

# MICROSATELLITE MARKER ANALYSIS OF MACEDONIAN GRAPEVINES (*VITIS VINIFERA* L.) COMPARED TO BULGARIAN AND GREEK CULTIVARS

Natasa Stajner<sup>1</sup>, Elizabeta Angelova<sup>2</sup>, Zvonimir Bozinovic<sup>2</sup>,  
Mihail Petkov<sup>2</sup> and Branka Javornik<sup>1</sup>

1: University of Ljubljana, Biotechnical Faculty, Centre for Plant Biotechnology  
and Breeding, Jamnikarjeva 101, 1000 Ljubljana, Slovenia

2: University Saints Cyril and Methodius, Faculty of Agricultural Sciences and Food,  
Department for Wine-growing and Wine-producing, Aleksandar Makedonski  
BB P.O. Box 297, 1000, Skopje, Macedonia

## Abstract

**Aims:** Eleven Macedonian grapevine accessions were genotyped by microsatellite profiling at 9 microsatellite loci, in order to identify Macedonian cultivars and to evaluate the relationships among them. The comparison with grapevine cultivars from two neighbouring countries was also performed.

**Methods and results:** Clustering analyses based on the proportion of shared alleles resulted in two clusters containing all accessions except cultivar « Vranec », which was distant from the others. Comparison of genotyping results of Macedonian accessions with 76 Bulgarian and 298 Greek accessions revealed no identical genotypes. In the dendrogram, Macedonian accessions are dispersed among Greek and Bulgarian grapevines, suggesting a common genetic background. Additionally, the synonyms « Smederevka » = « Dimyat » = « Zoumiatiko » and « Belo Zimsko » = « Karatsova Naousis » were also evaluated.

**Conclusions:** Clustering analyses showed that authentic Macedonian cultivars are distant from two widespread cultivars « Vranec » and « Smederevka ». Comparison of Macedonian cultivars with their synonyms from Greece and Bulgaria revealed differences in allelic profiles at some loci, but further analyses are needed to confirm their unique allelic profiles.

**Significance and impact of study:** This work is a first step towards the genetic characterization of Macedonian grapevine germplasm, thus contributing to the molecular investigation of grapevine germplasm within the Balkan region.

**Keywords:** *Vitis vinifera*, Macedonian grapevine, genotyping, microsatellite, synonymy

## Résumé

**Objectif :** Les auteurs ont caractérisé neuf loci de microsatellites, chez onze accessions de vigne de Macédoine afin d'identifier les cépages macédoniens et d'évaluer les relations entre eux. La comparaison avec les cépages de vigne de deux pays voisins a également été effectuée.

**Méthodes et résultats :** Des analyses de groupement basées sur la proportion d'allèles partagés ont résulté en deux groupes contenant toutes les accessions, sauf pour le cépage « Vranec », qui était éloigné des autres. La comparaison entre les accessions de Macédoine avec les 76 accessions bulgares et les 298 accessions grecques, n'a pas révélé de génotypes identiques. Dans le dendrogramme, les accessions de Macédoine sont dispersées entre les vignes de Grèce et de Bulgarie, ce qui suggère un fond génétique commun. En outre, les synonymes « Smederevka » = « Dimyat » = « Zamiatiko » et « Belo Zimsko » = « Karatsova Naousis » ont été évalués.

**Conclusions :** Les analyses de groupement ont montré que les cépages macédoniens authentiques sont éloignés des deux cépages répandus « Vranec » et « Smederevka ». La comparaison entre les cépages macédoniens avec leurs synonymes de Grèce et de Bulgarie ont révélé des différences dans les profils alléliques à plusieurs locus, mais d'autres analyses sont nécessaires pour confirmer leur profil allélique unique.

**Importance et impact de l'étude :** Ce travail est une première étape vers la caractérisation génétique du germoplasme de la vigne de Macédoine contribuant ainsi à l'étude moléculaire du germoplasme de la vigne dans la région des Balkans.

