Phenotypical, Sanitary and Ampelometric Variability within the Population of cv. Plavac Mali (*Vitis vinifera* L.)

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Summary

One hundred and sixty individual vines representative of the entire growing region for cv. Plavac Mali (Middle and Southern Dalmatia) were ampelographically analyzed *in situ* during a period of four years. High phenotypical variability was determined in: the maturity period (35.6% "early maturity" vs. 11.9% "late maturity"), colouring (>50% of vines had bunches that were not coloured completely) and density of bunches, and degree of firmness of flesh and size of the berries. In a sample of 36 vines harvested in 2005 high variability was determined in: parameters of fertility, sugar content (range 66-139°Oe), and the size parameters of bunches. The samples were tested for the presence of four viruses by ELISA test (Grapevine Fanleaf Virus (GFLV), Arabis Mosaic Virus (ArMV), Grapevine Leafroll associated Virus 1 and 3 (GLRaV-1 and GLRaV-3). 91% of all analyzed samples tested positive for GLRaV-3, while an infection with the ArMV (6%) was the least common. Only 5% of genotypes resulted as negative in virus testing. The results of the research point to a significant degree of intra cultivar genetical variability and the necessity of further individual clonal selection.

Key words

Vitis vinifera L., cv. Plavac Mali, ampelography, clonal selection

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Introduction

Cv. Plavac Mali is the major red wine cultivar (*Vitis vinifera* L.) in Croatia. It is mainly grown in the coastal and island regions, but the best quality is achieved on steep southern slopes next to the sea such as: Dingač and Postup (Pelješac peninsula), Ivan Dolac and Sv. Nedjelja (Island of Hvar), and Bol and Murvica (Island of Brač).

The long held belief that cv. Plavac Mali is a native cultivar of Dalmatia was recently confirmed by the research of Maletić et al. (2004) who determined, by the use of SSR markers, that cv. Plavac Mali is a progeny of two traditional Dalmatian cultivars Crljenak Kaštelanski (Zinfandel) and Dobričić.

The first ampelographic data on cv. Plavac Mali were recorded by Trummer (1841), who described it as a cultivar (Palvanz mali zerni) raised in The State Economic Garden in Maribor, and was brought there from Dalmatia. Goethe (1887) published short descriptions of 26 Dalmatian cultivars utilizing the work of Novak from the Island of Hvar. Among them he describes Crljenak (Crjenak, Crnjenak – Blank blau), which probably pertains to cv. Plavac Mali.

The first detailed ampelographic description of cv. Plavac Mali was written by Bulić (1949), who describes it as a leading cultivar in Dalmatia with the widest geographical distribution and multiple synonyms.

Jelaska (1960) states certain characteristics of cv. Plavac Mali (good resistance to fungal diseases and drought, consistent and plentiful fertility), due to which it became the leading cultivar in Dalmatia. Jelaska also selected certain types within the population ("petite", "large", "highly fertile", and "grey"). Maleš (1981) conducted more detailed ampelographic research on cv. Plavac Mali, determined highly significant differences in morphological and physiological characteristics between selected types ("petite", "large", and "high yielding") and the cultivar standard. Unfortunately, neither one of these researches resulted in controlled propagation, agricultural evaluation, mother blocks of these types forming, so that they are not available today for nursery production.

If there is a lack of genetic and sanitary selection within mature populations, such as cv. Plavac Mali, than differences in individual morphological and physiological characteristics might appear despite the fact that vegetative reproduction is practiced. These differences, if they are passed on by reproduction are explained as spontaneous mutations, and the individuals carrying the changed genetic material (DNA) are called mutants. Results and experience of studies on mutagenic breeding tell us that the majority of mutants within a population have negative characteristics from the production standpoint, but that there is also a possibility of finding mutants with positive characteristics, which have the ability to improve the cultivar (Mullins et al., 1992). Besides genetic variability, general phenotypical variability can be influenced by effects (symptoms) of certain grapevine diseases, principally by viruses. Despite this, Konrad et al. (2003) assume that intra cultivar phenotypic variability is predominantly influenced by genotypic variability.

Many wine-growing countries in the European Union, primarily Germany, France, and Italy, significantly improved their viticulture and enology by improving their cultivars using individual clonal selection. This became obligatory procedure for producing certified propagating material (European Union Council Directive 92/34/EEC). Germany has about 400 registered clones ("clonal cultivars") from 28 traditional cultivars (Rühl et al., 2002). Croatia, unfortunately, does not have a single registered clone from a native cultivar.

The aim of this study was to quantify the morphological, sanitary, and ampelometric variability of cv. Plavac Mali by analyzing 160 vines chosen from multiple locations on the basis of *in situ* observed phenotypical differences within a specific population, which is the assumption for work on the individual clonal selection. Because the environment has a significant effect on the phenotypical expression of the genotype of grapevine, all of the analyzed vines are going to be vegetatively reproduced and planted in a single vineyard as a continuation of this research.

Material and Methods

Plant material

In order to sample most of the genetic variability within the population, the selection of samples was carried out when the grapes were in full maturity on the basis of phenotypical differences within the cultivar in commercial vineyards and taking into account all locations the cultivar is grown in. Special consideration was given to all geographically and historically important locations within Middle and Southern Dalmatia (Table 1). The oldest possible vines (majority were older than 30 years) were chosen for sampling, under the assumption that they were locally reproduced for a large number of years and as such represent a result of a selection forces of the specific geographic location. Because the phenotypical variability of cv. Plavac Mali is significantly influenced by its location (exposure, inclination, temperature, precipitation, etc.), all vine growing locations were divided into three classes (M, K, and R), and the samples labelled accordingly. Class M are the locations of favourable inclination, with a southern orientation and in close proximity to the sea, class K are the locations of favourable inclination not in proximity to the sea, and class R are the locations in the field. By grouping the samples into these classes the influence of

