EXAMINATION OF THE PHENOTYPIC VARIABILITY OF THE MUSCAT HAMBURG VARIETY IN THE SKOPJE VINEYARD AS A BASIS FOR ISOLATING CLONES

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ABSTRACT

This paper describes the experiments performed on the Muscat Hamburg variety in the Skopje vineyard. Initial examinations were made in 2013-2014 in the collection plantations of the Agricultural Institute where the Muscat Hamburg variety was grown. In the then plantation were found 3 individual grapevines (units) in which differences in morphological and physiological characteristics were identified**.** Such grapevines are marked as individuals, monitored and taken from them for propagation material (they are vegetatively propagated by individual producers, and the seeds are used for laboratory tests). In fact, the phenotypic variability of the variety was monitored. In the following years, from 2013-2019, phenotypic variations were observed in other individual plantations and individual grapevines in the conditions of the Skopje vineyard. Most of the changes in the individual units of the Muscat Hamburg variety are manifested in the bunch, grain, fertilization, phenology, frost resistance and some technological characteristics. For example, in these units the grape is more compact, with better fertilization, darker color, the phenological feature of maturation takes place earlier and so on. Freezing resistance of these units is higher. They have the potential for better technological characteristics that would be used further for better product quality - wine, spirits and more. These units are the basis for obtaining improved properties in clonal selection and hybridization.

Key words: variations, phenotype, phenology, clone selection, hybridization.

INTRODUCTION

The examined variety in this paper - Muscat Hamburg is one of the most common varieties in our country and since its introduction, it has been successfully grown in several vineyards in our country for many years. Also, this variety persists in our assortment, both at large vine and wineries, but also in a large number of plantations at individual agricultural producers. Muscat Hamburg is a variety that is still represented on the macedonian market primarily as a table variety, but it is also used in the production of certain muscat wines, strong alcoholic beverages, etc. Such a condition is due to its good characteristics - pleasant muscat smell, beautiful appearance, the favorable chemical composition of the grain, the favorable combinatory abilities with other varieties in the finished products, the modest demand for agrobiological measures, etc.

Despite the representation of many new varieties on a global scale, the Muscat Hamburg variety still occupies a significant place in the assortments of several countries and quite a lot of

work is being done on the utilization of its positive properties and on its clonal selection in order to improve the existing properties.

Since in our experimental plantations in the Skopje vineyard, individual vines with slightly different properties from the standard variety were discovered, we decided that the topic of this paper would be the examination of the expression of the different properties in the Muscat Hamburg variety and their use for further selection to obtain clones with improved properties.

MATERIALS AND METHODS

Muscat Hamburg is an old variety, that is, it represents an intervarietal hybrid obtained by crossing the varieties - Muscat Alexandria and Tyrolan black, in the greenhouses of England. It belongs to the Black Sea ecological-geographic group of varieties (Convarietas pontica, subconvarietas georgica) (Božinović, 2010). It ripens in the III epoch and is a late table variety. It was introduced to us a long time ago and is grown in several vineyards. The tests were done in the experimental areas of the Institute of Agriculture in Skopje and with individual grapevines with some individual producers. The first different characteristics were observed already in 2013 and 2014, and then the grapevines that are different were propagated and their properties were monitored in more detail. Our investigations are mainly in the analysis of the characteristics between the standard variety Muscat Hamburg and its variation (Fazinić & Fazinić, 1990).

First, the phenological characteristics were examined, by observing and recording the dates for the phenophases – sprouting, flowering, the beginning of maturity and full maturity and the average total number of days from sprouting to full maturity (Božinović, 2010; Fazinić & Fazinić, 1990; Verries et al., 2000)

The weight of the bunch and the grain of the representative samples were analyzed according to the method of Prostoserdov. The weight is expressed in grams, and the length and width of the bunch in centimeters. The number of fertilized grains in the bunch was also analyzed (Fregoni, 1985; Sharif et al. 2015; Torregrosa, 1995).

The chemical composition of must is represented by the content of sugar and total acids. The sugar was measured with a refractometer in the field and with an Exlov odometer in laboratory conditions and was calculated according to Seileron's table. Total acids are obtained by titrating with n/4 NaOH and multiplying the amount of base by the factor 0,75. Also, analyzes of sugar and total and separate acids were made with HPLC methods. The measurement unit of sugar and total acids is g/dm^3 (Božinović, 2010; Crespan & Milani, 2001; Fazinić & Fazinić, 1990).