**Keywords:** *Vitis vinifera*, vigne macédonienne, génotype, microsatellite, synonyme

manuscript received the 15th of May 2008- revised manuscript received the 10th of February 2009

## INTRODUCTION

Grapevine has been grown in Macedonia since ancient times. Today, two-thirds of Macedonian grapevines are cultivated for wine production, with 38 % of these used for red wines and 62 % for white wines. The total annual grape production in Macedonia averages 240,000 tons on an area of over 22,400 ha. The widespread cultivars « Smederevka » and « Vranec » comprise 80 % of the total grape production. « Smederevka » is the leading cultivar for the production of white wines and « Vranec » is the leader for production of red wines (Efremov *et al.*, 2001). About 20 different cultivars are cultivated in Macedonia today, including some local ones but only to a minor extent. There has been a general trend of a falling number of local grapevine cultivars in viticulture in recent years, which may reduce the genetic resources available in the future (Martínez de Toda and Sancha, 1997), so old autochthonous grapevine cultivars should be preserved before they completely disappear from vineyards.

Grapevine cultivars have traditionally been characterized by standard ampelographic descriptions, e.g., comparison of the morphological characters of leaves, shoot tips, fruit clusters and berries (IPGRI, UPOV, OIV, 1997). Mistakes in cultivar characterization and identification can occur with the use of this approach, since the expression of morphological character is influenced by environmental factors. Some genetically related cultivars are morphologically very similar and difficult to differentiate by visual comparison (Aradhya *et al.*, 2003). Equally, intravarietal clones can differ considerably in phenotype even though they have identical DNA profiles (Vignani *et al.*, 1996; Franks *et al.*, 2002). As a result, some cultivars now have up to 100 synonyms, and numerous homonyms exist (This *et al.*, 2004). The introduction of molecular markers has allowed more

accurate identification, since the results are independent of environmental factors. Of the various molecular markers applied in grapevine DNA analysis, microsatellites have been mostly used (Sefc *et al.*, 2001), although their development is expensive and time consuming. A combination of SSR (short sequence repeat) and AFLP (amplified fragment length polymorphisms) markers has been recommended for the assessment of clonal variability (Labra *et al.*, 2001) although there are reports of the analysis of clonal variability using only microsatellites (Franks *et al.*, 2002; Riaz *et al.*, 2002; Kozjak *et al.*, 2003). Microsatellites are easy to score, thus allowing accurate analysis of many loci in multiple individuals (Stajner *et al.*, 2005) and enabling accurate comparison of genotyping results between different laboratories when reference varieties are included.

We report in this paper on the characterization of 11 Macedonian grapevine accessions using nine microsatellite markers. The resultant microsatellite profiles were further compared to grapevine genotypes of two neighbouring countries, Greece and Bulgaria, in order to evaluate the relationships among cultivars and discover possible synonyms or homonyms.

## MATERIAL AND METHODS

### 1. Plant material

Plant material consisting of 11 Macedonian grapevine accessions (table 1) was gathered from vineyards in the Povardarie - Vardar Valley (Central Region) region, the largest wine growing area in Macedonia, where 85 % of Macedonian wine is produced. Among 20 cultivars cultivated today in Macedonia we chose only local ones for DNA analysis. Two cultivars, « Chardonnay » and « Malvazia Istriana », were included in the genotyping analysis as reference cultivars for the standardization of

