

AN ANALYSIS OF MODERN WORKS OF DECIPHERING VITIS VINIFERA L. GRAPE GENOME

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ANALIZA LUCRĂRILOR CONSACRATE DESCIFRĂRII GENOMULUI VIȚEI DE VIE VITIS VINIFERA L.

Autorii prezintă o analiză succintă și consistentă a majorității covârșitoare de lucrări științifice consacrate descifrării **genomului istoric** al viței de vie care s-au efectuat în lume pe parcursul ultimilor 20 de ani. Soluționarea problemei a presupus sequenșarea deplină a genomului *Vitis vinifera* L., utilizând diferite sisteme de microsatellite-markeri (AFLP, S-SAP, SSR, RAPD, Inter-SSR). Astfel, vița de vie a devenit o plantă-model pentru investigațiile genetice de ultimă oră, în urma cărora s-au obținut date despre consecutivitatea a sute și mii de perechi de nucleotide. Importante sunt cunoștințele despre distribuția genelor structurale în ADN și acțiunea unor gene sau sisteme de gene asupra individului, soiului, speciei, genului *Vitis* L. Totodată, se atenționează asupra importanței descifrării **genomului nou**, sintetizat din genomii sps *Vitis vinifera* L. și *V. rotundifolia* Michx.

In recent years, scientists from Germany, France, Austria, the USA, Italy, India, the PRC, the Russian Federation, Australia, Turkey, Croatia, Hungary, Japan, and Chile have been conducting intensive research on deciphering the grape genome. Their **task** is *Vitis vinifera* L. complete genome sequencing, which includes using molecular markers in the vast majority of studies; this task is so alluring and ambitious that its solution will certainly require international efforts of many scientists all around the world.

In this context, the work of S. Molnar et al. (Germany, 2007), clarifies the role of the presence of a marker for selection (MAS) for resistance

to mildew in several hybrid families (Molnar et al., 2007), and is of great scientific interest. The practical application of genetic pyramid stability through the use of molecular markers was studied in the offspring of F₁, derived from crosses of VHR 3082-1-42 x 'Regent'. In fact, VHR 3082-1-42 is an intergeneric hybrid produced by crossing *Muscadinia rotundifolia* (Planch.) Small x *V. vinifera* L. as a result of a 4-grade backcross by another sort of *V. vinifera* L. This hybrid is the carrier of *Run 1* gene, which encodes resistance of grapes to mildew, and *Rpv1* gene, which is loosely associated with resistance to mildew. Both these genes have been introduced from the species of *Muscadinia rotundifolia* (Planch.) Small. 'Regent', in its turn, is a new sort having quantitative, comprehensive resistance to mildew; it is created in Germany for commercial purposes (Eibach, Töpfer, 2003).

Phenotypic evaluation for mildew has been implemented on the basis of the data of the artificial leaf surface inoculation and natural mildew infection in greenhouse conditions. The comparison of this estimate with the results of the molecular marker analysis has revealed an obvious correlation between the degree of stability and the presence of resistance related to alleles. It is shown that an equal number of seedlings (20 on the basis of phenotypic data and based on the presence of markers during the selection process) corresponded to 4 introduced resistance alleles, which reveals an effective combination of resistance genes of both parents (Eibach et al., 2007).

In this aspect, the work by F. Pelsy (2007) is of special interest; according to the data presented in it, Tvv1 are revealed by UTL-area amplification of the elements identified in 94 *Vitaceae* representatives; each of them reveals a single parent in the UTL derivative bands, inherited by offspring and fixed by clones by vegetative reproduction method. During their research, the authors have used 15 *microsatellite loci*. The analysis of the 7 sequenced UTL-regions has revealed the signs lost before and those conserved in evolution. In general, this marker system is original; it has not yet been tested on *Vitis* L., but may be of great help for identifying its representatives.

To detect agrobacteria which cause the growth of painful gall on grapes, F. Bini et al. (2008) have used new virulent and oncogenic specific combination of PCR- primers tested on *Azotobacterium vitis* and *Agrobacterium tumefaciens* strains, including other types occurring in the vineyards. Despite the fact that various attempts with a single primer or their

complex combination have been taken, they have failed to reliably detect all types of agrobacteria found in the vineyards. The objective of this work is completing the study, together with already published and new combinations of primers, which allows to detect and identify tumorigenic agrobacteria which can potentially infect the vineyards, with only one PCR (Bini et al., 2008).

Different climatic and geological conditions contributed to a large increase in diversity of *Vitis vinifera* L. varieties in Slovenia. According to the O.I.V., among 50 well-known and rare cultivars of Slovenia, there are three synonyms. To clarify the number of synonyms, N. Jbtajner et al. (2008) have analyzed landraces; 33 cultivars have been analyzed for 21 microsatellite-loci for the identification and evaluation of their synonyms and homonyms, including cultivated varieties of the adjacent countries. Among the Slovenian sorts, some previously considered synonyms have been confirmed, and a number of new ones have been discovered: 'Poljškica Drovnik' = 'Istrska Malvzija', 'Picolit Italy' = 'Picolit Vienna', 'Vitovska grhganja' = 'Racuk' and 'Prosecco' = 'Glera' = 'Jbteverjana'. Zelen sort types ('Zelen Pokov', 'Zelen 66', and 'Zelen 2.4') have been first detected by some microsatellite-loci and, therefore, can also be considered homonyms. Two 'Picolit' types match 'true-to-type' on the basis of the comparison with the 'Picolit' clone from Italy. However, the synonymy between 'Heunisch' and 'Belina' has not been confirmed, whereas 'Belina Pleterje' is different from 'Heunisch weiss' only by 13 out of the 19 loci. Comparisons of 'Vitovska grganja' sort from Slovenia with 'Vitovska' from Italy also demonstrate differences in the majority of the analyzed loci (Jbtajner et al., 2008).

