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AMPELOGRAPHIC AND AGRONOMIC VARIABILITY WITHIN THE 'TĂMÂIOASĂ ROMÂNEASCĂ' CULTIVAR

Daniela Doloris CICHI[1], Felicia STOICA[1], Ramona CĂPRUCIU[1], Mihai CICHI[2]

[1]University of Craiova, Faculty of Horticulture, Horticulture & Food Science Department, 13 A.I. Cuza Street, Craiova, Dolj, Romania
[2]University of Craiova, Faculty of Agronomy, 19 Libertății Street, Craiova, Dolj, Romania

Corresponding author email: danielacichi@yahoo.com

Abstract

The 'Tămâioasă românească' variety (syn. Muscat à petits grains blancs) is one of the oldest and most famous varieties for aromatic wines in Romania. This prospective study aimed to evaluate the variability of some morphological, agrobiological and qualitative features of 'Tămâioasă românească' cv. and of the two clones 'Tămâioasă românească' 104 Dg. and 'Tămâioasă românească' 36 Pt., in the specific conditions of the Drăgășani vineyard, Romania. Also, it aimed to identify some ampelographic descriptors useful for the discrimination between the two clones on the one hand and between the clones and the 'Tămâioasă românească' variety on the other hand. Based on the 48 ampelographic and ampelometric descriptors used and analyzed, our partial results show that there is an important phenotypic variability within the population of the 'Tămâioasă românească' variety.

Key words*: ampelometric descriptors, clones, grapevine, phenotypic variability.*

INTRODUCTION

The genetic diversity of the *Vitis* genus has always been a very valuable source for obtaining new genotypes, useful from a viticultural and oenological point of view, but also an alternative for adapting the varieties to the effects generated by disturbing abiotics and biotics factors from the viticultural ecosystem (Eibach and Töpfer, 2015; Riaz et al., 2018).

The conservation, assessment and use of genetic diversity in grapevines are a topical concern both worldwide (Lacombe et al., 2004; Žulj Mihalević et al.,2013 ; Marković et al., 2017; This et al., 2006) and nationally (Bodea et al., 2009 ; Bucur and Dejeu, 2018; Cichi et al., 2015; Popescu et al., 2017; Stroe, 2016).

Intra-varietal variability in grapevines is a valuable germplasm source, on which clonal selection and breeding of grapevines are based (Hajdu et al., 2011; Mannini, 2000; Oprea & Moldovan, 2007; OIV, 2017).

Various international studies and programs are being conducted to test and validate grapevine phenotyping methodologies, as well as to identify simple, fast, and low-cost methods to identify a large volume of grapevine accessions (Boursiquot et al., 1995; Rustioni et al., 2014; This et al., 2004; Volk, 2010).

Several phenotyping methodologies have been used and tested, including ampelometric characteristics of leaves (Bodor et al., 2013, 2018; Chitwood et al., 2014), bunch and berry morphology (Diago et al., 2013; Ferreira et al., 2018; Grimplet et al., 2019) as well as grape biochemical composition (Bigard et al., 2018; Escudier et al., 2016; Russo et al., 2009).

The grown of grapevines and the production of wine have a very long history and tradition in Romania (Teodorescu, 1964). However, the number of native varieties and the cultivated areas with these varieties are currently low (Antoce and Călugăru, 2017; Cichi et al., 2020). There is a real risk of diminishing the national viticultural germplasm fund, with all the consequences deriving from it in the current context of the challenges posed by climate change (Bucur et al., 2016; Cichi, 2006; Duchene et al., 2010), the limited number of varieties currently used for planting in Romania, the interest of many grape growers oriented towards international varieties, the lack of autochthonous planting material and of a deficient financing regarding the conservation and efficient use of the local genetic resources (Cichi et al., 2019).

The Drăgășani vineyard has a very old viticulture history and tradition in Romania.

According to Teodorescu I.C. (1943) the existence of vine on the territory of the current vineyard is related to the existence of the first plantations made by the Geto-Dacians (1st century BC). It is located in the Muntenia and Oltenia Hills wine-growing region, between the Getic Subcarpathians to the north and the Romanian Plain to the south and south-east, being located between 44°30' and 44°55' parallels north latitude and between 23°55' and 24°15' meridians eastern longitude (Olteanu et. al., 2002).