**Table 1 - Macedonian grapevine genotypes at nine SSR loci represented by allele sizes in base pairs (bp).**

Cultivar / Loci	VVS2	ssrVrZAG21	ssrVrZAG47	ssrVrZAG62	ssrVrZAG64	ssrVrZAG79	ssrVrZAG83	ssrVvUch11	ssrVvUch29
'Belo Zimsko'	134:142	202:214	159:163	185:199	159:159	252:260	190:194	242:242	209:297
'Crven Drenok'	138:142	202:214	159:163	185:187	139:159	252:260	188:194	242:242	209:213
'Vranec'	132:132	204:206	159:159	193:199	159:163	260:260	190:194	242:242	209:209
'Koncanka'	134:142	190:202	161:165	185:193	139:143	244:252	188:188	242:246	213:297
'Smederevka'	142:142	200:202	157:159	187:203	143:159	238:260	188:194	244:248	209:213
'Kratosija standard'	132:142	200:206	157:159	199:203	159:163	238:260	188:194	242:248	211:211
'Kratosija straggly'	132:142	200:206	157:159	199:203	159:163	238:260	188:194	242:248	211:211
'Kratosija fruitless'	132:142	200:206	157:159	199:203	159:163	238:260	188:194	242:248	211:211
'Stanusina standard'	134:142	202:206	159:167	193:203	137:159	244:266	194:194	246:262	209:309
'Stanusina straggly'	134:142	202:206	159:167	193:203	137:159	244:266	194:194	246:262	209:309
'Stanusina green'	134:142	202:206	159:167	193:203	137:159	244:266	194:194	246:262	209:309
'Chardonnay'	136:142	200:206	159:167	187:195	159:163	242:244	188:200	248:262	289:297
'Malvazia Istriana'	142:142	194:202	157:157	187:187	159:163	236:254	188:194	242:248	215:297

genotyping results and comparison with Greek and Bulgarian data.

## 2. DNA isolation

DNA was extracted from young leaves of individual vines using the modified CTAB method described by Kump and Javornik (1996).

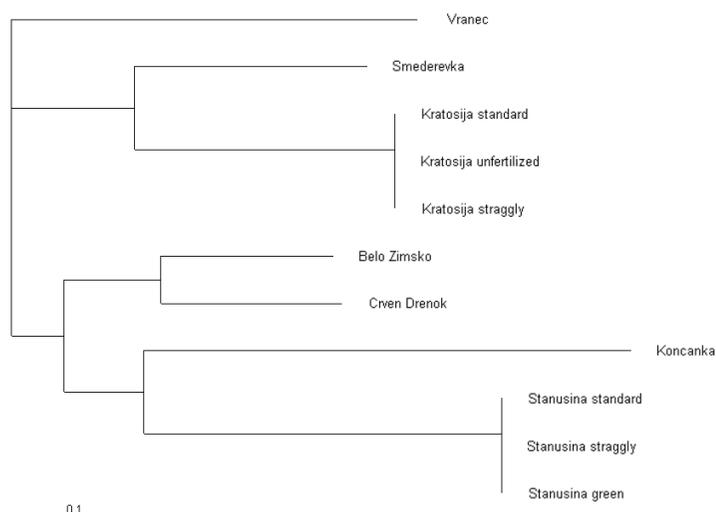
## 3. Microsatellite analysis

Nine microsatellite loci were chosen for the identification of Macedonian grapevines: VVS2 (Thomas and Scott, 1993), *ssrVrZAG21*, *ssrVrZAG47*, *ssrVrZAG62*, *ssrVrZAG64*, *ssrVrZAG79*, *ssrVrZAG83* (Sefc *et al.*, 1999), *ssrVvUch11* and *ssrVvUch29* (Lefort *et al.*, 2001). The choice of these loci made the results comparable to genotyping results of Greek and Bulgarian grapevines.