The work by French researchers F. Martinez et al. is very interesting; they have applied the new PCR-primers for the characterization of the distribution of *Botrytis cinerea* fungus in populations in French vineyards. One of the main tasks has been comparing the detection of replaced elements, in particular, *Flipper*, by means of using various PCR-primers. *Botrytis cinerea* phytopathogenic fungi represent a complex of the two main genetic groups: Group-I and Group - II. The latter group includes various TE types, which differ by the presence or absence of the two moving elements (TE): *Boty* and *Flipper*. In two grape populations from Bordeaux, the distribution frequency between these genetic subgroups shows that Group-I is isolated – radish (2.3 per cent), whereas, in Group-II, 4 TE types

have been identified in various ratios: II-*transpoosa* (59.8 per cent), II-*boty* (21.3 per cent), II-*vacuma* (15.5 per cent), and II-*flipper* (1.1 per cent). Both in Bordeaux and the Loire Valley, the vineyards, with significant differences between the main types of proven TE-types are described (Martinez et al., 2008).

To date, intensive research on decoding the grape genome is being practiced in many European countries, including Bulgaria. In this regard, the work by T. Dzhambazova et al. is of great theoretical and practical interest; it is dedicated to the genetic diversity of germplasm of Bulgarian local grapes (*Vitis vinifera* L.) based on nuclear and chloroplast polymorphic microsatellite-markers. Forty-one wild vines collected in different areas of Bulgaria and nineteen local Bulgarian cultivars have been studied genetically with 7 nuclear and 5 chloroplast SSR-markers. In this case, based on microsatellite allelic profiles, 6 wild vines gathered in the Danube valley, have been considered as the genotypes not related to *V. vinifera* L. species. Genetic diversity detected on the basis of nuclear loci in grape cultivars has been compared to that of other varieties cultivated in the collection. However, the lowest level of genetic diversity is observed in the range of wild specimens. The dendrograms obtained by nuclear SSRs-markers in cultivated grape sorts are very different from those detected in wild specimens. The most common chlorotype among wild samples is A type, while the cultivars have C-type. The differences between Bulgarian grape chlorotypes in the context with the differentiation of Eurasian grape flora chloroplasts are being discussed (Dzhambazova et al., 2009).

In the work by M. Meneghetti et al. (2009), it is indicated that 60 samples of 'Wildbacher' grape cultivar brought from various European collections and repositories, as well as from private Styrian and Italian vineyards have been genetically examined by SSR-markers using print-fungi. 5 different nuclear profiles confirmed that 'Wildbacher' is a common name and is used as a homonym. Some of the new genotypes could be related to the previous list of 'Wildbacher' families. In particular, it has been found that, in the Italian industrial vineyard, the two main 'Wildbacher' types are determined by A and B genetic profiles. They correspond to the two cited genotypes from the Styria, 'Wildbacher Blau' ('Blauer Wildbacher') and 'Wildbacher Spätblau'. 'Wildbacher Blau' is the most common and best grape sort in both countries due to its early ripening fruit compared to 'Wildbacher Spätblau' cultivar. They are very similar morphologically and

genetically, and they share the smallest allele from each of the 11 SSR-loci analyzed for identifying cultivars. Three 'Wildbacher' genotypes from the German collection different from the others are identified as individual sorts. At the same time, 'Rotblüttriger' (C genotype) and 'Früblau' from Germany (D genotype) have shown only loose genetic similarity with A and B genotypes, whereas E genotype of 'Wildbacher' sort, imported from Hungary, has quite a distinctive genetic profile. The close relationship with 'Heunisch' key sort (for increasing the European grape variety) demonstrates the division of one allele at all the loci studied to date (Meneghetti et al., 2009).

The application of genetic research, in particular, the analysis of the variability of *AFLP*-*SAP*-markers to identify clones of 'Malbec' and 'Syrah' cultivars is described in detail in the collaboration work by Slovenian and Argentinean scientists, N. Stajner et al. (2009). The clonal variation in 14 'Syrah' sort clones and 22 'Malbec' sort clones (*Vitis vinifera* L.) is evaluated with the help of *S-SAP*- and *AFLP*-marker systems. The usefulness of *S-SAP*-markers is compared with that of *AFLP*-markers. It is proven on the basis of the results obtained by the authors that *S-SAP* is a more informative marker system, and it shows the average higher number of polymorphic bands in the group of cultivars than *AFLP* does. The relationship between the clones is analyzed according to the cluster system. As a rule, the average arithmetic data is used; in both cases, it is possible to detect a well-defined group of clones, in which 'Malbec' sort clones are separated from 'Syrah' sort clones. The high variability of some clones may have been led into a cluster. Different levels of polymorphism obtained during this research for 'Malbec' and 'Syrah' sorts suggest that 'Malbec' sort shows a greater frequency of mutations than 'Syrah' sort. The research results indicate that a high ratio of polymorphic bands in *S-SAP*-markers makes them less intense and more effective for the further development of the identity approximate for clones (Stajner et al., 2009).

The work of Italian scientists D. Torello Marinoni et al. (2009) is dedicated to the problem of identification of grape sorts from Liguria (north-western Italy). To identify the grape varieties of the Italian region of Liguria, 51 samples have been collected and compared to collection varieties and the varieties that grow in the collections of the surrounding areas. The synonyms are confirmed with the help of *SSR*-markers in 9 loci. Only 36 samples have unique genetic profiles. This fact reveals the occurrence of synonyms within the group of varieties

from the region of Liguria and the other areas of Northern Italy. The six nameless varieties belong to the obvious homonyms. A significant number of sorts with unique genotypes from the region of Liguria represent an important and valuable factor of hereditary genetic diversity, which should be stored and preserved. Collections of grape sorts play an important part in solving this problem. Further development of ampelographic and genetic databases will contribute significantly to the comparative analysis of cultivars and samples from any grape cultivation or grape collection world centers, and improve general and accurate identification of grape sorts (Torello Marinoni et al., 2009).

