Characterization of grapevine (*Vitis vinifera* L.) cultivars from northern Portugal using RAPD and microsatellite markers

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**Summary**

Twelve grapevine cultivars from northern Portugal were characterized with RAPD and microsatellites. Nine primers were used in the RAPD analysis; 8 of the varieties showed monotypic patterns. With the 6 microsatellite loci a total of 38 alleles was obtained. Relationships among the studied varieties were observed. Both types of molecular markers have proved useful for identification. Existence of synonymies is discussed.

**Key words:** grapevine, identification, molecular markers, STMS, synonymies.

**Introduction**

Ampelographic characters are very often not sufficient to identify a given variety. The use of alternative methods based on DNA markers has proven a valid tool for characterization and detection of synonymies among grapevines (Bowers et al. 1993; Stanvakakis et al. 1997). Among these molecular markers, RAPDs (Qu et al. 1996; Ulanovsky et al. 2002), as well as microsatellites (Bowers et al. 1996), recently used for characterization of a Portuguese collection (Lopes et al. 1999), have provided positive results.

The present study includes 12 grapevine varieties that are representative of the ‘Douro’ and ‘Vinhos Verdes’ D.O. regions in Portugal. The varieties were characterized by both RAPD and microsatellite markers in order to identify each of them and to detect possible synonymies to other varieties.

**Material and Methods**

Four adult plants of each variety were kept under normal cultivation practices (Table). Four to 8 young leaves were sampled and stored at -80°C. DNA was extracted from the frozen leaves with the “Plant Leaf DNA Purification Kit” (Epiconic Technologies, Madison WI, USA); the DNA was quantified and a 10 ng µl-1 DNA working solution was prepared.

Decamers OPA-8, OPA-9, OPA-10, OPA-11, OPA-16, OPA-19, OPE-10, OPE-16 and OPE-17 from Operon Technologies Inc. (Alameda CA, U.S.A.), were selected for the amplification of RAPD sequences. The amplification was performed in a 25 µl reaction volume containing about 45 ng of template DNA, 10 mM Tris-HCl pH 9.0, 50 mM KCl, 2.5 mM MgCl2, 0.2 mM of each dNTP (Boehringer), 0.8 µM of a single primer and 1.5 units of Taq-DNA (MBI Fermentas, Lithuania) polymerase. The thermal cycler (Biometra UNO II) was programmed with an initial step of 5 min at 94°C, followed by 45 cycles of 2 min at 94°C, 2 min at 36°C and 3 min at 72°C, and finally a 7 min extension at 72°C. Fragments were separated according to size on a 2% agarose gel, run in 1X TBE buffer, at 3 V cm-1 for 4 h, stained with ethidium bromide, and visualized under UV light. RAPD profiles were photographed and captured using a BIO-CAPT system. The molecular size of fragments was estimated by reference to a DNA ladder mix (MBI Fermentas, Lithuania).

For the microsatellite analysis, 6 STMS loci were used: VVS2 (Thomas and Scott 1993), VVMD5 and VVMD7 (Bowers et al. 1996), and ssrVrZAG47, ssrVrZAG62 and ssrVrZAG79 (Swiec et al. 1999). Primer pairs were fluorescently labeled with Perkin Elmer Applied Biosystems fluorophores, 6-FAM (blue), TET (green) or HEX (yellow). Two multiplex PCR reactions were carried out (Martín et al. 2003). The amplified products were separated in capillary electrophoresis using an automated DNA sequencer ABI PRISM model 310 (Perkin Elmer Applied Biosystems), and the labeled fragments were detected by GENESCAN software.

Microsatellite results were expressed as allele size in base pairs. Allele frequencies were quantified. The observed heterozygosity was calculated as the ratio between heterozygote genotypes and the total analyzed genotypes for each locus. A similarity matrix was calculated by the simple matching coefficient, and a dendrogram was obtained by the UPGMA method from NTSYS-pc version 2.02 package (Rohlf 1998).

