS 21; the third group Lax PLS 13, and Safir PLS 18; and the fourth group Bluetta PLS 45, Northland PLS 52 and Duke PLS 20.

CONCLUSIONS

The first data about the hybrid serie obtained from mother plants by free pollination are valuable for further research. A database of images and data was obtained and consist as a useful base for an extended tool in performing phenotyping research with the help of digital tools.

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REFERENCES

Asănică, A., Delian, E., Tudor, V. & Teodorescu, R., I. (2017). Physiological activity of some blueberry varieties in protected and outside conditions. *AgroLife Scientific Journal*, *6*(1), 31-39.

Babiker, E., Stringer, S.J. & Sakhanokho, H.F. (2023). Combining high-throughput phenotyping and multivariate analysis to assess fruit quality traits in southern highbush blueberry (*Vaccinium corymbosum* Interspecific Hybrids) germplasm collection. *Hortscience 58*(7):750–755.

Cândea-Crăciun, V.C., Rujescu, C., Camen, D., Manea, D., Nicolin, A., L. & Sala, F. (2018). A non-destructive method for determining the leaf area of the energetic poplar. *AgroLife Scientific Journal*, *7*(2), 22-30.

Edger, P.P., Iorizzo, M., Bassil, N.V., Benevenuto, J., Ferrao, L.F.V., Giongo, L., Hummer, K., Lawas, L.M.F., Leisner, C.P., Li, C., Munoz, P.R., Ashrafi, H., Atucha, A., Babiker, E.M., Canales, E., Chagne, D., DeVetter, L., Ehlenfeldt, M., Espley, R.V., Gallardo, K., Gunther, C.S., Hardigan, M., Hulse-Kemp, A.M., Jacobs, M., Lila, M.A., Luby, C., Main, D., Mengist, M.F., Owens, G.L., Veazie, P.P., Polashock, J., Pottorff, M., Rowland, L.J., Sims, C.A., Song, G., Spencer, J., Vorsa, N., Yocca, A.E. & Zalapa, J. (2022). *Horticulture Research*, 9: uhac083

Farneti, B., Emanuelli, F., Khomenko, I., Ajelli, M., Biasioli, F. & Giongo, L. (2020). Development of a novel phenotypic roadmap to improve blueberry quality and storability. *Front. Plant Sci. 11*:1140.

Hera, O., Teodorescu, R. & Sturzeanu, M. (2021). Blueberry (*Vaccinium corymbosum*) breeding programe in the main cultivating countries. *Scientific Papers. Series B, Horticulture. LXV*(1), 82-89.

Lobos, G.A. & Hancock, J.F. (2015). Breeding blueberries for a changing global environment: a review. *Front. Plant Sci*. 6:782.

Mladin, P., Mladin, Gh., Ancu, I. & Chitu, V. (2008). Results of the blueberry breeding at the Research Institute for Fruit Growing Piteşti. *Bulletin UASVM, Horticulture 65*(1), 300-303.

Patrick, A. & Li, C. (2017). High throughput phenotyping of blueberry bush morphological traits using unmanned aerial systems. *Remote Sens*. *9*, 1250; doi:10.3390/rs9121250.