Similar problems are discussed in the work by Spanish scientists E. Diaz Losada, A. Tato Salgado, A. M. Ramos-Cabrera et al. (2010), who used twenty microsatellites of the (*SSR*s-) marker for discovering and studying the two main sources of variability of grape sorts of the north-western Spain. Grape germplasm bank at "Estacion de Viticultura y Enologia de Galicia, Xunta de Galicia" contains 15 varieties of grapes with 98 common samples: 'Brancellao', 'Albarello', etc. 'Syrah' and 'Pinot noir' sorts are included as reference sorts. Two different sources (pedigrees) have been discovered; one descends from 'Caiso Astureses', whereas the second one descends from 'Merenzao', a synonym of the French 'Trousseau' cultivar. The cultivars of the north-western Spain are derivatives of these two cultivars, obtained by hybridization and selection of genotypes adapted to local climatic conditions and fixed by inoculation for domestication and production. Both genealogical sources are different from each other by allele frequency and different distribution in the north-western Spain: the first genealogical source is found in the west, while the second one, associated with the French 'Trousseau' cultivar, is found in the east. 'Caiso Astureses' is the most common genotype, obtained by hybridization; this fact demonstrates the importance of the sort in the origin of Galicia grapes. In addition, 13 different genotypes have been identified. The identity of 'Brancellao' and 'Albarello' sorts is confirmed by *SSR*-markers. The other synonyms include 'Caiso Astureses', 'Caiso Bravo', and 'Albarin Negro'. 'Caiso Redondo' cultivar has revealed two different genotypes, one of which is associated with 'Caiso Astureses', and the other one is associated with 'Merenzao' sort. Two cultivars are included in the EVEGA collections, which have not been previously reported: 'Verdello' and 'Caiso Longo' (Diaz Losada et al., 2010).

The research by J. F. Vouillamoz and C. Arnold (2010) is dedicated to the application of microsatellite-analysis in explaining the origin of some grape sorts, in particular, for reconstructing the origin of the well-known technical grape sort, 'Müller-Thurgau', which obviously originates from such parents as 'Pinot' and 'Schiava Grossa'. 'Müller-Thurgau' sort has recently been proven to be the result of interbreeding between 'Riesling' and 'Madeleine Royale' sorts. In the 19th century, the parents of the breeding combination this sort originates from were considered to be unknown. The authors analyze the relationship line based on 93 grape cultivars from central Europe, which have been genetically examined using 57 microsatellite-markers. The results clearly demonstrate that 'Madeleine Royale' sort is obtained by crossing 'Pinot' x 'Schiava Grossa'. Since 'Riesling' is known to be a descendant of 'Gouais Blanc', hence 'Muller Thurgau' is related to 'Pinot noir, gris, blanc' group of sorts and 'Schiava Grossa' sort, or 'Heunisch Weiss' sort (Vouillamoz et al., 2010).

In the work by M. J. Poupin et al. (2007), it is noted that, despite the widespread use of *B class PI / GLO* and *AP3/DEF* for characterizing the model species, the role of *TM6* subgroup within the framework of *AP3* is completely unclear. A detailed chronological analysis of grape flowers in their progressive development with the help of the light microscope and temporal expressiveness analysis through the early and later stages of development shows that *VvPI* expressivity increases during the maturation of pollen and decreases in the process of pollination and fertilization. On the other hand, *VvTM6* manifests itself at the later stages of anther development, too. Specific *VvAP3* and *VvPI* gene expression is detected in petals and anthers inside the flower, while *VvTM6* expression is also detected in carpels. P. Abbal P. et al. (2007) have worked according to the same scheme. Using the expression of the sequenced (*EST*) with the label and the analysis of gene base, *Rop* family members, *Vitis vinifera* L. is first characterized as a species. It is stated that the grape genome contains 7 expressive *VvRops*. According to the phylogenetic analysis data, *VvRops* could be divided into 4 groups according to their description in the literature on the basis of model plants. Genetic mapping is successfully done for 5 *VvRops*-genes localized independently from the associated groups. Both permanent and modified areas are identified by sequencing proteins or pairs of nucleotides.

Nevertheless, obtaining the data about the

sequence of 100 thousand pairs of nucleotides (can be > or <) in the grape DNA should not be regarded as an end in itself. The knowledge of the nucleotide sequence, embedded in the memory of the computer, would not be able to solve essential problems of viticulture alone. Even greater work aimed at determining the structural and functional organization of the **grape genome** is necessary. In its turn, it requires **knowledge of the distribution of structural genes** within the DNA: only then the **task of revealing the impact of individual genes** and / or their **integrated systems** (Dubinin, 1988) on the development of **individuals**, varieties, hybrids, varieties, subspecies, **species**, and **genus** *Vitis* L. will emerge. Of course, in the 21st century, it will be possible to put in the computer's memory, as one would expect, the data regarding the alternation of nucleotides in grapes (in their belt of several hundred thousand pairs).

Taking into account that there are no common organs specifically related to *VvRop* expressivity, which could be detected, *VvRop* showed high expressiveness in the development of berries only. The potentiality of the involvement of *VvRop*-genes' specificity in the development of grapes is being discussed (Abbal et al., 2007).

An interesting relevant research has been done by C. Espinoza et al. (2007) due to viral infection. Almost simultaneously with this report in *Genome* magazine, a work by E. Falistocco, V. Passeri, and G. Marconi (2007), is published; they have presented the first results of studying *5S rDNA* in *Vitis vinifera* L. species. Sequencing of the *5S rDNA* from 7 genotypes has been PCR-amplified and cloned, while 3 re-types are found; two of them differ both in length and nucleotide composition, and the third one is classified as a short *DEL* repeat, which differs from the latter by the wide erased space. The authors consider it to be the newest re-type discovered since it is identified in only one genotype. According to the researchers, *FISH*-analysis shows that the *5S rDNA*-genes are localized in a single locus. The variability of the *5S rDNA*-repeats is being discussed in connection with the alleged origins of *Vitis vinifera* L.