**Results and Discussion**

RAPD primers yielded bands which were clearly identified and informative patterns in all cases and generated a total of 99 polymorphic bands from a total of 111 reliable fragments. The average number of bands per primer was 12.3, varying from 9 to 16. The size of the fragments ranged from 100 to 3000 bp. Eight cultivars had at least one monotypic pattern (Table), which is very useful from the point of view of cultivar identification. It agrees with the results from Qu et al. (1996) in a study with Muscadine and American bunch grapes, and Stanvakakis et al. (1997) with...
**Table**

Allelic sizes (in base pairs) of twelve Portuguese varieties at six microsatellite loci. Boldface numbers are unique alleles.

<table>
<thead>
<tr>
<th>Variety</th>
<th>M&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Berry colour&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Region of origin</th>
<th>Location&lt;sup&gt;3&lt;/sup&gt;</th>
<th>VVMD5</th>
<th>VVMD7</th>
<th>VVS2</th>
<th>ssrVrZAG47</th>
<th>ssrVrZAG62</th>
<th>ssrVrZAG79</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aragonez</td>
<td>X</td>
<td>N</td>
<td>Douro</td>
<td>R1 c7-8 (Q)</td>
<td>232</td>
<td>232</td>
<td>251</td>
<td>140</td>
<td>159</td>
<td>195</td>
</tr>
<tr>
<td>Malvasia Fina</td>
<td>B</td>
<td>Douro</td>
<td>R13 c1-2 (Q)</td>
<td>222 236</td>
<td>237</td>
<td>255</td>
<td>140</td>
<td>155</td>
<td>157</td>
<td>187 187</td>
</tr>
<tr>
<td>Moscatel Galego Branco</td>
<td>X</td>
<td>B</td>
<td>Douro</td>
<td>R21 c3-4 (Q)</td>
<td>224</td>
<td>232</td>
<td>231</td>
<td>247</td>
<td>130</td>
<td>157</td>
</tr>
<tr>
<td>Tinta Barroca</td>
<td>X</td>
<td>N</td>
<td>Douro</td>
<td>R1 c3-4 (Q)</td>
<td>224</td>
<td>232</td>
<td>231</td>
<td>140</td>
<td>142</td>
<td>157</td>
</tr>
<tr>
<td>Tinta Francisca</td>
<td>N</td>
<td>Douro</td>
<td>R1 c5-6 (Q)</td>
<td>234 236</td>
<td>237</td>
<td>237</td>
<td>130</td>
<td>157</td>
<td>157</td>
<td>185 165</td>
</tr>
<tr>
<td>Tinto Cão</td>
<td>X</td>
<td>N</td>
<td>Douro</td>
<td>R2 c7-8 (Q)</td>
<td>228</td>
<td>230</td>
<td>237</td>
<td>247</td>
<td>130</td>
<td>157</td>
</tr>
<tr>
<td>Touriga Nacional</td>
<td>N</td>
<td>Douro</td>
<td>R2 c1-2 (Q)</td>
<td>222 232</td>
<td>237</td>
<td>237</td>
<td>140</td>
<td>157</td>
<td>157</td>
<td>185</td>
</tr>
<tr>
<td>Touriga Franca</td>
<td>X</td>
<td>N</td>
<td>Douro</td>
<td>R2 c3-4 (Q)</td>
<td>222</td>
<td>224</td>
<td>237</td>
<td>140</td>
<td>140</td>
<td>157</td>
</tr>
<tr>
<td>Viosinho</td>
<td>X</td>
<td>B</td>
<td>Douro</td>
<td>R20 c3-4 (Q)</td>
<td>228</td>
<td>228</td>
<td>237</td>
<td>130</td>
<td>161</td>
<td>165</td>
</tr>
<tr>
<td>Amaral</td>
<td>X</td>
<td>N</td>
<td>Vinhos Verdes</td>
<td>R8 c4 (A)</td>
<td>222</td>
<td>228</td>
<td>237</td>
<td>132</td>
<td>140</td>
<td>157</td>
</tr>
<tr>
<td>Alvarinho</td>
<td>X</td>
<td>B</td>
<td>Vinhos Verdes</td>
<td>R2 c4 (A)</td>
<td>218</td>
<td>228</td>
<td>237</td>
<td>132</td>
<td>165</td>
<td>165</td>
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<tr>
<td>Borraçal</td>
<td>N</td>
<td>N</td>
<td>Vinhos Verdes</td>
<td>R5 (A)</td>
<td>228</td>
<td>234</td>
<td>237</td>
<td>130</td>
<td>157</td>
<td>193</td>
</tr>
</tbody>
</table>