'Tămâioasă românească' cv. (syn. Muscat à petits grains blancs, Tămâioasa alba românească, Tămâioasa alba de Drăgasani) is one of the oldest and most famous varieties for aromatic wines in Romania, being considered a local variety and a reference variety for the Drăgășani vineyard (as it appears from the synonymy with the variety). The Cotnari vineyard and the Pietroasa viticultural center are also the Romanian traditional cultivation areas of the 'Tămâioasă românească' variety (Constantinescu et al., 1960).

At present there are still controversies regarding the geographical and the genetic origin of this variety (Popescu et al., 2017). About the 'Tămâioasă românească' cv. and the wines produced from this variety in Drăgășani, there are written documents that attest to its cultivation a long time before to Phylloxera (Istocescu et al., 2004). These documents refer to the wine producers, the quality of the wine and the medals obtained by the wines at international and national wine exhibitions and competitions. In this regard, Iorga N. (1925), quoted by Teodorescu (1943), in the paper *History of Romanian Trade* (published in Bucharest, 1925, p.131), mentioned that in 1545 Greek merchants in Wallachia were stopped to sell in large quantities a sweet wine like *Malvazia*, specific to the Drăgășani region, in Sibiu (across the border at that time). Given the specifics of the qualitative potential of the 'Tămâioasă românească' cv. to produce natural semi-sweet and sweet wines, we appreciate that, most likely, the sweet Drăgășani wine mentioned above came from the 'Tămâioasă românească' variety.

Although it is a very old variety in culture in Romania, only four clones were obtained for the 'Tămâioasă românească' variety: 'Tămâioasă românească' 104 Dg. in 1982 at the Research and Development Station for Viticulture and Oenology Dragasani, 'Tămâioasă românească' 36 Pt. in 1982 and 'Tămâioasă românească' 5 Pt. in 1989 (at S.C.D.V.V. Pietroasa) and 'Tămâioasă românească' 24 Cot. in 2009 at S.C. Cotnari S.A.

Various researches carried out at national level regarding the 'Tămâioasă românească' variety focused mainly on the agronomic, technological characteristics and on the quality of the wines obtained from this variety in different cultivation areas (Popescu et al., 2009; Stoica et al., 2008, 2009; Vișan et al., 2014), but also on the SSR markers (Ghețea et al., 2010; Popescu et al., 2017) or several ampelographic characteristics (Giugea et al., 2019; Gorjan, 2012; Rotaru, 2009). Research on the evaluation of the phenotypic traits of the 'Tămâioasa românească' clones is limited (Stroe et al., 2009; Stoica et al., 2017).

In this context, this prospective study aimed to evaluate the variability of some morphological, agrobiological and technological features of 'Tămâioasă românească' cv. and of the two clones 'Tămâioasă românească' 104 Dg. and 'Tămâioasă românească' 36 Pt., in the specific conditions of the Dragasani vineyard. Also, it aimed to identify some ampelographic descriptors useful for the discrimination between the two clones on the one hand and between the clones and the 'Tămâioasă românească' variety on the other hand.

MATERIALS AND METHODS

Plant material. The plants used in the present study were the cultivar of *Vitis vinifera* L. 'Tămâioasă românească', and 'Tămâioasă românească' 104 Dg. and 'Tămâioasă românească' 36 Pt. clones. The vines were 18 years old. Ten vines per cultivar and clones were selected for the study, in three replications. The vines were cultivated under the same growing conditions using rootstock Kober 5 BB, with the 2.0 x 1.2 m spaces, semi-tall shape of the stem (with a trunk of 0.6 m), Double Guyot pruned, 12 bud/m^2, without irrigation.

Location and climatic characteristics. The study was conducted for three consecutive years (2018-2020) in the Drăgășani vineyard,

Drăgășani -Dealul Olt area. The main climatic characteristics during the experimentation period are shown in Table 1. The weather data were obtained from the Drăgășani meteorological Station, located at approximately 3 Km from the experimental site. In terms of heliothermal resources, the studied years were particularly favourable for the grapevine. Although the volume of precipitation was within normal limits in relation to the multiannual values, there is still a semi-arid aspect during the growing season based on De Martonne Aridity Index (Table 1).