Turkey is known to be rich in local grape sorts, mainly those intended for table use (for instance, such world-famous sorts as Chaush White, Pink, Afuz Ali Pink, etc.). Turkish researchers have decided to provide the genetic characteristics for local assortment, using for this purpose 6 polymorphic microsatellite-loci, intending to clarify the relationship between homonyms brought to

Turkey from other regions of the world. These results have revealed a wide range of genetic variability among cultivars, as expected. The number of alleles per locus varies in a fairly wide range (10-21), as well as a variety of genes (0.85 -0.93) (Karatat et al., 2007).

The results of genetic research obtained by scientists Moncada and Hinrichsen in their study of 'Carmenere' grapes are of particular interest. The authors note that 'Carmenere' is a splendid technical sort of grapes with red berries, which spread to other countries from France. It was brought to Chile before the crisis caused by phylloxera in Europe, i.e. much earlier than 1868. There, it was confused with 'Merlot' sort and other grape sorts with red berries until the mid-1990. In their research, the authors present the results of genetic diversity among the studied 26 sorts from Chile, France, and Italy. To make genetic analysis, *microsatellite* (SSR) - and *AFLP*-markers have been used. While using 20 SSR-markers as the standard genotype, three different haplotypes have been discovered (presumably they got into this list as the mutations of *VVMD7* and *VMC5g7*-loci). In the case of AFPL, with only 11 primer combinations 5 groups have been identified. Identifying 5 of the 26 analyzed sorts has become possible by means of combining both techniques. The results obtained suggest that 'Carmenere' sort has low level of genetic diversity compared with other French red-berry sorts (Moncada, Hinrichsen, 2007).

In recent years a significant number of works on deciphering the grape genome was published. In this aspect, the work by the Turkish researcher F. Selli et al. (2007) is very interesting.

Turkey is known to have played an important role in the evolution of the modern assortment of table grapes, mainly due to its unique location on two continents and bordering with the regions of the genetic diversity of grapes. However, the rich grape germplasm found in Turkey has not been analyzed genetically yet. In their work, F. Selli et al. present the results of studies of 31 'Dimrit' - and 'Gemre'- samples – grape groups, which are analyzed genetically on 8 *SSR* (*microsatellite*)-loci and a number of ampelographic signs. Analyzes have allowed identifying sufficient genetic diversity between these grape groups. Despite the unique geographic distribution and genetic relationships, the researchers have not found any significant correlations. In two cases, 'Gemre'-groups are defined as genetically identical. A case of a synonym and a few cases of homonymous genotypes have also been identified (Selli et al.2007).

In this sphere, the work by L. J. Welter et al. (2007), aimed at improving the genetic map of F₁ population by crossing 'Regent' sort resistant to fungi and 'Lemberger' sort susceptible to them, and using it for localizing the factors affecting the resistance to the pathogen and the leaf morphology, is of great interest. As a result, the map of 122 microsatellite markers, also used for localizing *QTL* (*quantitative trait loci*), which render resistance to mildew pathogens, has been re-made (Welter et al., 2007).

Nowadays, the research on deciphering the grape genome is given increasing attention. In this aspect, the works by Tsoлова et al., 2007; Ruel, Walker, 2006; Dhanokar et al., 2005; Barker et al., 2005; Bavaresco et al. 2005; Van Sluyter et al. 2005; Andersen et al., 2005; Kellow et al., 2002; Pauquet et al., 2001; Ramming D., 2000; Wang et al., 1999; Walker, Jin, 1998; Lin, Walker 1997, dedicated to genetic research are of great interest.

The research by C. B. Agüero, C.P. Meredith, A.M. Dandekar (2006) is dedicated to genetic transformation of *Thompson Seedless* and *Chardonnay* cultivars of *Vitis vinifera* L. species by using *PGIP* and *GFP* genes. *Vitis vinifera* L. transgenic plants of these sorts have been obtained by somatic embryogenesis.

The report presented by Benjak et al. (2006) includes the data stating that different methods of grape DNA extraction can cause different *AFLP* profiles. The authors analyzed 62 Pinot Noir clones, 6 Pinto White clones, and 4 Pinot Grey Clones using 2 *AFLP* sets. The profiles obtained after DNA extraction by three different methods are compared. They have turned out to be different within the same genotype depending on the DNA extraction method (Benjak et al., 2006).

The information regarding the rapid extraction of genomic DNA from juvenile plant leaves for sequencing is presented in research by Bertsch et al. (2006). It is known that the isolation of genomic DNA is essential for a number of manipulations in molecular biology. The authors have developed a simple and rapid protocol that can be used for *PCR-DNA* isolation, and is qualitative from 5 mm of a young leaf of grapes and other plant species (Bertsch et al. 2006). The work by J.P. Péros et al. (2006) is actually dedicated to the same issue.

The work by Bulgarian researchers Genov et al. (2006), who have studied the genetic diversity of strains of *Agrobacterium vitis*, isolated in the industrial vineyards and wild grape vines of Bulgaria and estimated by means of *CAPS*-analysis of *16S-23S*

rDNA CAPS is of great theoretical and practical interest to viticulturists and molecular geneticists. The research on these strains isolated in wild vines (*Vitis vinifera* ssp. *Silvestris*) has demonstrated that their *CAPS*-profiles are identical to the *CAPS*-profiles collected in commercial vineyards in different regions of Bulgaria. The authors suppose that first *Agrobacterium viti* infected wild grape vines, and then moved to the cultivated vineyards.

To characterize and identify *Vitis vinifera* L. sorts in the north-western Spain, Santiago et al. (2005) have used *microsatellite*-markers and ampelometric methods. 9 sorts from this region known as *Caico* type, and one type known as *Tinta Femia* have been characterized by the typical leaf construction and determining the genetic profile by using 6 *microsatellite*-markers. This procedure included comparing the leaf morphology and revealing the similarity between the sorts. Thirty-three alleles have been discovered in 6 analyzed *microsatellite*-loci. Different sorts have been identified by both methods. The synonyms between the Spanish sorts studied and Portuguese cultivars are being discussed (Santiago et al., 2005).

The work of M. Kocsis et al. (2005) is dedicated to the genetic diversity of the local grape varieties of the Carpathian Basin opened with the help of *RAPD*-markers. **The purpose of the work** is to find the optimal primers for the further analyses of the local grape of the Carpathian Basin, to identify the grape varieties and to make a research on them through *RAPD*-markers, if it is necessary to obtain the information for the systematical purposes.

