

Evaluation of the ampelographic characteristics of the new accessions introduced in grapevine germplasm collection (*Vitis vinifera* L.)

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ABSTRACT

The characterization of 22 grapevine accessions (*Vitis vinifera* L.), considered old, autochthonous and in danger for extinction, was performed using 49 OIV descriptors. The evaluation of the ampelographic descriptors for the young shoot, the young and mature leaf, the bunch, as well as some production and quality parameters, highlighted the diversity of the epigenetic characteristics of the newly introduced accessions into collection. The morphological description revealed the main features of each accession and the applied statistical approaches confirmed the possible relatedness among studied genotypes linked to all 49 descriptors.

Keywords: grapevine, ampelography, identification, morphological description, dendrogram.

INTRODUCTION

The grapevine is considered an important crop due to its profit income for the national economy and its nutritional value for food and human health (Bounab and Laiadi, 2019). Grapevine is one of the oldest known fruit species in the world and therefore researchers have always wanted to know more about it (Galet, 1956, 1998; Khouni, 2023). Ampelographic studies are essential for differentiating grapevine genotypes and are considered indispensable for their characterization. For a complete characterization, beside the morphological description, the molecular genetic methods are now added and bring accurately and certitude for grapevine genotypes identification (Maul *et al.*, 2015; Popescu *et al.*, 2017). In 1984, the International *Vitis* Varietal Catalog (VIVC) was founded (Alleweldt, 1988) and according to Maul *et al.* (2014), this represents a database containing approximately 23,000 primary names and 42,000 synonyms of various grapevine species and cultivar/cultivars. In addition, the International Organization of Vine and Wine (OIV) published a guide for varietal identification (2009) which is

considered a guide unanimously accepted by ampelography specialists. Through these publications, it was established a consensus for applied descriptors by the International Plant Genetic Resources Institute (IPGRI), the International Union for the Protection of New Cultivars (UPOV) and the OIV.

In this study, we set out to present a more accurate description of the ampelographic characters of some old vine genotype, using the internationally accepted methodology. Our objective was to verify the authenticity of the new accessions by comparing them with the old, initial descriptions (in the case of those for which we found information), representing the important initial stage for the complete identification of the old genotype, considered autochthonous and valuable for enriching the varietal assortment in our collection.

MATERIALS AND METHODS

Plant materials.

Were analysed 22 accessions (Table 1) grown and maintained into grapevine germplasm collection belonging to the National Research and Development Institute for Biotechnology in Horticulture Stefanesti-Arges (NRDIBH).

Ampelographic description.

Forty-nine OIV features were monitored over two consecutive growing seasons in 2021 and 2022 (OIV, 2009, Cost action FA1003-GROPENET). The most important ampelographic characteristics for the morphological characteristics of young shoots, young leaves, shoots, flowers, mature leaves, grapes and berries and grape yield per m² were registered. The ampelographic characterization of each accession was carried out according to the ampelographic methodology, to specific plant organs in certain stages of development and maturation.

Statistical analysis.

Table 1. The accessions analysed in this study and newly introduced into germplasm collection

No.	Accessions name	Berry colour	No.	Accessions name	Berry color
1	'Balaban alb'	White	12	'Muscat t�m�nios'	White
2	'Bacator'	White	13	'Negru de C�u�ani'	Black
3	'Bicane'	White	14	'Negru tinctorial'	Black
4	'Cabasm� alb�'	White	15	'Om r�u'	White
5	'Cabasm� neagr�'	Black	16	'Papasc� neagr�'	Black
6	'Coarn� aromat�' neagr�	Black	17	'P�rciu'	White
7	'Coarn� t�m�ioas�' neagr�	Black	18	'Rar� alb�'	White
8	'Frumoas� de Ghioroc'	White	19	'T�ţa caprei alb�'	White
9	'Galben� m�runţ�'	White	20	'T�ţa vacii alb�'	White
10	'Chasselas crocant'	White	21	'Tigvoas�'	White
11	'Morostin�'	White	22	'Zghihar� rar�'	White

The 49 descriptors evaluations were used to obtain a dissimilarity matrix among all descriptors for all accessions according to "Euclidean distance". According to the generated matrix, the morphological distance coefficients were calculated by NTSYS-pc

version 2.2 (Rohlf, 2009). The dendrogram was obtained using unweighted pair group method with arithmetic (UPGMA) (Sneath and Sokal, 1973) for further evaluation of ampelographic differences between accessions.