Table 1. Main climatic indexes of the experimental site

Climatic Index	Average 2018-2020	Class
Winkler Index	1810	Moderate temperate
Huglin`s heliothermal index (IH)	2525	IH5-Warm
Annual Rainfall (mm)	750	Normal for region
Rainfall in the growing season (mm, April 1st to September 30th)	425	Normal for region
De Martonne Aridity Index (IDM)	33	Slightly- arid
De Martonne Aridity Index growing season (IDM, April 1st to September 30th)	14.53	Semi-arid
Cool nights Index (IF)	13.04	CI_{+1}- Cool night

Ampelographic descriptors. The observations were carried out for three consecutive years. The ampelographic descriptors were recorded in accordance with OIV standardized descriptors and methods (OIV, 2009), at different stages of the growth cycle : 25 for mature leaf (OIV 067- 068, OIV 077-080, OIV 093-094, OIV 601- 617), 2 for inflorescence (OIV 152-153); 7 for bunch (OIV 202-204, OIV 206-209); 8 descriptors for berry (OIV 220-223; OIV225-226; OIV 236, OIV 238), 2 for vegetative growth (OIV 353-354) and 4 for grape yield (OIV 502-503, OIV 505-506).

Sampling measurement and analyses. Ten bunches for each clone/cultivar, 10 berries from the middle part of bunches, in 3 replicates, were used for measurements and analyses of bunch and berry traits, at full maturity. Sugar content (°Brix values) was measured using Kruss Optronic Hand Refractometer Hrot 32. Total acidity of must (g/L H_2SO_4) was determined by the titration method, NaOH 0,1N until pH 7.0. Sugar content and total acidity of must measurements were done in five replicates.

Fertility. The observations were made after flowering, targeting the number of inflorescences/vine (Ni), number of total shoots/vine (Nts), number of fertile shoots/vine (Nfs) and it was expressed by the relative fertility index (Rfi) and absolute fertility index (Afi). The two fertility indices were calculated according to the following formula:

Rfi = Ni/Nts;

Afi = Ni/Nfs.

Statistical analysis. Each variable was examined by analysis of variance (ANOVA). All variables that were significant in the F test were analysed by HSD Tukey`s test to means separation and to establish if there were significant differences among the clones and among the clones and 'Tămâioasă românească' cv.

RESULTS AND DISCUSSIONS

Along with SSR markers, the adequacy of the use of ampelographic descriptors of adult leaves, grapes and berries has been demonstrated as valuable tools in the identification of grapevine varieties and the evaluation of clonal polymorphism (Atak et al., 2014). The leaf is one of the most important vegetative organs used in phenotypic descriptions and morphological identification of grapevine cultivars (Bodor et al., 2013).

Regarding the phenotypic homogeneity of the ampelometric characteristics of the adult leaf in the 'Tămâioasă românească' cv. population, an obvious variability can be recorded. The coefficient of variation had values between 8.65% (OIV 601- lenght of vein N1, 104.91 ±9.08 mm) and 33.15% (OIV 611- lenght of vein N5, 24.05±7.98 mm). An important variability in 'Tămâioasă românească' cv. was also found in terms of the depth of the upper lateral sinuses, CV% = 23.66 for length petiole sinus to upper lateral leaf sinus (OIV 605, 43.79±10.36 mm). Chitwood et al. (2016) mention a variability in distal sinus depth associated with colder, drier climates during the growing season.Lenght of tooth of N2 (OIV 612) and width of

tooth of N2 (OIV 613) had a high degree of variation both in 'Tămâioasa românească' cv.

and in the two clones, the coefficient of variation recording values between 20.07% (OIV 613 at 'Tămâioasa românească' 36 Pt.) and 31.84% (OIV 613 at 'Tămâioasa românească' cv.).
The lenght of vein N1 (OIV 601), CV% with values between 7.78% ('Tămâioasa românească' 36 Pt.) and 8.72% ('Tămâioasa românească' 104 Dg.) had the lowest degree of variation in both the 'Tămâioasa românească' variety and the two clones. In Table 2 there are presented only the coded OIV descriptors with numerical evaluation showing the distinct features among 'Tămâioasă românească' cv., 'Tămâioasă românească' 36 Pt. and 'Tămâioasă românească' 104 Dg. clones.