The Hungarian researchers have made the *RAPD*-marker analysis of 12 local grape varieties *Vitis vinifera* L. to evaluate the genetic variety among these genotypes and to discover the genetic interconnections between them. By the way, Hungary has a rich assortment that includes more than 100 local grape varieties. The research has been made at the high level, since 28 primers, which generated 120 polymorph fragments, participated in it. The dendrograms showed that the studied varieties can be identified by the high degree of stability. According to the authors, the technique with *RAPD*- markers is useful upon identification and description of the local grape varieties. (Kocsis et al., 2005).

The interesting research has been made by the Croatian and Turkish scholars on discovery of the genetic interconnections among the local grape varieties of Croatia, Greece and Turkey. On this purpose 3 sets of the varieties have been studied that

included 6 Croatian, 5 Greek and 9 Turkish grape cultivars. All varieties are tested with the molecular markers *RAPDs* with 8 primers and *SSRs*-genes in 8 loci. The DNA profiles of the grape varieties have showed exactly that there are no synonyms among the tested varieties. Only the *AMOVA* analysis has detected the genetic similarity between the Greek and Croatian varieties in opposition to the Turkish varieties (Benjak et al., 2005).

The work of the Bulgarian and the Cypriot researchers is dedicated to the analogous issues. They have made an attempt to study 12 local varieties of the Cyprus by means of the *microsatellite*- marker. The allelic profiles have been obtained for all studied varieties that enabled to identify and to evaluate them from the aspect of the genetic diversity. The homonyms of the varieties of the Cypriot 'Sideritis' and the Greek 'Sideritis', the varieties 'Mavro' (from the Cyprus) and 'Mavrud' (from Bulgaria) showed that we cannot be sure of their genetic similarity (Hvarleva et al., 2005).

In the work of Dhanokar et al., (2005) the results of the genetic researches of 43 grape varieties cultivated in India are given and evaluated. *ISSR PCR*-markers have been used for their characteristics. From 139 reproduced fragments, 96 were polymorph from the species of *Vitis vinifera* L., but the variety of *Vitis labrusca* L. was homozygote, on the contrary. The results showed that *ISSR* is an effective and veritable marker system for the genetic analysis of the grape cultivars.

The work of C.L. Barker et al. (2005) is dedicated to the same problem. The authors have asserted that the grape resistance to the mildew is controlled by the gene *Run1*, a unique dominant gene that is present in the wild species of *Muscadinia rotundifolia*, but which is absent in the variety *Vitis vinifera*. According to the authors, the comparative data on the physical and genetic mapping show that the recombination located nearby presses the gene *Run 1*. This happens, probably, thanks to the differences in the sequencing, which contain inside the introgressive fragment of the species of *Muscadinia rotundifolia*, a bearing gene *Run1*.

In the work of L. Bavaresco et al. (2005), the issues that have been covered are close to the subject. The grape variety 'Cabernet Sauvignon' or its *R5* – clone have been grafted on the stem of *VR 043-43*, which is in its essence is a distant hybrid *V. vinifera* L. x *Muscadinia rotundifolia* Small, which grows in the noncarbonated and carbonated soils. **The work task** is to study the receptivity and resistance of this stock to the diseases.

S. Sluyter et al. (2005) have compared the values of resistance of the varieties of the subgenus of *Euvitis* to the fungus diseases with the resistance values of the species of *Vitis rotundifolia* ('Fry' variety) to the same diseases. Finally they have detected that the high resistance of the 'Fry' variety to the pathogens is conditioned by the other factors in comparison with the varieties belonging to the subgenus of *Euvitis*.

In Russia, 60 primer pairs *AFLP* have been studied for the search of the genetic diversity among the clones of Chardonnay, Cabernet Sauvignon, Merlot, Pinot group. According to the data of the researchers, only the clones of the varieties of the Pinot group (blanc, noir and gris) have showed the authentic differences from the test sample (L.P. Troshin, A. S. Zvyagin, P.P. Podvalenko, 2008).

In Australia, the scholars A.V. Kellow et al. (2002) have studied the resistance of the grape to two local races (biotypes) of phylloxera *in vitro*. As a result of the numerous experiments, the authors have come to the conclusion that the use of the *in vitro* methods for identification of the resistance of the stocks to phylloxera and identification phylloxera biotypes are disputable.

The researches of J. Pauquet et al. (2001) are devoted to the development of the local genetic maps through *AFLP*-markers on the *Run1* resistance gene in the grape and the evaluation of their practicality upon the selection. The authors point out that the monogenic dominant definitiveness in the issue on the full resistance to mildew, which the species of *Muscadinia rotundifolia* has carried in the genotype of the species of *Vitis vinifera*, has been identified and evaluated for the first time in the derivatives while crossing with *V. rotundifolia* – (BC₄ and BC₅ full-sib families). BC₅ population that covers 157 seedlings has been used for selection of the *AFLP*-markers connected with the gene of mildew resistance, (*Run1*). Thirty *AFLP*-markers have been selected and the local maps on the *Run1* resistance gene have been constructed. Ten markers from thirteen have been detected as participants of segregation with the resistance gene. The recombination as an event among the markers that were considered to be the participants in the segregation was established in one resistant seedling that shows the possibility of recombination in this sphere, but it may be noticed only in the rather big-number population.

The intensive research on creation of the resistant stems to the root nematodes has been made during

the recent years in the USA (Walker et al. 1998). To fight against the viruses spreading through the root nematodes, the stem was created in California (Davis) based on the distant hybrid of *Vitis vinifera* x *Muscadinia rotundifolia*. Unfortunately, it was potentially receptive to phylloxera and nematodes, that's why as a result of the numerous researches through the heuristic method based on the distant crossings they have created another stem, only not with the European grape, but with the species of *Vitis rupestris*, which proved to be more satisfactory for these purposes.