RESULTS AND DISCUSSIONS

Phenotypic variability of the main morphological characteristics.

The ampelographic characterization with standard descriptors applied for the 22 accessions grown in the same collection (Table 1), highlighted the similarities and differences among grapevine genotypes. All the morphological characteristics and evaluations carried out during the growing seasons in our *ex situ* germplasm collection were analyzed in comparison to the description of each genotype from the reference documents (the ampelographies published between 1960 and 1966). Excepting the seven genotypes ('Balaban alb', 'Galbenă mărunță', 'Chasselas crocant', 'Morostină', 'Muscat tămâios', 'Negru tinctorial' and 'Zghihară rară') for which no detailed information of their ampelographic characteristics was found, all the other accessions showed to have the specific characteristics as they were initially described.

With all accessions no differences were recorded for the following descriptors: 1) young shoot - opening of the shoot tip (OIV 001) and distribution of anthocyanin coloration on prostrate hairs of the tip (OIV 002); 2) mature leaf with absence of the teeth in the petiole sinus (OIV 81-1) and absence of teeth in the upper lateral sinuses (OIV 083-2); 3) berries with complete formation of the seeds (OIV 241); 4) phenological stages for budburst (OIV 301) and flowering (OIV 302). The other 42 descriptors and their evaluations revealed the differences and distinctiveness among accessions (data not shown).

For the young shoot aspect, most accessions showed none or very low intensity of anthocyanin coloration on prostrate hair (OIV 003) of the shoot tip (81.8%), with a distinct high intensity recorded for 'Țița vacii albă'. Concerning the presence and density of prostrate hairs on the shoot tip (OIV 004) were noted different degree of variation, from non or very low density of prostrate hairs for 'Cabasmă neagră', 'Negru tinctorial', to very high density for 'Om rău'. The shoots characteristics regarding the coloration on dorsal (OIV 007) and ventral (OIV 008) side of shoot internodes, our registrations for the analysed accessions showed variation from green (36.4%), to green with red lines (50.0%) and uniform red (13.6%). The mean value of the length of the tendrils (OIV 017) varied from very short, or up to 10 cm ('Galbenă mărunță' and 'Papască neagră'), to long tendrils, or about 25 cm ('Bicane', 'Cabasmă neagră', 'Chasselas crocant' and 'Morostină').

With the young leaves were registered different quotation for color of the upper side of the 4th blade (OIV 051) from green (31.8% of the accessions) to copper - reddish color for 9.1% of the studied accessions ('Pîrciu' and 'Rară albă').

The mature leaves presented specific features for *V. vinifera*, with a medium size of the blade (OIV 065) for most of the accessions (63.6%), and also with pentagonal shaped (OIV 067) and five lobes (OIV 068) leaves for the most genotypes (81.1%). Anthocyanin coloration of main veins on the upper side of blades (OIV 070) were generally weak, or absent (45.5% of the accessions), distinct being the accessions 'Bacator' and 'Rară albă' with the presence of anthocyanin coloration beyond the second bifurcation of the veins.

The main characteristic of the flower for grapevine is the development of the reproductive organs. Similarly to our previous studies (Popescu *et al.* 2018, Iliina *et al.* 2023), was noticed a high proportion of accessions developing female-type flowers. Most of the analyzed accessions have hermaphrodite flowers, but six of them ('Bacator', 'Bicane', 'Morostină', 'Negru de Căușani', 'Țița vacii albă' and 'Tigvoasă'), representing 27.3% of the

total, develop female flowers with anthers inclined to the outside of the ovary and produce pollen with reduced germination capacity, or infertile pollen.