The PCR reaction mixture in a total volume of 10  $\mu$ L contained 20 ng of genomic DNA, 1 x PCR buffer (Fermentas), 0.2 mM of each dNTP's (Sigma), 2 mM MgCl<sub>2</sub> (Fermentas), 0.5  $\mu$ M of each primer and 0.25 U of Taq DNA polymerase (Fermentas). One of the primers for each loci was labelled with fluorescent Cy-5 dye for fluorescent detection (IDT Inc., Bioscience). The amplification of microsatellite loci was performed in a Whatman Biometra T-Gradient thermocycler with the following steps: hot start for 5 minutes at 95  $^{\circ}$ C; 26-40 cycles of denaturation at 94  $^{\circ}$ C for 30-45 s, annealing at 50-58  $^{\circ}$ C for 30-45 s and an extension step at 72  $^{\circ}$ C for 90 s. Amplification of loci UCH11 and UCH29 was carried out using a tailing protocol (Schuelke, 2000.) with three different primers: 0.2  $\mu$ M of each unlabelled UCH primer and 0.075  $\mu$ M of 18 bp M13 tail sequence attached to the forward primer for subsequent fluorescent labelling (5'-3': TGTAACGACGGCCAGT). An optimized touchdown protocol was used for the PCR of these two loci: initial denaturation of 94  $^{\circ}$ C for 5 min; 5 cycles of 94  $^{\circ}$ C for 45 s, 60  $^{\circ}$ C for 30 s with a decrease of -1  $^{\circ}$ C per cycle and 72  $^{\circ}$ C for 1.5 min; followed by 25 cycles at the annealing temperature of 55  $^{\circ}$ C. Reactions were completed by incubating at 72 $^{\circ}$ C for 8 min.

After PCR optimization and amplification of individual loci, the amplification products were separated on 6 % polyacrylamide, 7M urea and 1 x TBE gels, running in 0.5 x TBE buffer on an ALFexpress DNA automated sequencer (GE Healthcare). The allele sizes were analyzed with AlleleLocator 1.03 software (GE Healthcare). Alleles were precisely sized against an ALFexpress sizer 50-500 (GE Healthcare) and by internal DNA standards of different sizes amplified from plasmid.

In order to obtain data of neighbouring genotypes, the genetic profiles of Greek grapevine cultivars were retrieved from the Greek Vitis Database (<http://gvd.biology.uoc.gr/>



**Figure 1 - A neighbour-joining dendrogram of Macedonian grapevine accessions based on the proportion of shared allele distances.**

[cgi-bin/webdata\\_nSSRdata.pl](http://cgi-bin/webdata_nSSRdata.pl)) (Lefort and Roubelakis-Angelakis, 2000) and of Bulgarian grapevines from the Bulgarian Grape nSSR Database ([http://bulgenom.abi.bg/cgi-bin/webdata\\_Grape%20nSSR.pl](http://bulgenom.abi.bg/cgi-bin/webdata_Grape%20nSSR.pl)).

Statistical analysis: expected heterozygosity ( $H_e$ ), observed heterozygosity ( $H_o$ ) and probability of identity (PI) were calculated using Identity 1.0 software (Wagner and Sefc, 1999). The expected heterozygosity was calculated according to Nei (1978):

$$H_e = 1 - \sum_{i=1}^l p_i^2$$

where  $p_i$  is the frequency of allele  $i$ .

Probability of identity (PI) was obtained using the formula according to Paetkau *et al.* (1995):

$$PI = \sum_{i=1}^n p_i^4 - \sum_{i=1}^{n-1} p_i^2$$

where  $p_i$  and  $p_j$  are the frequencies of the  $i$ -th and  $j$ -th alleles, respectively.

A genetic distance matrix based on the proportion of shared alleles was calculated by the program Microsat (Minch, 1997) and was applied to draw a dendrogram by using the Neighbor program from the Phylip ver. 3.6b software package (Felsenstein, 1993) and Treeview (Page, 1996) to visualize the obtained dendrogram.

