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IDENTIFICATION OF SOME AUTOCHTHONOUS AND REGIONAL GRAPEVINE VARIETIES ACCORDING TO O.I.V. DESCRIPTORS FOR CERTAIN CHARACTERISTICS

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ABSTRACT

Our studies included some autochthonous and regional grape varieties grown in the Republic of North Macedonia. The autochthonous varieties were examined - White winter, Konchanka, Stanushina, Ohrid black and Ohrid white, and the regional varieties - Vranec, Prokupec, Smederevka, Zilavka and Zupljanka were examined. The tests were divided into three methodological units. The first method was applied to all varieties - data collection, history and comparison with other tests. The second method was performed on all varieties - ampelographic identification because we had samples from all varieties. In some of the varieties, the third method was performed, that is, genetic (DNA) identification was made in the period from 2010 to 2020 in different examination centers and at different times. The results are compared with the results of a study by Professor M. Aradhya of the USDA Research Center, Davis CA, USA. He has performed a wide range of genetic identification on a number of autochthonous varieties in Europe and Asia, including varieties in the Balkans. With the second method of examination - ampelographic identification according to O.I.V. descriptors we got the most realistic results. An ampelographic description of certain characteristics of the individual organs of the vine was performed and it was concluded that the varieties according to their anatomical and physiological characteristics belong to 2 groups: ecological-geographical group Black Sea varieties, subgroup Balcanica and ecological-geographical group Eastern varieties, subgroup Antasiatica.

Key words: autochthonous, regional, ampelographic, DNA identification, descriptors.

INTRODUCTION

To improve viticultural production and obtain good results in the processing sector in viticulture and winemaking, it is necessary to follow tradition and protect those plant species and varieties that are characteristic of our climate and terroir here.

Therefore, in our study, several regional and autochthonous varieties of grapevine were selected as material for work, with the aim of greater adaptation to the changes that have occurred in the direction of improving the quality characteristics. It is an unwritten rule, that each variety gives its best in its growing area. The range or environment of cultivation of the grapevine represents its terroir – a composition of soil and climatic conditions that surround it. The grapevine, as a perennial and ennobled plant, can give the greatest adaptability and maximum quality if it is grown in the centers of origin or in regions where it was brought, cultivated and domesticated over a very long period of time. Therefore, by testing and experimenting with varieties that are domestic (autochthonous) or domesticated and adapted from a long time ago in the place where the test is

carried out, is the best way to get a superior variety. The genetic and selection methods applied to the autochthonous and surrounding - regional varieties achieve their results and varieties with the best and most desirable properties can be obtained.

MATERIAL AND METHODS

In this paper, we decided to examine several autochthonous varieties of grapevine - White winter, Konchanka, Stanushina, Ohrid black and Ohrid white and several regional varieties - Vranec, Prokupec, Smederevka, Zilavka and Zupljanka. These varieties are widespread here and in the surrounding countries in the region around us. They are registered in the list of approved and recommended varieties of the Republic of North Macedonia. The largest tests of these varieties were carried out in the period from 1998 to 2020. In that period, the regional varieties were the most widespread in our vineyards, and especially in the Agricultural Institute there were collection plantations for testing the varieties, where many world introduced varieties were represented, including many regional varieties. During that period, many old-new varieties were found throughout Macedonia that were probably forgotten, or were not studied at all and were neglected as individual grapevines. Today they are managed as newly discovered autochthonous varieties, their trials have been done and they have been introduced into the collection plantations at the institute or at some other collection location (Korunoska, 2007; Božinović, 2010; Maul, 2004; Galet, 1998).

The examination methods are in three methodological units: study of the origin according to written and narrative documents and knowledge, ampelographic and ampelometric identification and genetic identification. In our work, several varieties were successively taken and examined and their similarities and origins were determined, but for the time being it is still not possible to fully and accurately determine the parentage and exact origin of all of them.

The study of the origin of the studied varieties has been studied by finding old records, chronological notes, stories from the local population - natives, comparisons with other authors working on the same problem in the region, consultations with authors and their literature around the world, etc.