Table 1. Illustration of selected vines within the cv. Plavac Mali population. The vines were categorized according to a vine growing area and vine growing location classes. Class M are the locations of favourable inclination, with a southern orientation and in close proximity to the sea, class K are the locations of favourable inclination not in proximity to the sea, and class R are the locations in the field

Wine growing area	Number of genotypes according to classes of wine growing location			
	M	K	R	Total (M+K+R)
Brač	35	0	3	38
Pelješac	11	8	14	33
Hvar	16	11	0	27
Vis	0	7	11	18
Korčula	0	12	5	17
Kaštela	0	0	7	7
Šolta	0	0	5	5
Omiš	2	2	0	4
Čiovo	3	0	0	3
Biševo	0	0	3	3
Vrgorac	0	0	2	2
Sv. Andrija	1	0	0	1
Labin dalmatinski	0	0	1	1
Zemunik	0	0	1	1
Total	68	40	52	160

position on the level of expression of the observed characteristics will be correlated.

An exact location was recorded for each chosen vine for long term observation. Important details were photographed (vine, bunch, and leaf), information on the origin and age of the vineyard were recorded, and the expression of all observed characteristics was observed and/or measured by standard ampelographic methods for a period of 2-3 years.

Ampelographic analysis

Each chosen vine was determined as cv. Plavac Mali according to the standard description of the cultivar (ampelographic database of native grape vines Faculty of Agriculture Zagreb and Institute for Adriatic Crops and Karst Reclamation in Split) using Office International de la Vigne et du Vin descriptors (OIV, 1983) modified by the European Union Project GENRES 081 (2001). In case of larger phenotypical deviations a method of molecular markers (SSR) was used.

The following characteristics were specially observed because of the detection of phenotypical differences of the chosen genotypes: the time of full maturity, colouring, size and density, the existence of wings on the bunch, size and firmness of flesh of the berry, and the size of the leaf and the density of prostrate hairs on the lower side of the leaf. A scale of expression levels was created for each characteristic (subjective estimate of the breeder on the basis of the population average of each individual vineyard) (Table 2), similar to the scales done by Blanco et al. (2004). Data collecting in the field was done from 2002 to 2005. In 2005 a geographically representative sample of 36 vines was chosen out of available genotypes and their uvometric elements, fertility, and yield quality were analyzed when in full maturity.

Uvometric analysis was conducted on a sample of ten bunches from genotypes which had ten or more bunches, while for genotypes that had ten bunches or less an analysis was conducted on all of the bunches. Length, width, and mass of the berries and bunches were recorded, as was the number of berries in each bunch.

An analysis of the mechanical composition (partial) of the bunch and the berry was conducted according to Prostoserdov (1946). Skins and seeds were separated from the flesh of one hundred berries and subsequently dried on filter paper at room temperature. Structure of the bunch (percentage of stems, skins, seeds and flesh) was determined for each genotype.

Fertility parameters were determined using the number of buds and bunches on a vine, from which a coefficients of potential fertility (KpR) were calculated. Yield per vine (kg/vine) was calculated by weight.

Quality of must was determined by measuring: sugar content (°Oe), total acids (titration with n/4 NaOH), and pH value.

Table 2. Analyzed traits of the chosen genotypes of cv. Plavac Mali observed just before harvest.

Characteristic	Level of expression				
Full maturity time	1 – early	2 – standard*	3 – late		
Bunch coloration	1 – 100% coloration	2 – <10% green berries	3 – 10-15% green berries	4 – 15-20% green berries	5 – >20% green berries
Bunch size	1 – small	3 – middle	5 – large		
Bunch density	1 – loose	2 – semi loose	3 – standard*	5 – dense	
Bunch wing	0 – absent	1 – presence			
Berry size	1 – small	2 – semi small	3 – standard*	4 – semi large	5 – extra large
Berry flesh firmness	1 – soft	2 – semi firm	3 – firm		
Size of mature leaf from the medium third of shoot	1 – small	3 – standard*	5 – large		
Prostrate hairs density of mature leaf lower side	1 – weak	3 – standard*	5 – dense		

* expected middle (the most frequently) value, typically for cultivar, estimated on the basis of earlier ampelographic research activities

Sanitary analyses

108 genotypes were tested for the presence of four viruses deemed most economically destructive: (Grapevine Fanleaf Virus (GFLV), Arabis Mosaic Virus (ArMV), and Grapevine Leafroll associated Virus 1 and 3 (GLRaV-1 and GLRaV-3). The testing was done using ELISA tests (Boscia et al., 1997).

Table 3. Degree of infection of the chosen genotypes of cv. Plavac Mali by different virus types (ELISA test)

Number of vines	ArMV	GFLV	GLRaV-1	GLRaV-3
5	-	-	-	-
30	-	-	-	+
1	-	-	+	-
2	-	+	-	-
0	+	-	-	-
24	-	-	+	+
24	-	+	-	+
1	+	+	-	-
0	+	-	+	-
0	+	-	-	+
0	-	+	+	-
16	-	+	+	+
2	+	+	-	+
1	+	+	+	-
1	+	-	+	+
1	+	+	+	+
108	6 (6%)	47 (44%)	44 (41%)	98 (91%)

Results

Phenotypic variability

Results of phenotypic evaluations of vines are shown in Figures 1 to 5.

In full maturity phenophase for cv. Plavac Mali (IV period, beginning of October) unequal maturity of certain genotypes was observed (Fig. 1). "Early" type appears with high frequency within the population (36.5%), with vines found in all cv. Plavac Mali regions regardless of the location category.

An average cv. Plavac Mali bunch is categorized as a dense bunch. Figure 3 shows that about half of the tested genotypes has a dense to semi-dense bunch, while the other half has a more or less loose bunch.