For the bunches and berries characteristics, the notations carried out revealed a high level of variability among accessions. For the length of the bunches (OIV 202), were registered accessions with short bunches ('Om rău' and 'Pîrciu', or 9.1% from the total), with medium sizes (68.2%) or long length ('Bicane', 'Cabasmă neagră', 'Frumoasă de Ghioroc', 'Papască neagră' and 'Țița caprei albă', or 22.7% from the total).

Concerning the compactness of the bunches of grapes (OIV 204) and its shape (OIV 208) among studied accessions, all types of quotations were registered, standing out 'Galbenă mărunță' and 'Frumoasă de Ghioroc' for funnel shaped of the bunch, and 'Cabasmă neagră' and 'Negru de Căușani' with a very high density of the berries in the bunch. Looking for the number of wings in the bunch (OIV 209), more than half of the grapevine cultivars (59.1%) have one to two wings, another 36.4% (8 accessions) produced bunches without wings, and only 'Galbenă mărunță' distinguished by bunches with 3-4 wings.

A high heterogeneity among the studied accessions was registered through the ampelographic descriptors for berry characteristics. Thus, was noticed berry length (OIV 220) variations from short to long, with narrow to medium width (OIV 221), only 'Om rău' differing by a wide average around 23 mm of the berries. The shape of the berry (OIV 223) was established and recorded after the measurements of lengths and widths of normally and health berries taken from the middle part of 10 bunches. Were registered the following types of shapes: 1) globose shape registered for 63.6% among analyzed accessions; 2) obtuse ovoid for 'Coarnă neagră aromată', 'Coarnă neagră tamîioasă' and 'Frumoasă de Ghioroc'; 3) ovoid berry for 'Om rău', 'Țița vacii albă' and 'Tigvoasă'; 4) broad ellipsoid shaped for 'Bicane' and 'Țița caprei albă'.

At the maturity were made observations and evaluations of the color of the berries (OIV 225) from the middle part of 10 bunches. Were distinguished four groups: 1) green yellow for the 68.2% of the accessions; 2) blue black colour of skin for four genotypes ('Muscat tămâios', 'Negru de Căușani', 'Negru tinctorial', 'Papască neagră'); 3) red colour for 'Coarnă neagră aromată' and 'Coarnă neagră tamîioasă'; 4) dark red violet for 'Cabasmă neagră'.

A very important feature for breeders and wine makers is the particular flavor of the berries at the maturity (OIV 236). Among the 22 accessions, the majority (86.4%) were characterized by the lack of a special taste, only one ('Muscat tămâios') highlighted a muscat flavor and two genotypes ('Coarnă neagră aromată' and 'Coarnă neagră tamîioasă') had special flavors, others than muscat, foxy or herbaceous.

Analyzing the main moments of plant development, we found that 'Frumoasă de Ghioroc', in comparison to all the other accessions, is distinguished by an earlier time of beginning of berry ripening (OIV 303) and also an earlier time of full physiological maturity of the berry (OIV 304).

All the studied accessions, are characterized by single bunch weight (OIV 502) and single berry weight (OIV 503) varying between very low and medium values, and also by a medium sugar content and low acidity of the must. Among the 22 accessions, are standing out 'Bacator' producing must with a high sugar content (OIV 505) and 'Papască neagră' producing must with a high acidity (OIV 506).

Statistical analysis applied to evaluate the diversity of ampelographic descriptors.

For a visual presentation of the differences between the accessions (each to each other) according to the ampelographic descriptors presented previously, we used the dissimilarity matrix based on Euclidian distance, and the dendrogram obtained with these Euclidean distance values. In table 2 is presented the dissimilarity matrix based on Euclidian distance showing the degree of variability of morphological evaluations between

each accession to all the others. The data showed an epigenetic difference ranging from 0 in the case of 'Coarnă neagră aromată' and 'Coarnă neagră tămîioasă' (no ampelographic differences were recorded) to 12.97 between 'Frumoasă de Ghioroc' and 'Pîrciu' (differences for 23 out of 49 descriptors).

The highest differences were noticed between 'Frumoasă de Ghioroc' and all the others accessions (total 232.6), and the lowest differences between 'Muscat tămîios' and all the other accessions (total 166.4).