For the analysis of the number of chromosomes in mitosis and for the detection of certain anomalies, material was prepared from germinated seed pods and their cutting and preparation when they reach a size of 5-10 mm. Previously, the germination, which is very difficult, is carried out by standing the grape seeds on water filter paper alternately for 6 hours at a temperature of -2ºC to -3ºC in a refrigerator and 6 hours at a temperature of 25ºC in a thermostat, for several days until they germinate. For counting the chromosomes and observation of some phases of the mitosis the cytological technique of Thio and Levan (Božinović, 2010) was used, as well as the standard „Squash“ method of Battaglia (Markovska, 2001; Fenoll et al., 2009).

The cytogenetic status of varieties are examined according with description lists of primary and secondary OIV Code descriptors by the EU-PROJECT GENRES 081 - 09/2001 and 2009 (OIV, 2007). A special computer program for measuring chromosomal parameters and for the preparation of the karyotype and grapevine cariogram was used. A special microscope with a computer program with measuring units for measuring chromosomes in micrometers (μm) was also used (OIV, 2007; Sabır et al., 2008). The resulting chromosome pairs and single chromosome

numbers are compared with the given standard chromosome pattern and ploidy grade, given in table 1.

The organoleptic evaluation was carried out by a tasting evaluators, according to the list of ratings and standards for table grapes, and it evaluates: the taste, the external appearance, the consistency and the typicality of the grapes. At the end, a total grade is obtained (Riaz et al., 2004; Božinović, 2010; Fazinić & Fazinić, 1990).

The statistical processing was performed using the standard method with the coefficient of variation and the SPSS program.

Table 1. Chromosome constitutions in a normally diploid organism of grapevine with 2*n* = 38 chromosomes (labeled A, B, and C) in the basic set

Name	**Designation**	**Constitution**	**Number of chromosomes**
Monoploid	*n*	ABC	19
Diploid	2*n*	AABBCC	38
Triploid	3*n*	AAABBBCCC	57
Tetraploid	4*n*	AAAABBBBCCCC	76
Monosomic	2*n* − 1	ABBCC	37
		AABCC	37
		AABBC	37
Trisomic	2*n* + 1	AAABBCC	39
		AABBBCC	39
		AABBCCC	39

RESULTS AND DISCUSSION

The tests in this paper obtained the results that are followed in the following text. When analyzing the phenology, it can be said that in the variation of Muscat Hamburg, almost all phenophases take place earlier and the number of days from sprouting to full maturity is less. These are significant predispositions for avoiding some unfavorable temperatures and choosing a variety with earlier ripening (Božinović, 2010; Crespan & Milani, 2001). In the Muscat Hamburg variety, the total number of days from sprouting to full maturity is 177, and in the variation it is less and amounts to 172 days (table 2, figure 1, 2, 3 and 4).

Table 2. The phenophases are presented separately and the total number of days from sprouting to full maturity

Phenophases	Sprouting	Flowering			Beginning of ripening	Full maturity	Total days from sprouting to full maturity
		beginning of flowering	full flowering	finishing flowering			
Muscat hamburg	14.04	29.05	02.06	05.06	01.08	07.10	177
Variation of the Muscat hamburg	11.04	29.05	03.06	07.06	30.07	29.09	172

Figure 1. Old plantation where first vines noted

Figure 2. Bud awakening

Figure 3. Phenophase sprouting

Figure 4. Phenophase flowering

Examinations of the mechanical composition of the bunch showed that the weight of the cluster in the Muscat Hamburg variety is average 334,44 grams, length is average 16,15 cm, width is approximately 11,42 cm, ratio length / width is 1,41 and the number of fertilized grains in the cluster (bunch) averages 94,77 (Crespan & Milani, 2001; Fenoll et al., 2009; Loureiro et al., 2011). The weight of the cluster in the variation of Muscat Hamburg is average 367,16 grams, length is average 17,80 cm, width is approximately 12,17 cm, ratio length / width is 1,46 and the number of fertilized grains in the cluster averages 99,07 (Alleweldt, 1992; Aradhya et al., 2003; Arregui et al., 1988). The average parameters of the mechanical composition of the bunch and grain of the Muscat Hamburg variety are slightly better, but very significant for further use in the selection of clones (Table 3)