Significant variability was observed in colouring of bunches (Fig. 2). This is often mentioned as a characteristic of cv. Plavac Mali, and is the result of different times of flowering, fertilization, and maturity. This results in characteristic "multi-coloured bunch" on which a portion of the berries is completely green, a portion turning blue, a portion completely blue, and a portion overripe (raisin).

Somewhat less than half of the tested genotypes had a fully collared bunch (absence of green berries), while

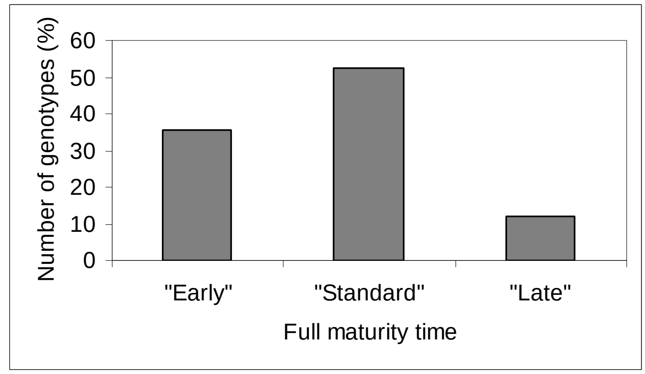

Figure 1. Frequency distribution of the timing of full maturity of different genotypes of cv. Plavac Mali. Subjective estimation based on observation of phenotypic characteristics and comparison to other individuals of the vineyard. Observed just before harvest during 2 or 3 successive years (n=160)

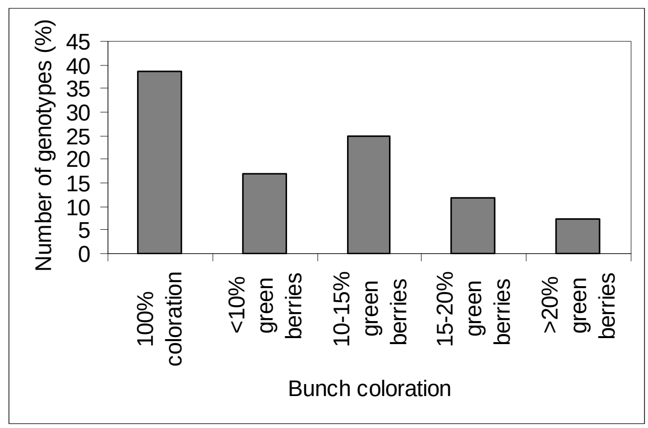

Figure 2. Frequency distribution of the bunch coloration of sampled cv. Plavac Mali genotypes. Subjective estimation based on observation of phenotypic characteristics and comparison to other individuals of the vineyard. Observed just before harvest during 2 or 3 successive years (n=160)

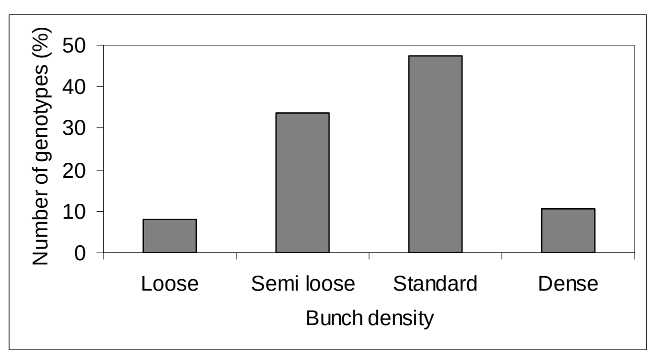

Figure 3. Frequency distribution of the bunch density of sampled cv. Plavac Mali genotypes. Subjective estimation based on observation of phenotypic characteristics and comparison to other individuals of the vineyard. Observed just before harvest during 2 or 3 successive years (n=160)

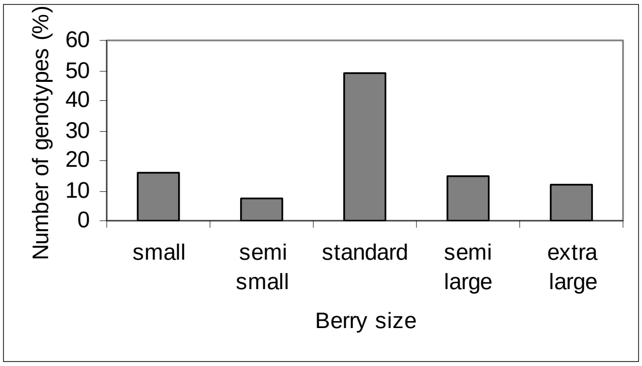

Figure 4. Frequency distribution of the mean berry size of sampled cv. Plavac Mali genotypes. Subjective estimation based on observation of phenotypic characteristics and comparison to other individuals of the vineyard. Observed just before harvest during 2 or 3 successive years (n=160)

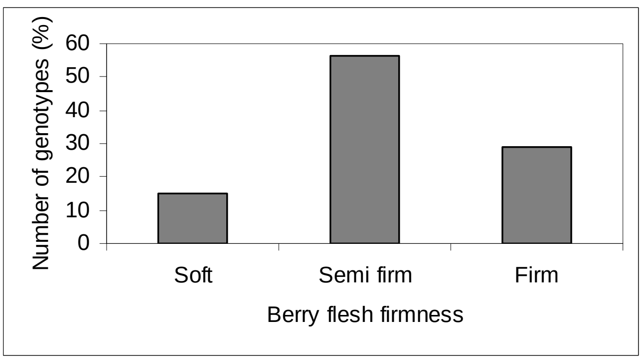

Figure 5. Frequency distribution of the firmness of flesh of berries of sampled cv. Plavac Mali genotypes. Subjective estimation based on observation of phenotypic characteristics and comparison to other individuals of the vineyard. Observed just before harvest during 2 or 3 successive years (n=160)

the rest had an unevenly collared bunch (>10% had green berries).