Using Euclidean distance and Ward's method was obtained a dendrogram showing the relationships among the studied accessions (Fig. 1). The dendrogram based on ampelographic characteristics for 49 descriptors revealed three distinct groups.

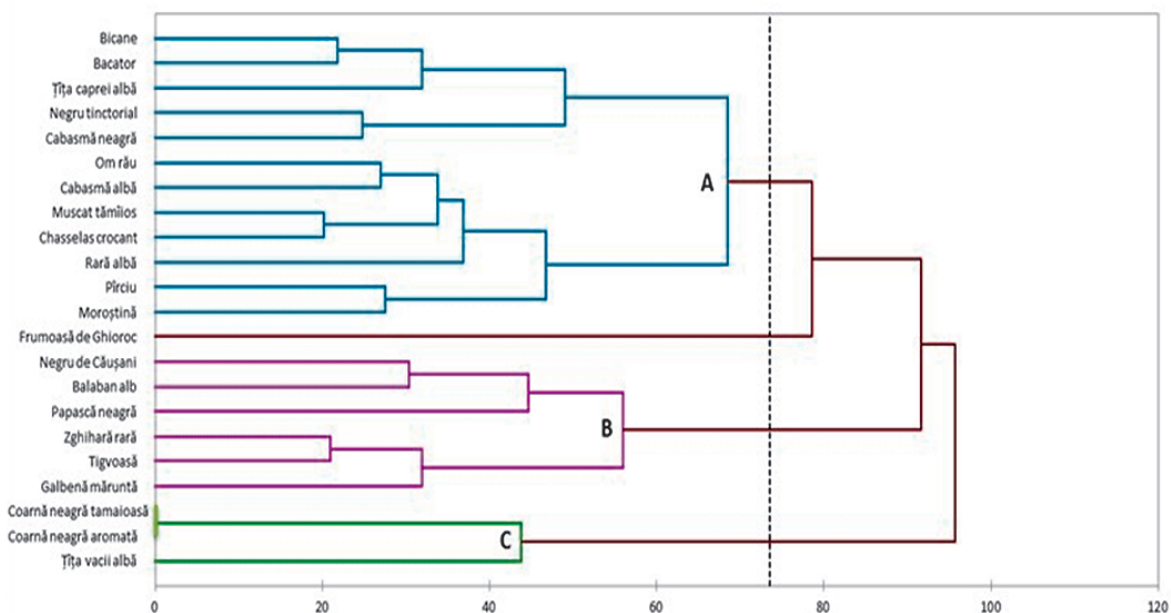


Figure 1. Representation of Agglomerative hierarchical clustering (AHC) dendrogram of Euclidian distance showing the relationships among the 22 accessions based on 49 OIV descriptors

Group A comprises 12 genotypes, 9 of them producing white color skin berry and 3 of them black color. Accessions with black grapes ('Negru tinctorial', 'Cabasmă neagră' and 'Muscat tămîios'), are similar each other by 23 descriptors, but 'Muscat tămîios' is remarked in comparison with the other two accessions by density of prostrate hairs on the shoot tip, yellow color of the young leaf, and conical shape of the bunch. A Euclidian distance of 7.42 was obtained between 'Pîrciu' and 'Moroștină', due to only 15 descriptors with different quotations, most of these for mature leaf and grape features. Both genotypes are characterized by very large blade with 3 lobes and wedge-shaped, with short berry length, of globose shape and without flesh anthocyanin coloration. The second group (B) put together four genotypes with white grapes ('Balaban alb', 'Zghihară rară', 'Tigvoasă' and 'Galbenă mărunță'), and two with black color grapes ('Papască neagră' and 'Negru de Căușani'). For all these accessions were registered the same quotations for 16 descriptors. Among them, 'Zghihară rară' and 'Tigvoasă' show the highest degree of similarity, and a very low value of distance (6.47), and 'Tigvoasă' and 'Negru de Căușani' are distinct by the type of functionally female flower.