In this article, we would like to focus our attention on another interesting fact. In Moldova, V.S. Codreanu (2006) suggests to make the subgenus of *Muscadinia* of the genus of *Vitis* L. to rank as the genus, basing on Small's attempt to do it already in 1913, (by the way, the taxonomists have not approved it) and, certainly, on his own anatomic researches. Of course, we could not agree for a long time with V.S. Codreanu's suggestion for a certain reason. Like the varieties of the species of the subgenus of *Euvitis* as well as those belonging to the subgenus of *Muscadinia*, the pollen-grains are similar according to their form and sizes, and consequently, the both subgenera have one progenitor, but the ways of their origin are different as well as their number of somatic chromosomes (**2n=38** and **2n=40** correspondingly). However, upon making this brief review of the sources, we have got assured that V.S. Codreanu has many supporters abroad. Naturally, we cannot oppose everyone, but in this case, the international agreement must be made that will be obligatorily agreed with the main systematics of plants, especially with the specialists on the family of the grapevines, on the fact that from 2008 the subgenus of *Muscadinia* is to be considered the **genus** of the *Vitaceae* Juss Family. If nevertheless the promotion to the genus rank happens, then this position must cover the other two genera that are included in the composition of the subgenus of *Muscadinia*, i.e. on *Vitis popenoei* Fennel. and *V. munsoniana* Simps. The analogous confusion may be, unfortunately, encountered on the pages of the prestigious scientific journals on the name of the Latin name of phylloxera. Currently, its synonyms are known: *Pemphigus vitifoliae* Asa Fitch, 1854; *Phylloxera vastratrix* Planchon, 1868; *Dactylosphera vitifolii*, Shimer, 1887; *Viteus vastator* Grassi et Foa, 1912; *Viteus vitifolii* Shimer (Fitch), 1955, and many others. In our opinion, the last name is the most opportune. By the way, P. H. Kiskin (1977) also supported the

last name. The time has come to introduce a proper order in this issue of the insects systematics. Since the phylloxera is known all over the world and it was the cause of the tremendous disaster in the agriculture history: it destroyed in Europe approx. 6 mln ha of the grapevines of the species of *Vitis vinifera* L. for 20-30 years, we believe that the name *Viteus vitifolii* Shimer (Fitch) is the fullest and it objectively corresponds to the essence of the binary nomenclature by K. Linney (1735).

In the work of M. S. Kristina et al. (1998) it is indicated that 66 cultivars and varieties –stems from the Austrian collection of the germ plasma are studied genetically with the help of 10 microsatellite-loci (*VVS1*, *VVS2*, etc., and *VVD5*, *VVMD28*). All cultivars (except two Portuguese) have been analyzed by the sole allelic profile. The phenogram was based on the establishment of similarity with two pairs of alleles, both at the cultivars of *Vitis vinifera*, and at the varieties-stems. To demonstrate the distinctive possibility of the microsatellite-markers they have counted the genetic diversity (GD) as well. Thus, to map the genome of *Vitis vinifera* and several vine stems, the authors have performed the simple repeated sequencing of the genome at 66 cultivars and stems to establish the sequence of the nucleotide pairs.

The report of P. L. Mancuso et al. (1998) is dedicated to the use of the artificial neural networks (ANN) in the ampelographic researches upon solution of the number of difficult questions connected to the specification of the distinct properties of the genotypes of the grapevine with the red-colored grapes. In the authors' opinion, they succeeded to define three pairs of the synonyms and to establish the distinct genetic properties of 9 genotypes with the biggest accuracy. Through this they have confirmed the advantage of their method in the ampelographic researches in comparison with the traditional methods.

In the work of R. Blaich and H. Grundhüfer (1998), the attention is focused on the establishment of influence of SiO₂ on the increase of the mildew resistance. G.N.Ye et al. (1998) has discovered very interesting interconnections between the grape varieties, sports and clones through their DNA analysis. For this purpose, they have used the polymorph *RAPD*. From 53 primers, total 464 bands have been generalized. It turned out that about one third was common for all the genotypes tested. As a result of the racemation analysis, as it had been expected, all varieties divided in two big groups (*Vitis vinifera* L. and *Vinifera* x *Labruscana* Bailey). The

polymorphism at the genetic molecular level is not recorded among the famous clones of Chardonnay (No. 7, 78), Pinot Noir (No. 29, Geneva clone and Pernand clone), Pinot Meunier, Пино Gris, Gamay Beaujolais, by which one may differentiate them from the parents, since all of them originate from Pinot Noir.

N.M. Stavrakakis and K. Biniari (1998) discuss the issues of the analogous character. They have carried out the genetic researches to map the genome of *Vitis vinifera* L. in the grapes with the muscadine aroma. As a result of the intensification of the polymorph genes, they tried to identify the distinct properties through the full sequencing of the muscadine grapes DNA to establish the sequence of nucleotides. More than 115 fragments are generalized, and on their basis the similarity degree and the dendogram of 14 varieties has been calculated. The results have showed the genetic variability among the muscadine cultivars within 0,666 - 1.00. The thorough researches of the bands have enabled to made a genetic identification of all muscadine varieties, except two varieties, which turned out to be genetically identical.

The range of the use of the genetic researches of the grapes is very wide. Especially the work of M.S. Kristina et al. (1998) is evidence of this fact. It was performed for identification and discovery of the origin of the grapes, which serve a source not only for food, but also for raw materials for the wine-making industry. Upon the genetic analysis of the grapes and racemations, the products of its processing the *microsatellite* DNA markers, minutes of DNA extraction to the grapes and racemations (by the way, DNA from the racemations was highly degraded) have been used. As a result of the sequencing of 18 varieties used for the commercial purposes as table grapes, it was asserted that only 11 from them (61%) match the genetic profiles obtained from the database, 4 samples were identified erroneously and 3 samples did not match any of the profiles from the database. The studied samples refer to the cultivar 'Sultanina'. Therefore, these researches have showed convincingly that grapevine genetic DNA database may be successfully used upon identification of the origin of the grapes and racemations after the collection.