Around 50% of tested genotypes had berries of standard size, while the rest had smaller or larger berries significantly deviating from the population average (Fig. 4).

56.3% of genotypes had berries with a characteristic semi-firm flesh, 22.8% with firm ("crunchy") flesh, and only 15.0% of genotypes had berries with soft flesh (Fig. 5).

No significant correlation was found between the results of phenotypic observations (colouration of the grape, maturity, and berry consistency) and the category of the location of the vineyard.

Ampelometric variability

The results of analysis of the phenotypic variables measured on 36 genotypes are shown in Table 4. A high variation in fertility parameters (from 0.4 to 2.5 bunches per bud) was recorded. More than 11 stocks had a coefficient of potential fertility that was less than 1. A very small number of bunches per vine (only three) was recorded in three genotypes (OB-016, OB-209 and OB-227), while the genotype OB-202 had the highest number of bunches per vine (39) which showed in the above average coefficient of potential yield (1.7) and the highest yield per vine (10.36kg).

The frequency distribution for bunch mass is shown in Figure 6, with the range from 79 to 351 g, the mean of 201 g, and the highest number of genotypes (18) in the 150 to 250 g range. The frequency distribution for the bunch length is shown in Figure 7, with the range from 10.8 to 18.0 cm, and the highest number of genotypes (10) with bunch length close to 15 cm. The frequency distribution for the bunch width is shown in Figure 8, with the range from 7.2 to 13.9 cm, and the highest number of genotypes (13) with bunch length close to 13 cm.

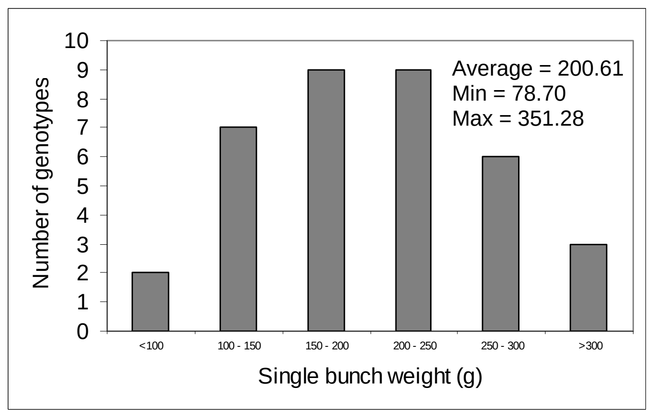

Figure 6. Frequency distribution of the average bunch weight of sampled cv. Plavac Mali genotypes. Measurements obtained from a geographically representative sample of 36 vines from the 2005 harvest

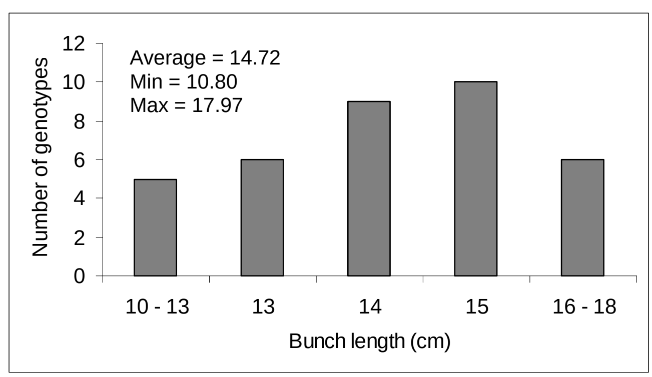

Figure 7. Frequency distribution of the average bunch length of sampled cv. Plavac Mali genotypes. Measurements obtained from a geographically representative sample of 36 vines from the 2005 harvest

Table 4. Ampelometric characteristics of 36 chosen genotypes of cv. Plavac Mali. Average values from the 2005 harvest.