In group C are gathered together three very appreciated table grape accessions ('Coarnă neagră aromată', 'Coarnă neagră tămîioasă' and 'Țița vacii albă'). These accessions are

characterized by berries with narrow ellipsoid shape and large size, fleshy and crunchy core with particularly pleasant taste. If 'Țița vacii albă' is considered as an autochthonous genotype, cultivated since ancient times in Romanian vineyards (Constantinescu *et al.* 1962), the other two accessions were selected in 1948 by Gherasim Constantinescu and Elena Negreanu in old vineyards as free-pollinated hybrids. Both are supposed to have the same origin parent, 'Coarnă neagră', and this could be the reason of their complete similarity regarding to their morphological features and technological parameters. We consider that only molecular analysis could bring new information and clarifications regarding the origin of these two accessions and relationships between them.

As it was found from the ampelographic determinations, and it is obvious from the dendrogram, 'Frumoasă de Ghioroc' is distinguished by the similarities of its characteristics with all other accessions. We know about this genotype that it was identified in the vineyards of Banat, and its origin is unknown.

Table 2. Euclidean distance obtained by Ward's method showing the relationships among accessions based on 49 OIV ampelographic traits evaluation

	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	A13	A14	A15	A16	A17	A18	A19	A20	A21	A22
A1	0	8.99	9.07	7.35	8.47	11.02	11.02	11.85	9.37	8.44	8.20	8.60	7.79	9.36	9.38	9.23	8.86	9.15	10.25	10.31	8.97	7.23
A2	8.99	0	6.60	8.32	8.16	8.81	8.81	10.61	9.76	7.15	7.43	7.00	8.92	7.91	7.46	9.66	8.13	7.29	7.72	8.49	8.83	8.76
A3	9.07	6.60	0	8.91	7.41	9.75	9.75	9.79	10.68	8.63	8.10	7.68	8.93	8.72	9.60	9.98	10.50	8.79	7.61	9.21	9.20	9.40
A4	7.35	8.32	8.91	0	8.89	8.74	8.74	10.88	7.91	7.25	7.43	7.44	7.95	9.29	7.35	8.90	8.15	8.42	9.41	8.32	7.97	7.20
A5	8.47	8.16	7.41	8.89	0	9.89	9.89	11.15	10.94	8.97	8.32	8.32	8.86	7.05	10.17	10.08	9.84	8.60	8.70	10.22	10.25	10.21
A6	11.02	8.81	9.75	8.74	9.89	0	0.00	11.23	9.68	7.39	9.94	7.45	9.71	9.40	8.73	9.66	11.17	10.41	8.96	8.10	8.06	9.18
A7	11.02	8.81	9.75	8.74	9.89	0.00	0	11.23	9.68	7.39	9.94	7.45	9.71	9.40	8.73	9.66	11.17	10.41	8.96	8.10	8.06	9.18
A8	11.85	10.61	9.79	10.88	11.15	11.23	11.23	0	11.45	10.34	10.96	9.30	11.26	11.45	11.08	11.98	12.97	10.46	10.69	11.40	11.51	11.04
A9	9.37	9.76	10.68	7.91	10.94	9.68	9.68	11.45	0	7.25	7.92	8.00	9.50	9.95	8.86	9.69	9.72	9.70	10.95	10.16	8.36	6.83
A10	8.44	7.15	8.63	7.25	8.97	7.39	7.39	10.34	7.25	0	6.61	6.36	8.24	8.66	7.51	9.13	8.55	7.79	7.89	8.87	8.09	7.00
A11	8.20	7.43	8.10	7.43	8.32	9.94	9.94	10.96	7.92	6.61	0	7.88	8.41	8.82	8.57	10.29	7.42	8.49	8.97	9.23	8.74	7.66
A12	8.60	7.00	7.68	7.44	8.32	7.45	7.45	9.30	8.00	6.36	7.88	0	7.78	7.86	8.08	8.07	9.10	7.80	7.82	8.10	8.63	7.70
A13	7.79	8.92	8.93	7.95	8.86	9.71	9.71	11.26	9.50	8.24	8.41	7.78	0	8.45	9.20	8.90	11.26	9.91	10.23	10.04	8.14	8.28
A14	9.36	7.91	8.72	9.29	7.05	9.40	9.40	11.45	9.95	8.66	8.82	7.86	8.45	0	9.38	9.93	9.88	8.54	9.58	10.70	9.88	9.25
A15	9.38	7.46	9.60	7.35	10.17	8.73	8.73	11.08	8.86	7.51	8.57	8.08	9.20	9.38	0	10.26	8.78	8.54	9.79	8.90	8.44	8.76
A16	9.23	9.66	9.98	8.90	10.08	9.66	9.66	11.98	9.69	9.13	10.29	8.07	8.90	9.93	10.26	0	11.80	11.02	8.97	10.30	9.82	9.16
A17	8.86	8.13	10.50	8.15	9.84	11.17	11.17	12.97	9.72	8.55	7.42	9.10	11.26	9.88	8.78	11.80	0	9.73	11.17	10.58	11.01	9.75
A18	9.15	7.29	8.79	8.42	8.60	10.41	10.41	10.46	9.70	7.79	8.49	7.80	9.91	8.54	8.54	11.02	9.73	0	8.60	10.26	10.17	9.48
A19	10.25	7.72	7.61	9.41	8.70	8.96	8.96	10.69	10.95	7.89	8.97	7.82	10.23	9.58	9.79	8.97	11.17	8.60	0	9.11	9.83	10.27
A20	10.31	8.49	9.21	8.32	10.22	8.10	8.10	11.40	10.16	8.87	9.23	8.10	10.04	10.70	8.90	10.30	10.58	10.26	9.11	0	7.98	8.81
A21	8.97	8.83	9.20	7.97	10.25	8.06	8.06	11.51	8.36	8.09	8.74	8.63	8.14	9.88	8.44	9.82	11.01	10.17	9.83	7.98	0	6.47
A22	7.23	8.76	9.40	7.20	10.21	9.18	9.18	11.04	6.83	7.00	7.66	7.70	8.28	9.25	8.76	9.16	9.75	9.48	10.27	8.81	6.47	0