Table 3. Mechanical composition of the cluster (bunch) of grape (weight, ength, width)

Variety	Weight of the cluster (g)	Length (cm)	Width (cm)	L/W	Number of fertilized grains in the cluster
Muscat hamburg	334,44	16,15	11,42	1,41	94,77
Variation of the Muscat hamburg	367,16	17,80	12,17	1,46	99,07
Average	350,80	16,98	11,80	1,44	96,92
sd	23,1	1,2	0,5	0,0	3,0
CV%	6,6	6,9	4,5	2,5	3,1

Figure 5

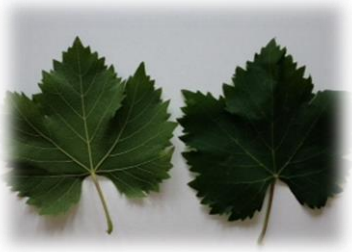

Figure 6

Figure 7

Figure 8

Figure 9

From figure 5 to 9 visible comparisons are made in leaf shape, cluster and grain size, and cluster compactness (number of fertilized grains) between cultivar and variation. Chemical composition of the grapes is represented by the amount of sugar and total acids in grape juice analyzed as appropriate fractions of the HPLC detector (Fenoll et at., 2009; Fregoni, 1985; Galet, 1998). Average sugar of Muskat hamburg variety is 203 g/dm^3 and total acids 5,6 g/dm^3. Average sugar of variation of Muskat hamburg is 199 g/dm^3 and total acids 5,2 g/dm^3 (Galet, 1993; Hocquigny et al., 2004). The variation of Muscat Hamburg is distinguished by a better composition of sugars and acids and has a better and more harmonious taste (Table 4).

Table 4. Chemical composition of grapes

Variety	Sugar g/dm^3	Index	Total acids g/dm^3	Index
Muscat hamburg	203	104	5,6	108
Variation of the Muscat hamburg	199	102	5,2	100
Average	201,0		5,40	
sd	2,8		0,3	
CV%	1,4		5,2	

Table 5 and Figures 10 and 11 show the results of analyzing the number of chromosomes, the degree of ploidy, certain anomalies in the division of chromosomes in mitosis, the possibility of the existence of a gigantic number of chromosomes and disturbance of the condition and ratio of the nucleus, cytoplasm etc. (Alleweldt, 1992; Galet, 1998).

Table 5. Chromosome construction in the examined grapevine cultivars

Diploid cultivars V. vinifera	Designation	Constitution	Number of chromosomes
Muscat hamburg	2*n*	AABBCC	38
Variation of the Muscat hamburg	2*n*	AABBCC	38

The examined Muscat Hamburg and its variation have a diploid number of chromosomes according to the standard scheme and the length of chromosomes in micrometers (μm**)** with statistical differences between individual chromosomes. Not observed any more concessions and abnormalities in chromosomes in the different cultivars. in the different variations (OIV, 2007; Riaz et al., 2004). The examined Muscat Hamburg and its variation belong to the sub-genus Euvitis and they are diploid cultivars (2n = 38). In them, the metaphase is normal, there are no anomalies in the structure and the number of chromosomes, there are 38 clearly differentiated chromosomes under microscope (Markovska, 2001; Fazinić & Fazinić, 1990; Riaz et al., 2004)