Code of genotype	Class of wine growing location	Number of buds per vine	Number of bunches per vine	Coeff. of potential fertility (KpR)	Weight of bunch (g)	Bunch lenght (cm)	Bunch width (cm)	Stem (%)	Skin (%)	Seeds (%)	Flesh (%)	Yield per vine (kg/vine)	Sugar (°Oe)	Total acid (g/l)	pH value
OB-011	M	15	6	0.40	253.32	15.48	10.3	2.91	13.55	1.43	82.11	1.79	105	5.2	3.46
OB-016	M	8	3	0.38	136.93	10.8	8.57	-	-	-	-	0.41	139	5.7	3.57
OB-010	M	10	10	1.00	217.7	15.43	10.94	2.32	6.36	0.99	90.34	2.18	102	7.0	3.19
OB-014	M	21	15	0.71	150.08	14.69	9.53	3.46	13.01	1.59	81.93	2.73	95	3.7	3.60
OB-018	M	13	8	0.62	198.27	14.73	11.91	2.09	4.6	0.76	92.55	2.88	98	5.3	3.35
OB-019	M	10	12	1.20	121.72	12.25	8.12	4.32	18.34	1.79	75.54	1.68	125	5.9	3.33
OB-251	M	8	15	1.88	201.02	16.53	10.99	2.15	7.2	1.05	89.59	2.68	96	3.6	3.76
OB-259	M	8	15	1.88	185.2	14.16	9.66	1.79	12.82	1.59	83.81	2.71	95	3.8	3.73
OB-260	M	7	12	1.71	79.08	13.26	8.74	15.38	10.35	1.04	73.23	0.98	99	2.4	4.12
OB-261	M	8	16	2.00	178.72	14.9	9.86	1.88	8.71	1.25	88.17	2.45	87	4.2	3.67
OB-262	M	8	13	1.63	221.52	14.84	10.53	0.97	7.7	1.28	90.06	2.68	85	2.9	3.90
OB-269	M	9	7	0.78	106.28	13.13	8.11	2.15	7.95	0.98	88.92	1.15	119	4.3	3.61
OB-271	M	8	20	2.50	111.98	13.37	7.47	2.07	6.55	1.39	89.99	1.95	90	4.5	3.45
OB-272	M	7	8	1.14	110.35	12.08	8.54	2.2	8.12	1.47	88.21	0.81	97	2.9	3.75
OB-275	M	10	20	2.00	225.65	15.99	11.76	2.34	6.34	1.65	89.67	4.18	91	3.1	3.74
OB-280	M	7	12	1.71	246.15	14.67	9.68	1.52	5.27	1.36	91.84	2.71	92	5.0	3.45
OB-032	K	6	9	1.50	212.93	14.71	10.39	2.27	8.9	2.3	86.52	1.86	87	4.1	3.51
OB-035	K	8	5	0.63	291.59	15.4	12.4	2.1	7.95	2.45	87.5	1.54	78	4.0	3.52
OB-042	K	22	16	0.73	221.04	16.88	10.03	2.89	4.7	1.36	91.05	3.29	68	6.5	3.16
OB-082	K	12	15	1.25	155.98	13.57	10.43	2.14	8.23	2.7	86.93	2.40	83	4.8	3.47
OB-093	K	8	11	1.38	241.86	15.69	9.39	2.96	5.62	1.52	89.9	2.71	97	3.9	3.14
OB-079	R	10	15	1.50	301.15	17.57	12.57	2.71	2.89	1.1	93.3	4.16	66	5.9	3.15
OB-098	R	8	19	2.38	241.55	15.71	10.84	2.31	6.16	1.24	90.29	3.17	81	6.6	3.50
OB-229	R	7	4	0.57	78.7	11.25	7.2	-	-	-	-	0.42	84	3.6	3.55
OB-036	R	10	17	1.70	302.8	17.16	13.9	2.6	4.99	2	90.41	4.70	79	6.5	3.34
OB-060	R	10	10	1.00	148.02	13.72	10.54	3.13	10.05	1.73	85.1	1.05	81	6.1	3.30
OB-078	R	12	11	0.92	188.66	14.28	10.59	2.75	6.7	1.52	89.04	2.14	89	4.7	3.35
OB-100	R	14	33	2.36	256.3	17.97	10.79	2.65	9.24	2.26	85.85	4.67	68	7.6	3.31
OB-201	R	11	22	2.00	282.37	15.87	13.47	3.75	5.14	2.17	88.93	4.74	80	6.8	3.26
OB-202	R	23	39	1.70	290.72	16.09	11.49	1.73	5.88	1.07	91.32	10.36	77	5.9	3.20
OB-209	R	-	3	-	138.77	12.5	8.43	-	-	-	-	0.64	122	8.1	3.34
OB-210	R	-	6	-	280.08	15.83	10.17	1.93	8.41	1.4	88.26	1.68	79	7.0	3.13
OB-211	R	-	6	-	177.23	15.75	9.77	1.95	9.04	1.66	87.36	1.34	99	6.1	3.52
OB-227	R	6	3	0.50	161.39	13.43	9.33	-	-	-	-	0.62	91	3.9	3.63
OB-228	R	15	14	0.93	155.64	14.39	10.74	3.46	12.07	1.75	82.72	2.49	84	2.8	3.61
OB-099	R	8	12	1.50	351.28	15.86	11.51	1.97	5.9	1.9	90.23	3.81	70	7.8	3.29
Average		10.52	12.83	1.34	200.61	14.72	10.24	2.84	8.09	1.55	87.52	2.55	91.06	5.05	3.47
	min	6.00	3.00	0.38	78.70	10.80	7.20	0.97	2.89	0.76	73.23	0.41	66.00	2.40	3.13
	max	23.00	39.00	2.50	351.28	17.97	13.90	15.38	18.34	2.70	93.30	10.36	139.00	8.05	4.12

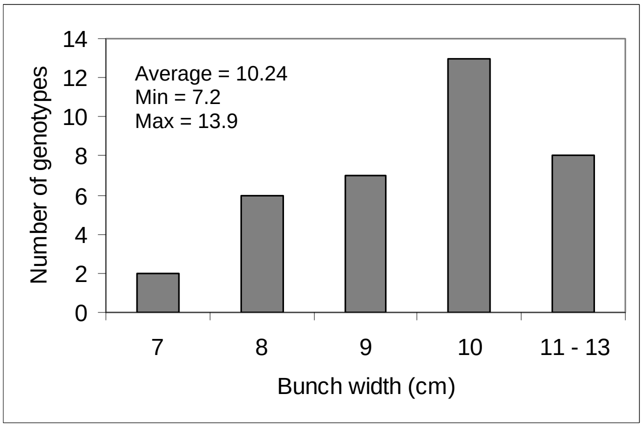

Figure 8. Frequency distribution of the average bunch width of sampled cv. Plavac Mali genotypes. Measurements obtained from a geographically representative sample of 36 vines from the 2005 harvest

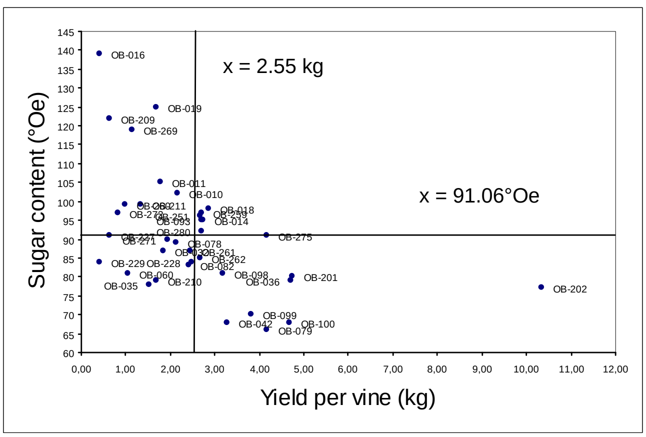

Figure 9. Scatterplot representing correlation between yield and sugar content of sampled cv. Plavac Mali genotypes. Measurements obtained from a geographically representative sample of 36 vines from the 2005 harvest

Sugar content had a range from 66 to 139°Oe, with the mean of 91°Oe. Figure 9 shows that the quantity of yield and sugar content are inversely related, which was to be expected, considering that this is a known relationship in general. A positive relation was shown between the category of location and sugar content and total acidity. Genotypes from the M category contained a significantly higher sugar content and lower total acidity (P=0.05) than those in the K and R groups. There was no significant difference between the K and R groups (Fig. 10 and 11).