Table 2. Main distinct phenotypical characteristics evaluated by OIV descriptors

OIV Code	'Tămâioasă românească' cv.	'Tămâioasă românească' 36 Pt.	'Tămâioasă românească' 104Dg.
067	4	5	4
078	7/5	7/5	3
093	1	3/5	3
094	7	7	5
152	3	3	3
153	2	2	2
204	7	7	7
208	2	1/2	1/2
209	2	2	2
223	2	2	2
236	2	2	2
601	3	3/5	3
602	3	5	5
603	5	5	5
605	3	3	5
606	3	3	5
607	7	7	5/7
608	3/5	5	5
609	5	7	5
610	3	5	3
611	3	1	3
612	5	5	3
614	3/5	3/5	3
615	3	5	3/5
617	3	5	5

Regarding the fertility, as a valuable trait of grapevine varieties to reproductive performance and capitalize on specific biotope conditions, one can notice a high variability of the relative fertility of the shoots (the ratio between the number of inflorescences/vines and the total number of shoots/vine) in the 'Tămâioasă românească' variety, in which CV% = 28 % . A medium variability of the absolute fertility (Afi) is observed both in 'Tămâioasă românească' cv. as well as in the two clones. This is partly explained by the response of genotypes to the variability of environmental conditions specific to the three years of study. Under the same experimental conditions, the 'Tămâioasă românească' 104 Dg. clone was noted in terms of relative (Rfi) and absolute fertility (Afi), the differences being statistically significant compared to 'Tămâioasă românească' 36 Pt. ($p \leq 0.01$).There are also differences in the length of internodes (OIV 353). 'Tămâioasă românească' 104 Dg. has the longest internodes, the differences being statistically significant compared to 'Tămâioasă românească' and Tămâioasa românească' 36 Pt. ($p \leq .01$).
The production of useful biomass and the efficiency of the use of pedoclimatic resources are important objectives in grapevine breeding programs, but also an important criterion in the choice of plant material by the vine growers. Results of various studies showed a genetic variability and different clonal responses to soil water availability (Tortosa et al., 2020), to use rootstock (Boso et al., 2010), to bud load or pruning system (Feitosa et al., 2018).Both the 'Tămâioasă românească' 104 Dg. clone and the 'Tămâioasă românească' 36 Pt. clone have a shorter length of bunch compared to the 'Tămâioasă românească' cv., the differences being statistically significant ($p\leq .01$). The weight of bunch is also lower in the 'Tămâioasă românească' 104 Dg. and 'Tămâioasă românească' 36 Pt. clones (Table 3), the differences being significant compared to 'Tămâioasă românească' cv. ($p\leq .01$).The biometric characteristics of the grape had an medium variation, except for the weight of berry (OIV 503) at 'Tămâioasă românească' 104 Dg., in which a high degree of variation was noticed (CV% = 28.87). 'Tămâioasă românească' cv. and 'Tămâioasă românească' 36 Pt. have berries larger in length (OIV220) and width (OIV 221) compared to the 'Tămâioasă românească' 104 Dg. clone, the differences between the means being statistically significant ($p \leq .01$). Of the two clones, the highest berries weight was recorded at 'Tămâioasă românească' 36 Pt., the differences being statistically significant

Table 3. Main morphological, agro-biological and quantitative characteristics

Charactheristics	U.M.	'Tămâioasă românească' cv.			'Tămâioasă românească' 36Pt			'Tămâioasă românească' 104 Dg.		
		Mean	SD	Min→Max	Mean	SD	Min→Max	Mean	SD	Min→Max
Relative fertility index (Rfi)	ratio	0.75^{ab}	0.21	0.43→1.31	0.67^{b}	0.14	0.40→1.31	0.81^{a}	0.16	0.46→1.23
Absolute fertility index (Afi)	ratio	1.25^{b}	0.25	0.81→1.77	1.13^{b}	0.23	0.73→1.65	1.47^{a}	0.22	0.97→1.81
Length of internodes	cm	8.48^{b}	1.59	6.53→12.6	8.86^{b}	1.49	6.82→12.54	12.14^{a}	1.98	9.21→14.3
OIVnotation	353	3		*1→5*	3		*1→5*	5		*3→7*
Diameter of internodes	mm	8.69^{a}	1.30	6.46→11.47	8.97^{a}	1.23	6.34→12.03	8.94^{a}	1.19	6.98→11.52
OIVnotation	354	3		*3→5*	3		*3→5*	3		*3→5*
Length of bunch (peduncle excluded)	mm	197.52^{a}	21.16	157.46→223.00	170.24^{b}	20.73	129.31→200.27	164.13^{b}	21.71	118.39→207.53
OIVnotation	202	7		*5→7*	5		*3→7*	5		*3→7*
Weight of bunch	g	266.85^{a}	45.68	183.5→359.51	196.10^{b}	49.67	138.89→368.20	184.07^{b}	24.15	138.31→286.20
OIVnotation	502	3		*3*	1/3		*1→3*	1/3		*1→5*
Length of berry	mm	14.14^{a}	1.73	11.45→18.6	14.71^{a}	1.33	12.66-16.76	12.34^{b}	1.5	8.94-15.23
OIVnotation	220	3		*3→5*	3		*3→5*	3		*1→3*
Width of berry	mm	13.82^{a}	1.39	11.06→16.10	14.15^{a}	1.36	11.7→16.24	12.29^{b}	1.43	9.47→14.60
OIVnotation	221	3		*3→5*	3		*3→5*	3		*1→3*
Weight of berry	g	2.15^{a}	0.32	1.24→3.28	2.29^{a}	0.27	1.61→3.25	1.42^{b}	0.41	0.69→2.2
OIVnotation	503	3		3	3		3	3		*1→3*
Length of pedicel	mm	4.32	0.69^{b}	3.16→5.94	4.78^{ab}	0.88	3.21→6.59	4.99^{a}	1.06	3.10→6.51
OIVnotation	238	1/3		1-3	1/3		*1-3*	1/3		*1-3*