## RESULTS AND DISCUSSION

The information content of nine microsatellite markers included in the present study was assessed as the

probability of identical genotypes (PI) and number of alleles amplified. The values of PI for each locus and overall are shown in table 2. The markers were not equally informative, because in some cases the information content of a locus was decreased by the prevalence of one or two alleles. This is the case, for example, for locus *ssrVrZAG83*, where one allele occurred at a frequency of over 50 %, increasing the PI value to 0.445. The cumulative probability of detecting identical genotypes from the analysed cultivars was in the order of 10<sup>-6</sup>. The results show that the level of observed heterozygosity is generally higher than expected (table 2), which may be due to natural and human selection against homozygosity in grapevine plants. Grapevines are known to be very sensitive to inbreeding depression so heterozygous individuals are preferred (Sefc *et al.*, 2000). The average similarity of all accessions is 38 % of shared alleles, which is close to the average similarity observed for mid-European cultivars (40 %, Sefc *et al.*, 1998).

Microsatellite analysis of 11 Macedonian grapevine accessions is presented in table 1. The allele sizes obtained are shown in base pairs. A neighbour-joining tree was constructed based on the allele sharing distances between the cultivars (figure 1). Overall, two clusters were obtained, containing all accessions except « Vranec », which was distant from the others. The cultivar « Vranec » and cultivars « Kratosija » and « Smederevka » are non-authentic Macedonian grapevines belonging to the ecological and geographic group Pontica, subgroup Balcanica. Two of them, « Vranec » and « Kratosija », originate from Montenegro and « Smederevka » is thought to originate from Serbia. They are shown to be separated from the group of autochthonous Macedonian cultivars which are represented by « Koncanka », « Belo Zimsko »,

« Crven Drenok » and accessions of « Stanusina ». Accessions of « Stanusina » have been raised in the Tikvesh viticultural region from ancient times. The place of origin of « Koncanka » is uncertain but it is thought to be an authentic cultivar from the village of Konche near the city of Gevgelija. In the Gevgelija wine region, 90 % of vineyards were planted with this cultivar before the appearance of phylloxera but nowadays the cultivation of « Koncanka » is greatly reduced. The cultivars « Belo Zimsko » and « Crven Drenok », which clustered together in the dendrogram, share the same synonym « Valandovo Drenok ». They both belong to the ecological and geographic group of Eastern varieties, subgroup Antaziatica and are known to be genetically related cultivars (Bozinovic, 2005).

Three types each of Kratosija and Stanusina, distinctive in morphological characters, were included in our analysis. These were « Kratosija standard », « Kratosija straggly » (the berries are so widely spaced on the main and lateral stems that the bunch is distinctly open), « Kratosija unfertilized » (with unfecundated berries, seedless), « Stanusina standard », « Stanusina straggly » and « Stanusina green » (with uncoloured, green berries). The standard types have the highest yield and the most positive productive properties. The straggly types give the lowest and very inconsistent yield, but the highest quality of vine (Bozinovic *et al.*, 2003). So far, our genotyping results showed no allelic differences among the types of Kratosija or Stanusina. The lack of difference among them at the molecular level might be due to the small fraction of the genome analysed. Point mutations affecting flower, berry or bunch characteristics may not therefore have been found. Such closely related genotypes probably require molecular analysis with a

**Table 2 - Number of alleles, observed heterozygosity (Ho), expected heterozygosity (He) and probability of identity (PI) of 9 SSR loci studied in 11 Macedonian *V. vinifera* accessions.**

Loci	No. of alleles	Ho	He	PI
VVS2	4	0.818	0.645	0.302
<i>ssrVrZAG21</i>	6	1 000	0.752	0.181
<i>ssrVrZAG47</i>	6	0.909	0.686	0.202
<i>ssrVrZAG62</i>	5	1 000	0.769	0.166
<i>ssrVrZAG64</i>	5	0.909	0.682	0.213
<i>ssrVrZAG79</i>	5	0.909	0.764	0.161
<i>ssrVrZAG83</i>	3	0.636	0.541	0.445
<i>ssrVvUch11</i>	5	0.727	0.707	0.209
<i>ssrVvUch29</i>	5	0.636	0.748	0.183
Average	4 889	0.838	0.699	*1,065x10 <sup>-6</sup>