The mean yield per vine was 2.25 kg. Six genotypes had yield lower than 1.00 kg, while another six had yield higher than 4.00 kg.

Total acidity varied from 2.4 to 8.1 g l^{-1}. Eleven vines had total acidity levels of less than 4.0 g l^{-1}, which is not good for making well balanced red wines.

Figure 10. The relation between mean values of sugar content (°Oe) and vine growing location classes

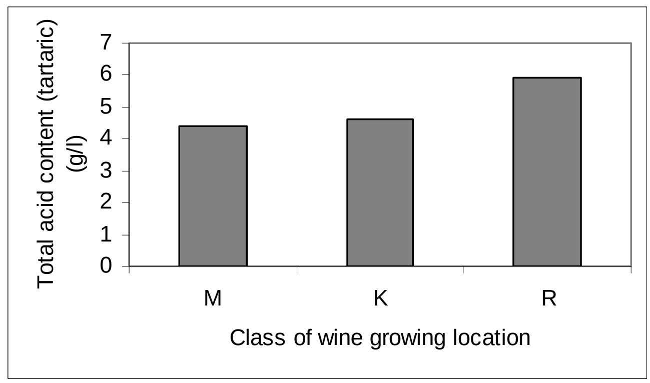

Figure 11. The relation between mean values of total acids (expressed as tartaric acid) and vine growing location classes

Sanitary analysis

Only five vines (5%) were free of the four viruses tested. GLRaV-3 was the most common virus, present in 90% of all analyzed vines, while ArMV was the least common, with a presence of only 6%. GLRaV-3 was the most common (28%) when it comes to single infections, but also regularly present in a combination with other viruses. High frequency of infections with GLRaV-3 and it's presence in the oldest vines tested (OB-031, OB-032, OB-033, OB-034 and OB-035) that are still raised on their own rootstock on the sandy soil close to Dol on the Island of Hvar (age of the vineyard higher than 140 years), points a long term presence of GLRaV-3 in Dalmatia. About 30% of analyzed vines was infected with only one virus, about 45% with two, and 19% with three harmful viruses. The presence of all four viruses was recorded in only one case. For the cuttings to have a certified healthy status it is necessary that they are free of all four viruses. Therefore it is evident that the current population of cv. Plavac Mali has a very small number of "healthy" vines (<5%) and that the current methods of reproduction (on the spot grafting or with cuttings of Standard category) are maintaining and spreading the viruses. These results, although gotten from a limited sample, suggest that a sanitary selection in the population of cv. Plavac Mali is essential.

There was no correlation found between frequency of infection and virus type, and geographical location. The exception was Vis Island where all of the twelve tested samples tested negative for GLRaV-1, despite the fact that on the whole over 40% of all samples tested positive.

Discussion

Distribution, names and ages

At present time, cv. Plavac Mali is a cultivar that can be found in the coastal and island regions of Croatia from Konavle (South Dalmatia) to Zadar. The main cultivation region is between Pelješac and Kaštela. Currently the highest production, considering the number of vines and the quantity of wine produced, is on the Pelješac, Hvar, and Vis regions. cv. Plavac Mali, the standard name for the cultivar, is also the most commonly used, altough there are some local names still in use: "Crljenak Mali", "Crvenak", "Plavac", "Greštavac", "Zelenac", and "Šarac". The oldest vineyard of cv. Plavac Mali was found in Dol on the Hvar Island, as a part of the vineyard of Šime and Ivo Šurjak (Borough of Stari Grad, cadastral plot 4643-1 "Draganjač"), which was, according to the cadastral excerpt and the testimony of the current owners, planted by Mate Stančić in 1860 (Photo 1). There are about 20 vines that are growing on their own roots and which were the only ones that survived a philloxera epidemic owing to the fact that they are located on top of a sloping parcel with sandy soil. These vines were used later for revitalization of the vineyard by grafting, and are still used today for propagation by the owner. The second oldest vineyard, planted about 110 years ago, is in the ownership of Mr. Ivo Bezek and can be found in Pijavičino on the Pelješac Penninsula.

The results of ampelographic analyses done on vines, representing the entire growing area, show high phenotypic variability within the population of cv. Plavac Mali. A normal distribution of the quantitative characteristics, such as yield per vine and mass and dimensions of the berry was observed, rather than different classes. Konrad et al. (2003) found the same kind of normal distribution for yield, while analyzing 42 clonal prodigies of 9 vines of the Pinot Noir cultivar. However, for total acids, total soluble solids, and botrytis infection, they found that there were distinctive classes of data. In our study we found that the data collected showed normal distributions, with the substantial representations of extremes. The exception is the colouring of the bunch where less than 50% of analyzed vines had an even ripening of bunches occur (completely coloured bunch). More than 50% of the examined vines

Photo 1. A cv. Plavac Mali vine on its own roots, about 140 years old. Vineyard on cadstral plot no. 4643-1 «Draganjač», k.o. Dol, Stari Grad on the Island of Hvar

had an uneven colouring of bunches (> 10% of green berries per bunch). These findings confirm the writings of Bulic (1949) who states that some synonyms (Zelenac and Šarac) originated because of this property. The "uneven colouring of bunches" is a property that can be caused by an infection by GLRaV-3 (Spranger-Garcia et al., 1989; Jackson, 2000), a virus that was found in more than 90% of the tested population. However, in several cases the vines were not infected with GLRaV-3 (OB-043, OB-056, OB-061, OB-100, OB-201, and OB-288), but still had a "multicoloured" bunch, which points to a conclusion that this trait is controlled genetically.