Note: Means separation by HSD Tukey`s test at p≤0.05. Means with the same superscript are not statistically significant

Table 4. Main qualitative characteristics at full maturity (Average 2018-2020)

Cultivars/Clone	Sugar content (° Brix)			Total acidity of must (g/L H_2SO_4)		
	Mean	SD	Min→Max	Mean	SD	Min→Max
'Tămâioasa românească' cv.	21.97[a]	1.78	19.33→24.56	4.71[a]	0.52	4.00→5.62
'Tamaioasa romanesca' 36 Pt.	22.17[a]	1.75	20.8→24.60	4.74[a]	0.57	3.72→5.48
'Tamaioasa romanesca' 104 Dg.	20.24[b]	1.16	18.98→22.78	4.17[b]	0.30	3.60→4.77

Note: Means separation by HSD Tukey`s test at p≤0.05. Means with the same superscript are not statistically significant

compared to 'Tămâioasă românească' 104 Dg. ($p \leq .01$). Similar results regarding the morphological traits of cluster and berries at 'Tămâioasă românească' cv. were reported by Popescu et al. (2015).

The phenotypic expression of different grapevine genotypes in terms of the complexity of the quality of grapes and wines obtained in various climatic contexts of the different viticultural regions, represent important research concerns in various fields (Moutinho-Pereira et al., 2009; Neethling et al. 2012; Neumann et al., 2014).

With a multiannual average over the three years of study of 22.17 ° Brix and an average total acidity content of 4.74 g/L H_2SO_4, the 'Tămâioasă românească' 36 Pt. clone had the highest sugar content in must and the highest acidity, the differences being statistically significant compared to 'Tămâioasă românească' 104 Dg. clones ($p \leq .01$) for both sugar content and acidity content. The 'Tămâioasă românească' 104 Dg. clone also reveals significant negative differences regarding the multiannual average of the sugar content in must ($p \leq .05$) and the average of the content in total acidity ($p \leq .01$) compared to 'Tămâioasă românească' cv. (Table 4).

CONCLUSIONS

Ampelographic and ampelometric descriptors can be useful tools in identifying many discriminating phenotypic characteristics between the grapevine variety and its clones.

Our partial results show that there is an important phenotypic variability within the population of the 'Tămâioasa românească' variety, but also of the two clones.

We consider it necessary to continue the investigation of intra-varietal and intra-vineyard variability in the 'Tămâioasa românească' variety both in the Dragasani vineyard and in other wine-growing areas in Romania, especially in the traditional ones (Cotnari, Pietroasa), in order to identify valuable clonal elites (with agrobiological and oenological performances, physiological and sanitary resistances), possible candidate clones, very useful in the context of current climate change and the growing and diversified demands of vine growers.

The use of polyclonal plantings of 'Tămâioasa românească' in the Dragasani vineyard can be a solution for vine growers, capitalizing in this way the bioproductive potential of the 'Tămâioasa românească' 104 Dg. clone, the high qualitative potential of the 'Tămâioasa românească' 36 Pt. clone and of the 'Tămâioasa românească' variety, thus creating the possibility of obtaining complex wines, with a high degree of typicality and specificity.

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