In the researches of E. Maletić et al. (1999) the grape varieties from Croatia have participated. They were a genetic characteristics for the purpose of the more accurate identification of the synonyms in Croatia and the neighbor regions. Twenty local varieties have been subject of the grape genome

sequencing to establish the sequence of nucleotides. **The research task** is to establish the similarity with the profiles by 9 properties – *SSR*-loci. The identity of genotypes by these marker properties detected at the cultivars of Plavin and Bradjik have confirmed the hypothesis on their belonging to one variety. As a result of the comparison of the *SSR* profiles of the species of Croatia with the profiles kept in the database (approx. 300 European varieties), three pairs of synonyms have been discovered. For example, the genotype ‘Terana Bijeli’ is close to the genotype of the Italian cultivar ‘Procesco’, ‘Muscat Rusa Porecki’ matches the cultivar ‘Rosenmuscateliu’ from the North Italy, and the cultivar ‘Moslavak’ is identical to the Hungarian cultivar ‘Furmint’. The similarity of the microsatellite markers at these varieties and their identification as synonyms have confirmed the ampelographic observations. The genetic variability inside the cultivars of Croatia is high and it is equal to the genetic diversity, which amounts to 75%. These researches are, of course, indicative of the perspective of the genetic researches upon solution of the number of the difficult ampelographic issues connected to the numerous synonyms of the grape varieties.

The issues of the use of the genetic researches, particularly, the (*EFA*) analysis and the artificial neural networks (*ANNs*) are presented in detail in the work of S. Muncuso (1999). The value of the seedlings from the self-pollination and sanguine grapevines based on the use of the ampelographic and microsatellite analysis is given in the work of I. Filippetti et al. (1999).

W.H. Wolfe has published one of the first works on the grapes genetic research in the American journal «Winemaking and Winegrowing» (1976). Then several other works have appeared sporadically, but only after the publication of the work of R. Bott et al. in 1995. The grapes genetic researches have acquired a purposeful character. The authors have performed the sequencing of 24 varieties and one clone from the Italian collection of the germ plasma through the *microsatellite*-marker.

The analysis has been carried out at the high scientific level, with the use of the primers and the GENESCAN device. The obtained results have been compared with the data of sequencing of several varieties from the Australian collection of the germ plasma. The general varieties from the collection of the germ plasma of Italy and Australia had the same genotype. It was the evidence of the appearance of the suitable, universal and reliable system for the

thorough analysis and identification of the grape varieties (Botta et al., 1995) by means of the *STS*-marker *microsatellite*.

Hence, the work of J. Bourquin et al. (1995) is very interesting. Methodologically, basing on the analysis of the DNA polymorphism, the authors have studied and described the cultivated varieties and the grapes stems. Using the *PCR-RFLP* system upon the analysis of the genera of *Vitis*, *Ampelopsis*, *Parthenocissus* of the *Vitaceae* Family, they have identified and described 17 varieties of *Vitis*, 3 forms of *Ampelopsis* and 2 forms of *Parthenocissus*. In their opinion, this technique does not take much time and through it one may receive the data obtained in the other laboratories (Bourquin et al. 1995).

The American scholar M.A. Lodchi et al. (1997) has performed the vast genetic researches for making the full characteristics by means of *RAPD*-markers by their use in the identification and description of the representatives of the genus of *Vitis*. He has set the purpose: to study the possibilities of the use of *RAPD*-markers upon description and identification of the kin populations from the genus of *Vitis* and to identify the primers that may enhance the Kosak’s sequencing as a means of obtaining of the maximal data from one DNA copy from the grape genome. The research have been carried out on 2 inter-variety hybrid populations (*Cayuga White x Aligote and Horizon x Illinois*) and the variety ‘Cabernet Sauvignon’ (*Vitis vinifera* L.). The results obtained indicate the perspective of the research method upon identification and description of the grape varieties (Lodhi, Weeden, Reisch, 1997).

O. Silvestroni et al. (1997) has reported of the discovery of the genetic diversity among the clones of the cultivar of Fortrana (*Vitis vinifera* L.) by means of the DNA *microsatellite*-analysis. The allelic polymorphism in 6 *microsatellite*-loci is analyzed at 5 clones of the Fortana variety that showed that by 5 loci they are polymorph, and by one locus (*VVMD7*) they had the identical DNA profiles (Silvestroni et al., 1997). K.A. Loulakakis’s work (1997) gives a detailed presentation on the genome organization and expressiveness of the grape gene *pVVOSMI*.

M. N. Stavrakakis et al. (1997) has reported of the identification and description of 8 grape varieties of *Vitis vinifera* L. by means of the DNA polymorph markers. Fifteen primers have been used to enhance the genome DNA through the polymerase reaction (*PCR-RAPD*) for identification and description of 8 grape varieties that grow on the Crete (Stavrakakis, Binari, Hatzopoulos, 1997). M.

Kristina et al. (1997) gives a thorough report on the use of the *microsatellite-markers* upon the study of the parent forms of the grapes. The use of the genetic *microsatellite-analysis* is possible not only upon the differentiation of the varieties, but also upon their identification. Many economically valuable grape varieties, for example, 'Cabernet Sauvignon' occupy the vast areas of the industrial plantations, but their origin is unknown. The Austrian scholars have performed the genetic analysis of 51 cultivars by 24 *microsatellite-loci*. The results received have confirmed the origin of the variety of 'Cabernet Sauvignon': it was obtained from the crossing of the varieties 'Cabernet franc' and 'Sauvignon blanc' (Kristina et al., 1997).

The genetic analysis by the polymorph *RAPD-PCR* markers has been used in the research of the grapes stems, such as 5BB and SO4 (Bauer, Zyprian, 1997).