Ampelographic and ampelometric identification is performed with a botanical description of the plant (grapevine) and comparison with certain standard plants according to description lists of primary and secondary OIV Code descriptors by the EU-PROJECT GENRES 081 - 09/2001 and 2009 (Bodor et al., 2012; Maul, 2004; OIV, 2007).

When analyzing the varieties, their description and their origin, DNA extraction attempts were also made from the tissue of the shoot, which is located just below the peeled shell, and it was treated accordingly (Riaz et al., 2004; Sabir at al., 2008; Vos, 1995). The analysis and determination were made according to the standard microsatellite locus determined according to the OIV descriptor list in the following order: SSR-marker, VVS2, SSR-marker, VVMD5, SSR-marker, VVMD7, SSR-marker, VVMD27, SSR-marker, VrZAG62, SSR-marker, VRZAG79, SSR-marker, data – coded fragment sizes (in our case with Marker fragments sizes from 100bP to 10000bP) (Blaich et al., 2007, Sabir et al., 2008; Vos, 1995).

DNA extraction was performed first by isolating it from the woody tissue of the shoots, then it was chopped and mashed according to the method of Lodhi et al. (1994) using a buffer medium – 2% PVPP. Then the DNA concentrate was separated with chloroform and octanol (24:1), treated with NaCl and dried at room temperature. Amplification was performed with polymerase in cyclic processes at a temperature of 45^{o}C to 95^{o}C. The analysis sequences are: 5'-CCC ACC ACA C-3'. After electrophoresis, formed bands (circular lines or rings in arrays) are

displayed in an agarose gel as a substrate and the same are observed in a dark chamber under UV light, where the bands are compared according to their similarity (Torregrosa, 1995; Zhang, 2021). The more similar bands there are in the arrays, the closer the varieties are in their origin, affiliation and characteristics (DNA analyzes of material from our varieties were performed at the Grape and Wine Institute in Gassenheim, Germany) (Blaich et al., 2007; Cretazzo 2010; Hocquigny 2004).

With computer processing and a suitable program, statistical processing in the form of dendograms showing the convergence and divergence of the varieties (similarity and differences) were made. Such dendograms were made after ampelographic characterization and after DNA identification (Hocquigny, 2004; Houben et al., 2014; Li et al., 2020). The obtained results have been compared with the results of several scientists around the world, but mostly with the investigations of Professor M. Aradhya from the USDA research center, Davis CA, America. During his research, according to genetic identification and ampelographic characterization, he created a huge database (in the form of a dendogram) for a large number of autochthonous varieties in Europe and Asia, including the varieties of the Balkans.

RESULTS AND DISCUSSION

Varieties - Stanushina, Ohrid black, Ohrid white, Vranec, Prokupec, Smederevka, Zilavka and Zupljanka belong to the Black Sea ecological-geographic group of varieties (Convarietas pontica, subconvarietas balcanica). The varieties - White winter and Konchanka belong to the Eastern ecological-geographic group of varieties (Convarietas orientalis, subconvarietas antasiatica). The varieties White winter, Konchanka, Stanushina, Ohrid black, Ohrid white are found and grown mostly in Macedonia and are considered our autochthonous (domestic) or domesticated varieties. The other varieties were found, obtained and cultivated in the surrounding countries of the region, but in a large percentage they are also represented here, in our assortment as leading varieties (Galet, 1998). The varieties White winter and Konchanka are table varieties, they were found in our country, they are recognized as autochthonous and they are mostly grown in the Gevgelija - Valandovo vineyard and in other vineyards (Arregui et al., 1988; Korunoska, 2007). The varieties Stanushina and Ohrid black are varieties for the production of red wines, they were found in our country, they are recognized as autochthonous varieties and are mostly grown in the Ohrid, Tikvesh, Bitola and other vineyards (Korunoska, 2007; Božinović, 2010). Ohrid white is a variety for the production of white wines, it was found here, it is recognized as an autochthonous variety and it is mostly grown in the Ohrid, Prespa and other vineyards (Korunoska, 2007). Vranec is a variety for the production of red wines, a Montenegrin autochthonous variety, it is mostly grown in Montenegro, Serbia, Croatia, and in our country, as an introduced variety, it is a leading variety in several vineyards (Božinović, 2010). Prokupec is a variety for the production of red wines, an autochthonous variety from Serbia, it is mostly grown in Serbia, Bulgaria, and in our country, as an introduced variety, it is one of the leading varieties in several vineyards (Božinović, 2010; Maul, 2004). Smederevka is a variety for the production of white wines, it is assumed to be a Serbian autochthonous variety, but its origin is still being questioned, it is mostly grown in Serbia, Bulgaria, Turkey, Romania, Hungary, and in our country, as an introduced variety, it is a leading variety for the production of white wines in several vineyards. Zilavka is a variety for the production of white wines, an autochthonous variety from Herzegovina, it is mostly grown in Herzegovina, Dalmatia, Serbia, and in our country as an introduced variety it is grown in Veles, Tikvesh and other vineyards (Božinović, 2010; Maul, 2004; OIV, 2007). Zupljanka is a variety for the production of white wines, created at the Institute of Viticulture and Fruit Growing in Sremski Karlovci (Serbia) by Prof. Dr. Milosavljevic, by crossing the Prokupec x Burgundy