\* Product of probability of identity (PI) calculated over all loc.

different marker system. However, there are reports available (Franks *et al.*, 2002; Riaz *et al.*, 2002; Hocquigny *et al.*, 2004) where clonal polymorphisms of grapevine cultivars were revealed using only microsatellite markers. They have identified periclinal chimerism as the main source of clonal differences, which resulted in triallelic profile at a heterozygous locus of some individuals. This phenomenon in long-lived clonally propagated crops, such as grapevine, may affect cultivar identification and pedigree analysis (Franks *et al.*, 2002), but many microsatellite loci need to be analysed to discover this kind of polymorphism. For instance, intravarietal polymorphism was observed at 5 loci out of 50 in the case of *Vitis vinifera* « Pinot gris » (Hocquigny *et al.*, 2004) and at 17 loci out of 100 in the case of « Pinot noir » and « Chardonnay » clones (Riaz *et al.*, 2002).

Macedonian accessions were further compared to Bulgarian and Greek cultivars, since these two countries border on Macedonia and are likely to share some common grapevine cultivars. Serbia is another bordering country, but does not as far as we know keep microsatellite genotyping data of their grapevines. The genetic characterization of Albanian grapevine cultivars by microsatellites has been already published (Ladoukakis, *et al.*, 2005) but we were unable to perform comparison since their data do not include any standard cultivars. We decided to do genotyping comparison with available data of bordering countries. Two reference cultivars, « Malvazia Istriana » and « Chardonnay », were used for the standardization of data. The « Chardonnay » accession in the Greek *Vitis* Database is completely different from true-to-type « Chardonnay », so « Malvazia Istriana » was used as standard. Comparison of the reference alleles between databases revealed locus specific deviations of allele lengths. Our reference alleles differed from the compared data by 1 to 4 bp, while the allelic profiles of reference varieties were identical for all loci. This shifting effect has also been found in some previous SSR genotyping approaches (Sefc *et al.*, 1998) indicating the need to standardise results when they have to be compared. Comparison of 11 Macedonian accessions with 76 Bulgarian and 298 Greek accessions at 9 microsatellite loci showed no similarity of the Macedonian cultivars with those from neighbouring countries, indicating their authenticity. On the other hand, Macedonian accessions did not cluster together in the dendrograms but were dispersed among Greek and Bulgarian grapevines (data available on request), which indicate their common genetic background. In addition, we compared the allelic profiles of « Smederevka », a leading white cultivar in Macedonia (Bozinovic, 2005), with two of its hypothetical synonyms « Dimyat » and « Zoumiatiko », cultivated in Bulgaria and Greece. Since only one reference variety was available in our comparison of synonyms, we compared only allelic profiles (homozygosity and heterozygosity) but not allele

sizes. Differences among these three hypothetical synonyms thus found at two loci. At locus VVS2 two allele sizes were reported (140:142 bp) for cultivar « Dimyat », while « Smederevka » and « Zoumiatiko » had only one allele present (142 bp). At locus *ssrVvUch11* one allele size (242 bp) was reported for « Zoumiatiko », while « Smederevka » and « Dimyat » revealed two allele sizes (246:250 bp). Comparison of another two synonymic cultivars Macedonian « Belo Zimsko » and Greek « Karatsova Naousis » showed a difference at one locus (*ssrVvUch11*), where one allele (242 bp) was obtained for « Belo Zimsko » and two alleles (242:244 bp) for « Karatsova Naousis ».

## CONCLUSIONS

Analysis of Macedonian grapevines showed that there are four authentic Macedonian cultivars (« Belo Zimsko », « Crven Drenok », Koncanka » and « Stanusina »), which are genetically distant from the widespread cultivars « Vranec » and « Smederevka ». Comparison of Macedonian cultivars with their synonyms from Greece and Bulgaria revealed differences at some loci. In the future more reference cultivars are planned to be included in the analysis which will enable accurate comparison of allele sizes between labs. However, this work is a first step towards the genetic characterization of Macedonian grapevine germplasm, thus contributing to the molecular investigation of grapevine germplasm within the Balkan region.