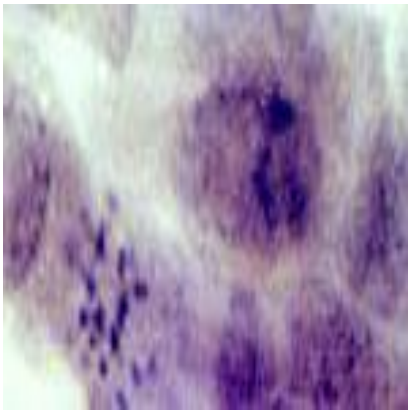

Figure 10. Chromosomes of cultivar

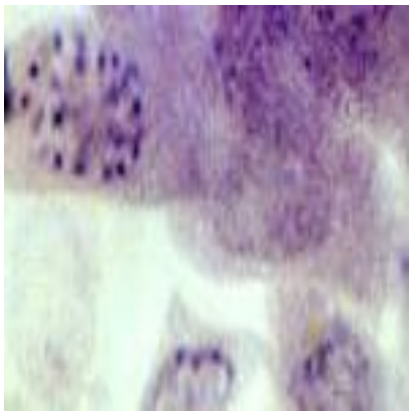

Figure 11. Chromosomes of variation Muscat hamburg

In continuation of the examination of the Muscat Hamburg variety and its variation, comparisons of DNA fragments were made to see their mutual similarity and origin (in case it is not a question of some other variety similar to Muscat Hamburg?). The DNA fragments were taken as part of another collaborative study of relatedness and parentage of a larger number of cultivars and a larger display of DNA fragments (Sharif et al., 2015; Torregrosa, 1995; Verries et al., 2000). The first two fragments are identical and show that it is the same variety, but that there are variations within the variety.

Comparisons are also made according to other authors, mostly with the author Aradhya, M.K. and col., 2003, who based on the similarity and origin of varieties and their variations have made centers of origin in many varieties (Aradhya, 1992; Sabir et al., 2008). According to them, Muscat Hamburg is in G15.

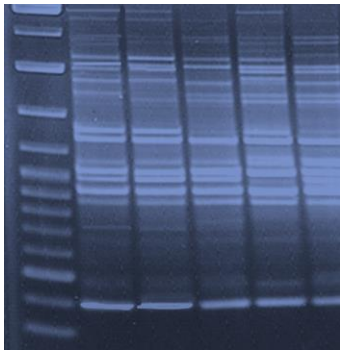

Figure 12. Comparisons with DNA fragments

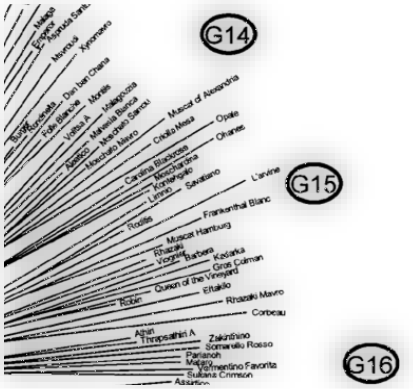

Figure 13. Pattern of origin of varieties (Muscat hamburg in G 15)

Figure 14

Figure 15

Figure 16

Figure 17

From figures 14 to 17 visible comparisons are made in the external appearance of the bunch and the grain.

Table 6. Organoleptic evaluation

Variety	Outdoor. appearance 1 - 3	Consistency 1 - 3	Taste 1 - 3	Specifics 0.1 - 1	A general impression to 10	Total
Muscat hamburg	1,5	2,0	1,5	0,7	5,7	11,4
Variation of the Muscat hamburg	1,0	2,0	2,0	1,0	6,0	12,0

Table 6 shows the organoleptic analysis, the individual evaluations and the total evaluation of Muscat Hamburg and its variation (Božinović, 2010; Fregoni, 1985; Galet, 1998). They were examined; the taste, the external appearance, the consistency, the typicality of the grapes and total grade (Markovska, 2001; Božinović, 2010; Fazinić & Fazinić, 1990). The evaluation is given by expert evaluators and random evaluators (Crespan & Milani, 2001; Fazinić & Fazinić, 1990) According to the organoleptic tasting, the variation of Muscat Hamburg received higher individual ratings and a higher overall rating.

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CONCLUSIONS

From the obtained results the following can be concluded:

- The Muscat Hamburg variation has almost all phenophases take place earlier and the number of days from sprouting to full maturity is less. These are significant predispositions for avoiding some unfavorable temperatures and choosing a variety with earlier ripening.
- The Muscat Hamburg variation has a larger grain, tighter cluster, better coloration and a higher number of fertilized grains in the cluster. In general, the variation has a more beautiful external appearance.
- The variation of Muscat Hamburg is distinguished by a better composition of sugars and acids and has a better and more harmonious taste.
- The examined Muscat Hamburg and its variation belong to the subgenus Euvitis and they are diploid cultivars (2n = 38). In them, the metaphase is normal, there are no anomalies in the structure and the number of chromosomes.
- By comparing DNA fragments, it cannot be said with certainty that a change in phenotype also has a change in genotype.
- According to the organoleptic tasting, the variation of Muscat Hamburg received higher individual ratings and a higher overall rating.
- Our investigations of provenance and DNA identification are consistent with the investigations of other authors who have also shown the Center of Origin of Muscat Hamburg (G15).

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