Black varieties, in 1970. She has parents with different centers of origin. It is mostly grown in Serbia, Bulgaria, Romania, and in our country, as an introduced variety, it is grown in Skopje, Kumanovo, Ovche Pole, Strumica and other vineyards (Božinović, 2010; Galet, 1998; Maul, 2004).

Our investigations were aimed at describing some ampelographic characteristics that are significant for defining the belongingness and similarity of varieties. Thus, several significant characteristics were singled out and compared using primary descriptors and two significant characteristics analyzed using a secondary list of descriptors. Based on the ampelographic characterization, dendograms were made that show the similarities and differences between the studied varieties and their close and distant, different affiliation (convergence and divergence).

From Table 1 it can be seen that the cultivars are most different in several characteristics from the OIV primary list of descriptors: bunch shape (208), grain length (220), grain shape (223) and skin color (225). From table 2 it can be seen that according to the OIV secondary list of descriptors the cultivars are mostly different according to the characteristic bunch length (206) (OIV, 2007; Galet, 1998). These characteristics are varietal (differences between varieties) and cannot fully reflect differences in origin and parentage (their antecedents).

Figure 1 shows formed bands (circular lines like flattened rings in arrays) in an agarose gel substrate, observed in a dark chamber under UV-light, in the investigated varieties and comparing the bands according to their similarity. The more similar band rings there are in the arrays of the studied varieties, the closer they are in terms of origin, affiliation and characteristics (Li et al., 2020; Sabir et al., 2008; Zhang et al., 2021). According to the comparative analyzes from the amplification of microsatellite loci and electrophoresis and according to the displayed bands, it was shown that a greater number of the examined varieties have similar DNA among themselves. The arrangement from left to right is as follows: Marker (Fragments sizes from 100bP to 10000bP), Vranec, Prokupec, Smederevka, Ohrid white, Zilavka, Konchanska, Stanushina, White winter, Ohrid black, Zupljanka, PCR control and again a marker (Anhalt et al., 2011; Aradhya et al., 2003; Blaich, 2007).

On the basis of the OIV lists of primary and secondary descriptors of the characteristics and on the basis of the DNA identification (the analyzed bands), schematic representations (dendograms) were made of the similarity of the varieties and their origin, i.e. their convergence and divergence were shown. From the presented schemes (dendograms – figure 2 and 3) it is possible to see the convergence and the divergence among the varieties examined. There are more pronounced similarities in characteristics and a greater coincidence in origin between the varieties Vranec, Prokupec, Stanushina and Zupljanka, then between the varieties Ohrid white, Ohrid black and Zilavka and between the varieties White winter, Konchanka and Smederevka. In that way, they are arranged in separate clusters according to convergence. Some of the investigated varieties have a similar DNA description (similar arrangement of rings - bands) which indicates that they have the same origin (same center of origin - convergent) or have common ancestors (one common parent, one variety is the parent of another variety, etc.). Some of the examined varieties have a different DNA description and even have different centers of origin (divergent) (Houben et al., 2014; Riaz et al., 2004).