## REFERENCES

- ARADHYA M. K., DANGL G. S., PRINS B. H., BOURSICQUOT J. M., WALKER A. M., MEREDITH C. P. and SIMON C. J., 2003. Genetic structure and differentiation in cultivated grape *Vitis vinifera* L. *Genet. Res.*, **81**, 179-192.
- BOZINOVIC Z., 2005. *Ampelografija. Code des caractères descriptive des variétés et espèces de Vitis*, O.I.V. (1984), Paris. Ed. Academic, Skopje.
- BOZINOVIC Z., BELESKI K. and BOSKOV K., 2003. The Influence of Morphological Characteristics on Productive Properties of *Cultivar Stanusina*. *Acta Hort.* (ISHS), **603**, 609-612.
- EFREMOV G., GALEV T., MURACALIEV A., ANAKIEV B., ANGELOV I., ANCEV E., BOZINOVIC Z., JAKIMOVSKI J., POPOVSKI H. and DZABIRSKI V., 2001. *Strategy for development of Agriculture in the Republic of Macedonia to 2005*. Macedonian Academy of sciences and Arts. Skopje.
- FELSENSTEIN J., 1993. *Phylogeny Inference Package (PHYLIP)*. Version 3.5. University of Washington, Seattle.
- FRANKS T., BOTTA R., THOMAS M. R. and FRANKS J., 2002. Chimerism in grapevines: implication for cultivar identity, ancestry and genetic improvement. *Theor. Appl. Genet.*, **104**, 192-199.