G. Yamamoto et al. (2006) has reported on the development and the use of the microsatellite-markers and the microsatellite-analysis, upon the study of the Eastern grape varieties. They have given a full characteristics of 9 new *microsatellite-markers* for the grapes and described 7 Western and 8 Eastern cultivars, including the Japanese and Chinese varieties of *Vitis vinifera*, two varieties of the genus of *Vitis labrusca* and per one variety of *Vitis riparia* and *Vitis rotundifolia*. According to the authors' conclusion, the micro-satellite analysis method has exactly showed the differences between the Western and Eastern grape varieties by the genotype. The results have given the Japanese scholars the idea that the Eastern varieties present a valuable genetic source (Goto-Yamamoto et al. 2006). The Chinese researchers have successfully used the *RAPD*-markers upon the description of the wild grapevines in China (Luo, He, 2001).

The genetic diversity of some valuable grape genotypes by means of the *RAPD* -markers has been evaluated in India. The researchers have obtained 250 bands, using 19 informative primers that have exactly showed the differences between the cultivated and wild genotypes. This is the first attempt of the Indian scholars to identify the genetic interconnections between the economically important grape genotypes by means of the molecular markers (Shubhada, Patil, Rao, 2001).

To learn the origin and the genetic affinities of the variety of 'Ansonica' cultivated on the Giglio (Italy), the Italian scholars M.Labra et al.(1999) have performed the phylogenetic research by means of the *AFLP*- and *SSR*-molecular markers,

i.e. they have used the *microsatellite-analysis*. To identify the similarity degree, the DNA polymorph bands presented as dendrograms have been studied on the variety 'Ansonica', 23 cultivars from the various regions of the Mediterranean Sea and 40 Greek varieties. According to the research results, 'Ansonica' from the Giglio and Tuscany is identical as well as the species 'Inzolia' from Sicily, 'Airen' from Spain, 'Clairette' from France and 'Roditis' from Greece are identical. The analysis based on the *SSR*-markers has showed that only the species 'Siderites' and 'Roditis' from the studied forty Greek species are genetically very similar to the species of 'Anasonica'. Based on this, M.Labra et al. (1999) have made the conclusion of the Greek origin of the variety 'Anasonica'.

F.Regner, E. Wiedeck, A. Stadlbauer (2000) have reported on the differentiation and identification of the clones of the variety of *Riesling blanc* by means of the genetic markers. For the genetic research of 10 clones of *Riesling blanc* the marker systems *RAPD*, *SSR* and *Inter-SSR* have been used. The DNA polymorph fragments have confirmed the genetic variability in the framework of the clones studied. As a consequence, one may perform the research by the genetic markers for the purpose of more precise determination of the origin and identification of the grape clones.

The genetic characteristics of variability of 35 table grape varieties based on the analysis by means of the *AFLP*-markers is given in the work of M.T. Cervera et al. (2000). The genetic similarity between the various table grape varieties varied within 0,65-0,90. In the authors' opinion, the analysis through the genetic markers of the *AFLP* system is quite useful upon identification of the various grape clones.

The work of the Italian researchers G. Fanizza et al. (2000) is devoted to the analysis of the genetic interconnections between the muscadine grape varieties by means of the *RAPD*- markers. They have determined the interconnection of all muscadine varieties by the similarity coefficient among the genotypes, except for 'Moscato Reale' и 'Moscato Canelli'.

J. Ibócez et al. (2000) reports of the allelic variation by one locus between two synonyms – 'Black Currant' and 'Mavri Corinthiaki'. These two synonyms are generally considered to be the synonyms of the variety 'Black Currant'. No differences have been noticed between the variety 'Black Current' and its synonyms at the molecular level. They are similar between themselves by 15 and 16 loci. Therefore, the variety 'Black Currant'

and its synonyms at the molecular level have proved to be identical. The difference was established only by locus (*VVMD7*). The consequences of such grape mutations are discussed.

A.F.Adam-Bondon et al. (2001) reports on the practicality of the use of two genetic markers of the *SCAP* system upon the grape selection for the purpose of raising of the seedless varieties. The preliminary researches of the practicality of these genetic markers connected to their participation in the selection of the grape seedless varieties showed that unlike the gene *SCP18* the gene *SCP8* has proved to be quite useful, at least, at the progeny acquired upon crossing of Seedless x Seedless (Adam-Blondon, Lanogue-Esnault, Bouquet, Boursiquot, This, (2001).

The genetic research of the industrial and table grape varieties in the province of Apulia (South Italy) by means of the microsatellite-markers has been carried out by the Italian scholars Zulini, Russo, Peterlunger (2002). They studied the grape varieties typical of Italy by 6 loci for the purpose of the synonyms discovery, and to confirm the origin of several varieties. All studied varieties have

been genetically described in detail, except for two varieties Regina (syn.Afuz Ali) and Mennavacca. The same genetic profile was discovered in them. Besides this information, the authors succeeded also to explore very important information on the origin of two varieties. For the first time, based on the genetic research with the microsatellite-markers, the parental pairs of the variety of Victoria (Cardinal x Afuz Ali) and the variety of Matilde (Italia x Cardinal) were confirmed.

So, *the 21st century is a century of the grape genetic research!* We are happy that the scholars from many countries of the world have altogether started mapping the grape genome (as it had been expected the internationalism of the scholars is very high). In any case, the geography of the scholars taking part in this process is quite vast, it covers the countries of the both hemispheres. There is still a lot to do for the full mapping of the grape genome, but the start has already been marked.

In conclusion it is necessary to note that the genetic research is to enlighten many unanswered questions of the winemaking. Among them we may enumerate: the rich synonymy of the grape

varieties, identification of the scion-rooted and grafted cultivars, a complex origin of the cultured grape and, finally, selection of the new varieties, which must approach in future maximally the ideal variety by for the economically valuable properties and by the biological properties. If the traditional genome aroused such great interest for the researchers, **the new grape genome** that we have made from two species of *Vitis vinifera* and *V.rotundifolia* will be much more interesting. We will only wait and hope that the corresponding researches will start in the countries of the CIS, Europe or in America in the near future. Its **mapping** opens great perspectives for the further economic prosperity of all winemaking countries. That's why we consider that these tasks are of priority in the work of each geneticist and selectionist of the 21st -century grape.



Iurie Matei. *Furnicile care au mâncat creierii unui matematician.*
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