It was often thought that the Ohrid black variety was actually the Stanushina variety. But our DNA tests showed that they are two separate varieties that have many similarities. Among the studied varieties, there is no completely identical DNA description, and therefore it can be said that all varieties are different and it is not a question of one and the same variety with two different synonyms (Houben et al., 2014; Riaz et al., 2004). Comparisons are made with the studies of other

authors, in the specific case with the studies of Professor M. Aradhya and his collaborators, who made a large schematic display - a dendogram of a large number of world grapevine varieties according to their similarities in characteristics and according to origin. The dendogram consists of several sections labeled as G1 to G15. A larger number of our examined varieties, although they belong to different ecological - geographical groups, according to greater similarities, we found them in G13 - Vranec, White winter, Zilavka and Stanushina (Aradhya et al., 2003).

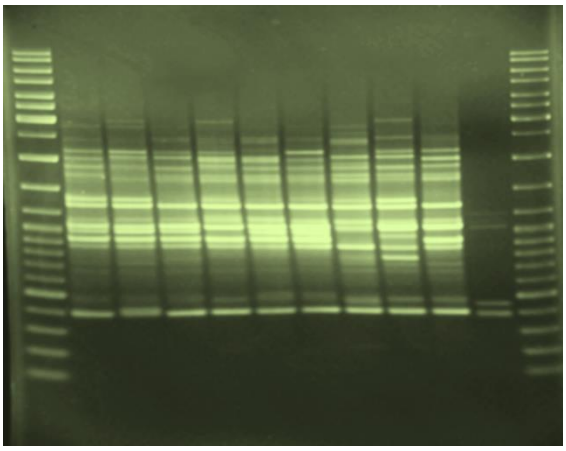
Table 1 Primary ampelographic descriptors with an assessment

OIV Code N°, Descriptor, Characteristics	Cultivar									
	White winter	Kon-chanka	Stanu-shina	Ohrid black	Ohrid white	Vranec	Proku-pec	Smede-revka	Zilav-ka	Zup-ljanka
OIV 151 Inflorescence: sex of flower	3	4	3	3	3	3	3	3	3	3
OIV 202 Bunch: length	5	5	5	5	5	5	5	5	5	5
OIV 208 Bunch: shape	3	3	2	1	1	2	2	3	1	1
OIV 209 Bunch: number of wings	2	2	2	3	3	2	2	2	3	3
OIV 220 Berry: length	7	7	5	3	3	5	5	5	3	3
OIV 221 Berry: width	7	7	7	5	5	7	7	7	5	5
OIV 223 Berry: shape	8	8	2	1	1	2	2	2	1	1
OIV 225 Berry: color of skin	1	5	5	5	1	6	5	1	1	1
OIV 236 Berry: particular flavor	1	1	1, 4,	1, 4,	1	4	4	1	1	1
OIV 241 Berry: presence of seeds	3	3	3	3	3	3	3	3	3	3

Table 2 Secondary ampelographic descriptors with an assessment

OIV Code N°, Descriptor, Characteristics	Cultivar									
	White winter	Kon-chanka	Stanu-shina	Ohrid black	Ohrid white	Vranec	Proku-pec	Smede-revka	Zilav-ka	Zupljan-ka
OIV 204 Bunch: density	7	5	5	7	7	5	5	5	7	7
OIV 206 Bunch: length of peduncle	7	7	5	5	3	5	5	5	3	5

V P S Ow Z K St Ww Ob Zu



V = Vranec, P = Prokupec, S = Smederevka, Ow = Ohrid white, Z = Zilavka, K = Konchanka, St = Stanushina, Ww = White winter, Ob = Ohrid black and Zu = Zupljanka.