As a rule, completely coloured bunches were found to have a correlation with "early maturing" type and a smaller berry size. "Multicoloured bunch" type has a larger bunch and a larger berry, and in the same timeframe has lower sugar content and total acids than the "early ripening" type.

Significant deviation from the population average of important phenotypic characteristics was found in some genotypes. Subsequent check using SSR markers showed these genotypes as being other cultivars (Vokurka et al. 2006). Types and level of expression of certain mutants, as well as the influence of genetic factors, have not been researched enough, which makes identifying as to which cultivar genotype a particular vine belongs difficult. An experienced ampelographer will be able to identify "intruders" of a foreign genotype in a population of a particular cultivar in majority of cases, although it is possible to do this with certainty only in certain phases of development, in a collection vineyard with a large number of individual vines, and referent genotypes. Intrusion of genotypes of phenotypically similar, often genetically related, cultivars is a common occurrence in vineyards of cv. Plavac Mali in Dalmatia. This is to be expected considering: the traditional method of propagation (on the spot grafting), the selection done by the wine growers themselves, and the fact that there are a large number of cultivars named Plavac (Kupusar, Sobotovac, Runjavac, etc.), which are not recognized as separate from cv. Plavac Mali by a number of vine growers. Change to the production of callused bench grafts has further aided in the spread of "atypical" vines within the cv. Plavac Mali population, because a positive selection is very rarely practiced during the maintenance of cultivars and the production of propagation material, and the buds are often taken from production vineyards without any selection.

A high variability has been found by ampelometric analysis, which coincides with the findings of Maleš (1981), who found highly significant differences between types "PM-high yielding", "PM-large", and "PM-petite". Eleven vines with very low coefficient of potential fertility (<1.0) were recorded, which corresponds to the type "PM-large". The number of vines that had total acid lower than 4.0 g l^{-1}, which is one of the characteristics of "PM-petite" (average total acidity 3.23 g l^{-1}), was also eleven. Six vines had a yield larger than 4 kg/vine, which can be related with the "PM-high yielding" type (average yield 4.64 kg/vine). However, average bunch mass, which was one of Maleš's basic criteria for categorization into types, had a wide range of values (78.7 to 351.3g), but the distribution was normal, which was expected. This study did not confirm the existence of distinct types "PM-high yielding", "PM-large", and "PM-petite" that were defined by Maleš (1981), because of the continuous variability exhibited by the values of the traits which were the basis of Maleš's categorization.

Unequal maturity of berries in a bunch (a type suggested by this study "Multicoloured bunch") is a trait that was not recorded as a type by Maleš (1981) or other authors who studied cv. Plavac Mali, although the authors of this study found it a typical and widespread occurrence for this cultivar, which from an enologic standpoint, has a large selection potential. This presents a possibility that unequal maturity of berries in a bunch is a result of recent spread of mutants favoured by a specific selection pressure (ex. preference of high yield, high vigour, etc.), or it can alternatively be a result of non genetic influences (ex. spread of specific viruses in recent times). Frequency distribution for this trait (Fig.1), and specifically the percentage of early maturing vines, gives hope that this trait is a result of a specific mutation, presenting a possibility of a successful selection and separation of clones of cv. Plavac Mali carrying the mutation. These would accumulate a sufficient quantity of sugar in conditions of less than favourable sum of temperatures, and produce a higher quality in "unfavourable" areas than current populations do. Because of late maturing, which is a typical trait of the cv. Plavac Mali population, cv. Plavac Mali shows lower quality (lower sugar content and unfavourable bunch coloration), while the "Late maturity" type (11.9%) can not reach full maturity (Gazzari and Pešut-Gligo, 1964; Maleš et al., 1980). There was no correlation found between virus infections and early, standard, and late types, although there are a few dozen grape vine viruses, which means that this possibility is not completely excluded.

It was impossible to credibly define distinctive phenotypic classes (characterization of mutants) for the majority of important agronomical characteristics observed *in situ*, although it was possible in the case of skin colour. Cv. Plavac Mali "Grey" is a mutant with a distinct skin coloration which was discovered in a cv. Plavac Mali population (Jelaska, 1960; Mirošević, 1988), an example of which is shown in Photo 2. Despite the fact that this mutant has a clearly distinct phenotype, a comparison of other morphological characteristics and analysis of SSR markers on 15 loci confirms that its genotype, for the most part, is the same as that of cv. Plavac Mali. This type of mutation was observed a long time ago with cv. Pinot (Viala and Vermorel, 1909), which besides a green-yellow (cv. Pinot Blanc) also has a grey (cv. Pinot Gris), and red (cv. Pinot Noir) skin colour variation. All three mentioned varieties have the same SSR genotype, which confirms that they are of the same generative origin; however, there is an obvious difference in their enologic properties (content of: sugar, organic acids, polyphenols, and aromatic compounds) which results in three distinct types of wine. This suggest a possible pleiotropic effect of the gene responsible for skin colour, which besides controlling the synthesis of anthocyanin, also affects the changes of some other properties. Kobayashi et al. (2004) have recently shown that the insertion of retrotransposon *Gret1* into the regulatory part of the skin coloration gene blocks the synthesis of anthocyanin and results in mutants with white coloration of the skin. Whether the skin coloration of cv. Plavac Mali "Grey" is controlled by the same genetic locus, remains to be seen, because in this particular case the skin