A1-'Balaban alb', A2-'Bacator', A3-'Bicane', A4-'Cabasmă albă', A5-'Cabasmă neagră', A6-'Coarnă neagră aromată', A7-'Coarnă neagră tămîioasă', A8-'Frumoasă de Ghioroc', A9-'Galbenă mărunță', A10-'Chasselas crocant', A11-'Morostină', A12-'Muscat tămâios', A13-'Negru de Căușani', A14-'Negru tinctoria', A15-'Om rău', A16-'Papască neagră', A17-'Pirciu', A18-'Rară albă', A19-'Țița caprei albă', A20-'Țița vacii albă', A21-'Tigvoasă', A22-'Zghihară rară'.

CONCLUSIONS

The ampelographic method by standardized description was used to obtain an up-to-date description of the 22 neglected old grapevine accessions. The evaluation of the morphological features and their comparison with the old reference documents were important to confirm the authenticity of newly plant material introduced into germplasm

collection. The ampelographic characterization of the accessions represents only the first step in preventing information for 'true-to-type' status, and also is essential in the management of germplasm collections.

The 49 OIV descriptors evaluated by us during two consecutive years were applied to obtain and complete the description file for each accession, and the applied statistical methods revealed the diversity of their morphological features and degree of similarity.

Starting from the consideration that viticultural germplasm collections represent a valuable source of biodiversity, correctly and completely characterized accessions represent a guaranteed biological material for scientific and economic activities. Among the accessions studied and presented in this article, the following can be distinguished: 'Frumoasă de Ghioroc' by precocity, 'Bacator', 'Bicane', 'Moroștină', 'Negru de Căușani', 'Țița vacii albă' and 'Tigvoasă' by the type of flower, 'Om rău', 'Țița caprei albă', 'Țița vacii albă' and 'Tigvoasă' for the size and commercial aspect of the bunch, 'Coarnă neagră aromată' and 'Coarnă neagră tămâioasă' for the special flavor of the berries, and 'Bacator' and 'Papască neagră' for the quality of the obtained must.

The existing genetic resources in the germplasm collections bring new opportunities for reconsidering the old grapevine genotypes, removed from the cultivated varietal assortment and for their efficiently used in breeding and winemaking programs. Moreover, the genetic resources are very important for ensuring the sustainability of grapevine production under climate change.

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