% Observed heterozygosity: 83%, 67%, 75%, 92%, 83%, 83%

Number of different genotypes: 11, 6, 7, 6, 11, 7

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<sup>1</sup> X = varieties with monotypical RAPD patterns.

<sup>2</sup> N = black; B = white.

<sup>3</sup> R = row number; c = column numbers. Grapevine collections: Q = Quinta Nª. Sra. de Lurdes, University of ‘Trás-os-Montes e Alto Douro’ (UTAD), Vila Real (Portugal); A = ‘Estação Vitivinícola Amândio Galhano’ (EVAG), Arcos de Valdevez (Portugal).
Grapevine cultivars. Aragonez and Borraçal were most distinct from the other varieties. Each variety can be distinguished from the others with these markers.

The alleles obtained in the microsatellite analysis are also shown (Table). A total of 38 alleles, ranging from 5 in VVMD7 and ssrVrZAG79 to 8 in VVMD5, were detected with an average of 6.3 alleles per locus. The most frequent allele was VVMD7-237, which showed a frequency >60%. On the other hand, 9 alleles (23.6%) were unique. Samples in which only one single allele per locus was detected were considered as homozygous genotypes instead of heterozygous with a null allele. The number of different genotypes varied from 6 in VVMD7 and ssrVrZAG47 loci to 11 in VVMD5 and ssrVrZAG62 loci. The level of the observed heterozygosity ranged between 67% (VVMD7) and 92% (ssrVrZAG47) (Table).

The clustering of the varieties using microsatellite results (Figure) indicates a first group including Touriga Franca and Tinta Barroca plus Touriga Nacional and Amaral. A second group has two pairs of varieties: Tinta Francisca and Viosinho, and Tinto Cão and Borraçal. Moscatel Galego has less alleles in common with the others. RAPD results also indicate that Tinta Barroca, Touriga Franca and Touriga Nacional are probably related, as are Viosinho, Tinta Francisca and Tinto Cão.

Results of the microsatellite analysis led to the detection of several synonyms in comparison with previously existing databases. The synonymy of Aragonez (Tinta Roriz) and the Spanish variety Tempranillo was confirmed, as had been shown earlier (O.I.V. 1996). Malvasia Fina is a synonym of Boal Cachudo and Boal da Madeira when compared with the results of López et al. (1999). Moscatel Galego Branco has the same alleles as Muscat à petit grins, confirming their synonymy (O.I.V. 1996). Amaral (Azal Tinto) is confirmed to be synonymous with Caíno Bravo from Galicia (Spain; O.I.V. 1996). Alvarinho is confirmed to be synonymous with the Spanish Caño (Martin et al. 2003).

In conclusion, both molecular markers are useful to identify grapevine varieties though the results from microsatellites are easier to compare with results from other laboratories.

References


Acknowledgements

This work has been supported by the Joint Integrated Action between Portugal (Acção N° E-65/01) and Spain (Ministerio de Ciencia y Tecnología, Acción HP00-32). We thank Prof. N. Magalhães, Eng. J. Baltazar and Eng. J. Garrido for their technical support and for the supply of the plant material.

Received July 15, 2002