Figure 1. Bands shown in a UV-dark chamber

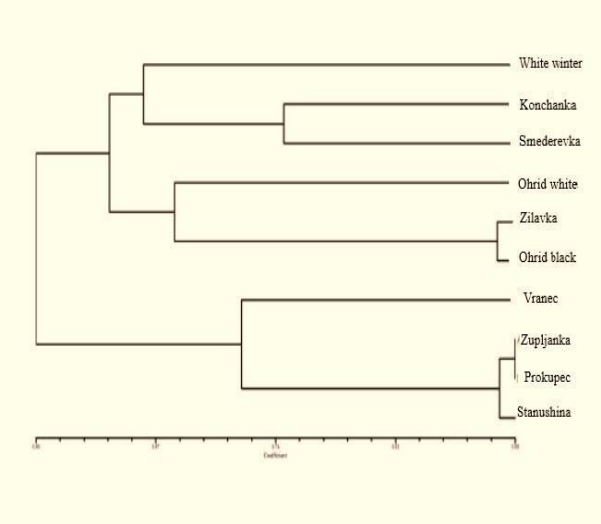


Figure 2. Schematic representation of the similarity and origin of all varieties, according to ampelographic characterization and DNA identification (dendogram)

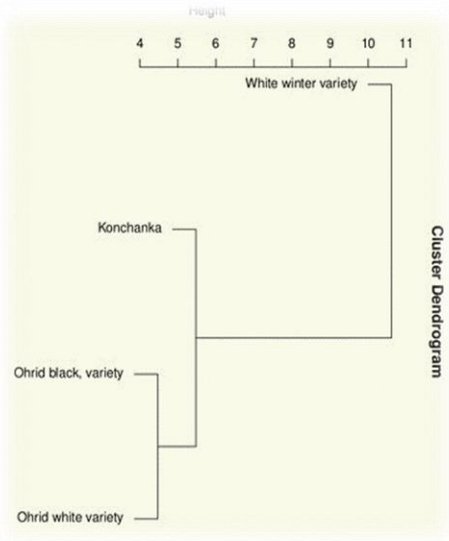


Figure 3. Schematic presentation of the similarity

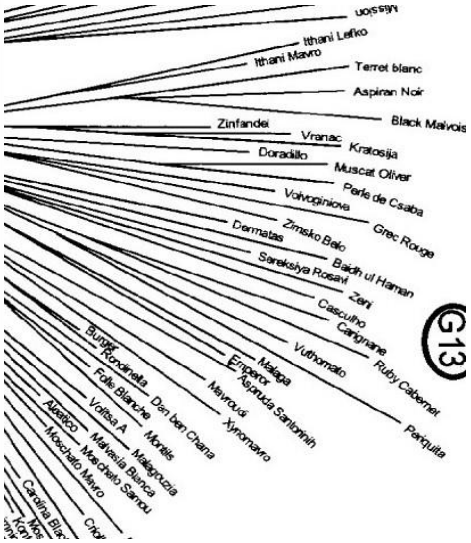


Figure 4. Schematic representation of the and origin of a part of the varieties according to similarity and origin of varieties, compared with the ampelographic characterization (dendogram) other scientists (Aradhya M. K. and col.2003

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CONCLUSIONS

Based on previous tests and analyzing the results can be draw the following conclusions:

- According to historical data, oral and written records, stories through generations and other types of anamnesis, it is argued that the varieties - Vranec, Prokupec, Smederevka, Ohrid white, Zilavka, Stanushina, Ohrid black, Zupljanka belong to Convarietas pontica, subconvarietas balcanica, and the varieties - White winter and Konchanka belong to Convarietas orientalis, subconvarietas antasiatica).
- The ampelographic characterization (ampelographic description) with OIV primary and secondary descriptors provides a clear representation of the similarity, affiliation and origin of the varieties, but this identification needs to be supplemented with another identification.
- With previous knowledge, then with ampelographic characterization and DNA identification of the tested varieties, their similarity and common origin (convergence and divergence) can be scientifically confirmed.
- There are more pronounced similarities in characteristics and greater similarity in origin between the varieties Vranec, Prokupec, Stanushina and Zupljanka, then between the varieties Ohrid white,

Ohrid black and Zilavka and between the varieties White winter, Konchanka and Smederevka. Our tests showed that the Ohrid black variety is not the same as the Stanushina variety.
- The obtained results in the studied varieties are in accordance with other studies by other scientists in the world (Aradhya M. K. et. al. 2003).

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