- HOCQUIGNY S., MERDINOGLU D., HELOIR M. C. and PELS F., 2003. Chimerism and genetic diversity within the cultivar group of pinots. *Acta Hort.*, **603**, 535-544.
- IPGRI, UPOV, OIV, 1997. *Descripteurs de la vigne (Vitis spp.)*. Union internationale pour la protection des obtentions végétales. Genève, Suisse/Office international de la Vigne et du Vin, Paris, France/Institut international des ressources phytogénétiques, Rome.
- KOZJAK P., KOROSK-KORUZA Z. and JAVORNIK B., 2003. Characterisation of cv. Refosk (*Vitis vinifera* L.) by SSR markers. *Vitis*, **42**, 83-86.
- KUMP B. and JAVORNIK B., 1996. Evaluation of genetic variability among common buckwheat (*Fagopyrum esculentum* Moench) populations by RAPD markers. *Plant Sci.*, **114**, 149-158.
- LABRA M., WINFIELD M., GHIANI A., GRASSI F., SALA F., SCIENZA A. and FAILLA O., 2001. Genetic studies on Trebbiano and morphologically related varieties by SSR and AFLP markers. *Vitis*, **40**, 187-190.
- LADOUKAKIS E.D. F. LEFORT P. SOTIRI A. BACU E. KONGJIKI and ROUBELAKIS-ANGELAKIS K. A., 2005. Genetic characterization of Albanian grapevine cultivars by microsatellites. *J. Int. Sci. Vigne Vin*, **39**, 109-119.
- LEFORT F. and ROUBELAKIS-ANGELAKIS K. A., 2000. The Greek *Vitis* Database: A Multimedia Web-backed Genetic Database for Germplasm Management of *Vitis* Resources in Greece. *J. Wine Res.*, **11**, 233 - 242.
- LEFORT F., KYVELOU C. J., ZERVOU M., EDWARDS K. J. and ROUBELAKIS-ANGELAKIS K.A., 2001. Characterisation of new microsatellite loci from *Vitis vinifera* and their conservation in some *Vitis* species and hybrids. *Mol. Ecol. Notes*, **2**, 20-21.
- MARTÍNEZ DE TODA F. and SANCHA J. C.; 1997. Ampelographical characterization of red *Vitis vinifera* L. cultivars preserved in Rioja. *Bull. OIV*, **70**, 220-234.
- MINCHE., 1997. MICROSAT, Version 1.5b. Stanford University Medical Center, Stanford.
- NEI M., 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*, **3**, 583-590.
- PAETKAU D., CALVERT W., STIRLING I. and STROBECK C., 1995. Microsatellite analysis of population structure in Canadian polar bears. *Mol. Ecol.*, **4**, 347-354.
- PAGE R. D. M., 1996. TreeView - an application to display phylogenetic trees on personal computers. *Comput. Appl. Biosci.*, **12**, 357-358.
- RIAZ S., GARRISON K. E., DANGL G. S., BOURSQUOT J. M., MEREDITH C. P., 2002. Genetic divergence and chimerism within ancient asexually propagated winegrape cultivars. *J. Amer. Soc. Hort. Sci.*, **127**, 508 - 514.
- SCHUELKE M., 2000. An economic method for the fluorescent labelling of PCR fragments. *Nat. Biotechnol.*, **18**, 233-234.
- SEFC K. M., LEFORT F., GRANDO M. S., SCOTT K., STEINKELLNER H. and THOMAS M. R., 2001. Microsatellite markers for grapevine: a state of the art, 433-463. In: *Molecular biology and biotechnology of grapevine*. Ed. K. A. Roubelakis-Angelakis, Kluwer, Amsterdam 500 p.
- SEFC K. M., LOPES M. S., LEFORT F., BOTTA R., ROUBELAKIS-ANGELAKIS K. A., IBANEZ J., PEJIC I., WAGNER H. W., GLOSSL J. and STEINKELLNER H., 2000. Microsatellite variability in grapevine cultivars from different European regions and evaluation of assignment testing to assess the geographic origin of cultivars. *Theor. Appl. Genet.*, **100**, 498-505.
- SEFC K. M., REGNER F., TURETSCHKE E., GLÖSSL J. and STEINKELLNER H., 1999. Identification of microsatellite sequences in *Vitis riparia* and their applicability for genotyping of different *Vitis* species. *Genome*, **42**, 367-373.
- SEFC K.M., REGNER F., GLOSSL J. and STEINKELLNER H., 1998. Genotyping of grapevine and rootstock using microsatellite markers. *Vitis*, **37**, 15-20.
- STAJNER N., JAKSE J., KOZJAK P. and JAVORNIK B., 2005. The isolation and characterisation of microsatellites in hop (*Humulus lupulus* L.). *Plant Science*, **168**, 213-221.
- THIS P., JUNG A., BOCCACCIP., BORREGO J., BOTTA R., COSTANTINI L., CRESPIAN M., DANGL G. S., EISENHELD C., FERREIRA-MONTEIRO F., GRANDO S., IBA\_EZ J., LACOMBE T., LAUCOU V., MAGALH\_ES R., MEREDITH C. P., MILANI N., PETERLUNGER E., REGNER F., ZULINI L. and MAUL E., 2004. Development of a standard set of microsatellite reference alleles for identification of grape cultivars. *Theor. Appl. Genet.*, **109**, 1448-1458.
- THOMAS M. R. and SCOTT N. S., 1993. Microsatellite repeats in grapevine reveal DNA polymorphisms when analysed as sequence-tagged sites (STSs). *Theor. Appl. Genet.*, **86**, 985-990.
- VIGNANI R., BOWERS J. E. and MEREDITH C. P., 1996. Microsatellite DNA polymorphism analysis of clones of *Vitis vinifera* « Sangiovese ». *Sci. Hortic.*, **65**, 163-169.
- WAGNER H. W. and SEFC K. M., 1999. IDENTITY 1.0. *Centre for Applied Genetics*, University of Agricultural Sciences, Vienna 5 p.