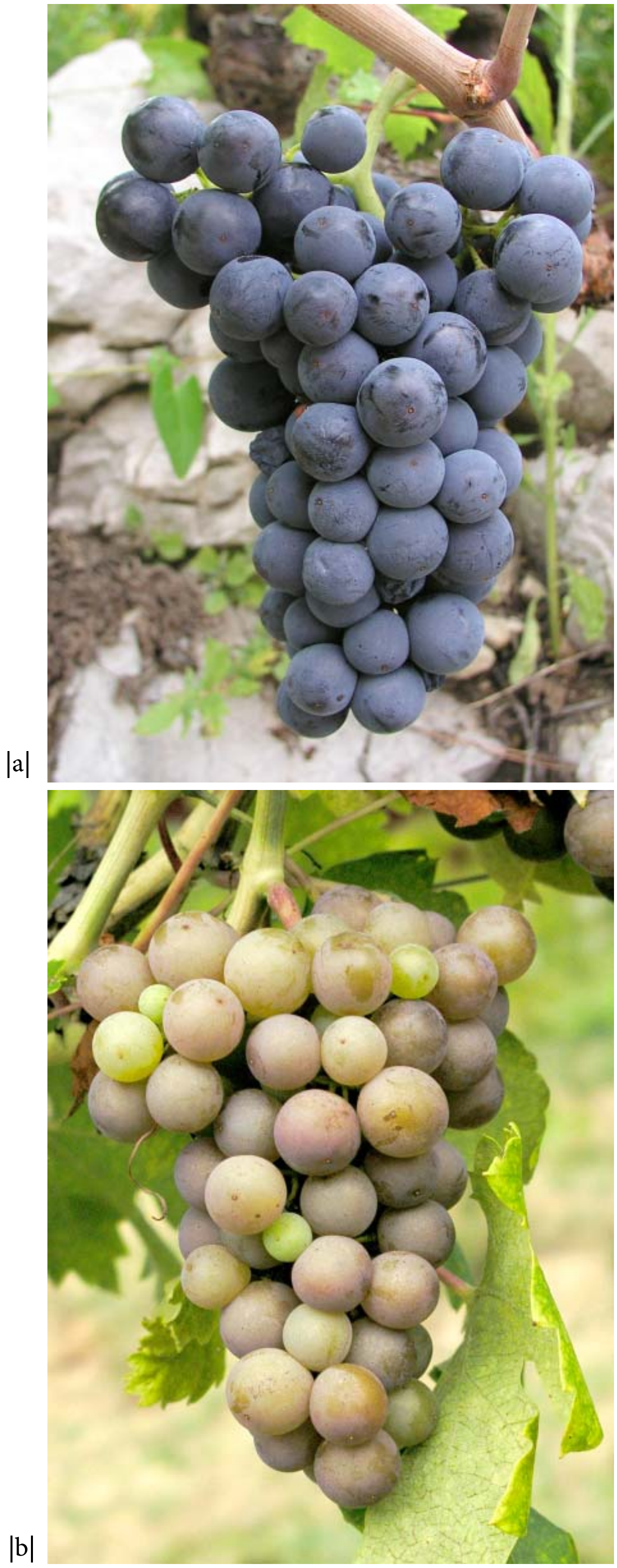

Photo 2. cv. Plavac Mali «Black» (a) and it's mutant with «Gray» skin colour (b). Photographed in 2005 in the collection of Croatian native cultivars of grape vine in the trial plantation of the Faculty of Agriculture of the Zagreb University

Photo 3. The most common phenotypic variability in the cv. Plavac Mali cultivar population: (a) «totally coloured» type, (b) «multi-coloured» type, (c) variation of berry colour and ripeness within a single bunch in multi-coloured type (d) loose and elongated bunch

Photo 4. «A collection of the cv. Plavac Mali legacy», research vineyard in Duilovo, next to the Institute for Adriatic Crops and Carst Reclamation in Split. The vineyard contains vegetative progeny from more than 100 mother vines chosen during «Project No 0178006 - Mutant detection within cv. Plavac Mali crni (*Vitis vinifera* L.) by DNA markers» (2001-2005) from the entire growing area of cv. Plavac Mali

coloration is not completely white, but grey. Photograph 3 illustrates phenotypic variability of cv. Plavac Mali by showing of several bunch shapes and several degrees of bunch maturity, which can be found in unspecified percentages in random vineyards, and locations.

Variability of the cv. Plavac Mali population is probably a result of interaction of genotypes, infections with certain diseases, and a multitude of abiotic environmental factors (location, climate, soil composition). Because of that, the experimental vineyard in Duilovo (Split) (Photograph 4) will be of particular value for the continuation of this study, and for monitoring the stability and type of genetic control over the characteristics. The vineyard contains 3-6 clonal progeny of most of the vines selected by this study. Only in an experimental vineyard of this type will it be possible to eliminate environmental effects, and thus determine the real genetic variability of cv. Plavac Mali and, with that, the possibility of success of clonal selection.

Taking into account the possible effect of environmental effects (location, level of nourishment, age of the vineyard, agricultural practices, etc.) on the phenotype, we conclude that in a population of cv. Plavac Mali there exists genetic variability conducive to the selection for: early and late maturity, size and shape of the bunch, size of the berry and firmness of flesh, colour and the intensity of colour of the skin, sugar and total acids content, and even bunch maturity (percentage of green berries). The results of this preliminary study confirm the necessity of clonal selection, because lack of knowledge about the population structure and lack of selection can lead to uncontrolled propagation of negative mutations, and thus lead to negative change of a population. The presumption gotten from the results obtained is that with individual clonal selection it would be possible to separate early maturing clones which would directly improve the quality of the wine from cv. Plavac Mali.

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