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Characterisation of related red-berried and white-berried grapevine cultivars

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Abstract

Eight presumably related dark red-berried (dR), light red-berried (lR) and white-berried (W) grapevine cultivars grown under similar environmental conditions were characterised on the basis of morphologic (phenotype) and molecular (genotype) parameters. The ampelographic characterisation was based on the main descriptors that correspond to the 'Primary descriptor priority list' suggested by the International Organisation of Vine and Wine (OIV). At the molecular level, the total genomic DNA of the cultivars was isolated from frozen grapevine leaves. Six microsatellite loci were used for characterisation of the grape cultivars: 'VVS2', 'VVMD5', 'VVMD7', 'VVMD27', 'VrZAG62', and 'VrZAG79'. This molecular characterisation allowed the confirmation, in seven of the eight studied cultivars, including dark red, light red and white-berried variants, of colour mutants with origin in three different varieties. 'Pinot Blanc' (W), 'Pinot Gris' (lR) and 'Pinot Noir' (dR) formed a group of colour mutants, and 'Malvasia Fina' (W) and 'Malvasia Fina Roxa' (lR) formed another group. 'Moscatele Galego Branco' (W) and 'Moscatele Galego Roxo' (lR) were confirmed as colour mutants, but their allelic profile was not related to that of 'Moscatele Galego Tinto' (dR).

Keywords: *Vitis vinifera*, grape berry colour, anthocyanins, microsatellite, SSR

INTRODUCTION

Grapevines (*Vitis vinifera*) are one of the most traditional and most important crops in Portugal where there are 343 different authorised wine production varieties, 169 of which are black, 152 white, and 22 grey. Occasionally the same variety is known under different names (synonyms) and sometimes the same name applies to different varieties (homonyms). In fact, several synonymies and homonyms were detected between these grape varieties by using different molecular markers, namely RAPDs, ISSRs and SSRs (Pinto-Carnide et al., 2003; Santiago et al., 2005; Martín et al., 2006; Castro et al., 2008, 2011; Martín et al., 2011), which are as the most indicated for biodiversity analysis and cultivar identification. However, no differences were observed between such varieties as 'Pinot Noir' and 'Pinot Blanc'.

There is no accurate information about exactly how many more cases exist of varieties like 'Pinot Noir' and 'Pinot Blanc', where the same variety suffered mutations that gave rise to different berry colouration. What distinguishes black from white varieties is the presence or absence of anthocyanins in several cell layers constituting the skin of the berry.

In grapevine, five very similar *VvmybA* genes were identified as putative regulators of anthocyanin synthesis in the red-berries cultivars (Kobayashi et al., 2002; Walker et al., 2007; Deluc et al., 2008). In the case of grape, extensive molecular physiology studies provide evidence that two adjacent transcription factors, *VvmybA1* and *VvmybA2*, are able to induce the *VvUGT* transcription needed for berry pigmentation (Ageorges et al., 2006; Walker et al., 2007). Somatic variation for berry skin colour has been associated with the



presence of Gret1, a retrotransposon, in the promotor region of VvmybA1 (Kobayashi et al., 2004). The presence of this retrotransposon on white grapes obstructs VvmybA1 gene transcription and hinders anthocyanin biosynthesis activation. Another similar gene, VvmybA2, which is physically linked to VvmybA1, was also implicated in this trait (Walker et al., 2007). A final transcription factor, Vvmyb5b, was also shown to marginally induce the VvUFGT (Deluc et al., 2008).

In the present study three groups of supposedly related red-berried and white-berried grapevine cultivars were characterised in order to confirm if they are actually colour mutants. The studied grapevine cultivars were installed in an experimental vineyard at the University of Trás-os-Montes e Alto Douro (UTAD) campus in Vila Real, in the Baixo Corgo sub-region of the Demarcated Douro Region, northern Portugal.

The cultivars were characterised on the basis of morphologic parameters (phenotype) and molecular markers (genotype). The ampelographic characterisation was based on the 14 main descriptors that correspond to the 'Primary descriptor priority list' suggested by the International Organisation of Vine and Wine (OIV) in the Second Edition of the OIV Descriptors List for Grape Varieties and *Vitis* Species (OIV, 2009). For the molecular characterisation, six SSR (Simple Sequence Repeats) markers identified in the project GENRES #081 were used as more suitable for the identification of grapevine cultivars ('VVS2', 'VVMD5', 'VVMD7', 'VVMD27', 'VrZAG62', and 'VrZAG79' and corresponding to OIV801-806 descriptors (OIV, 2009)).

MATERIALS AND METHODS

Vitis vinifera 'Pinot Blanc' (W), 'Pinot Gris' (lR), 'Pinot Noir' (dR), 'Malvasia Fina' (W), 'Malvasia Fina Roxa' (lR), 'Moscatele Galego Branco' (W), 'Moscatele Galego Roxo' (lR), and 'Moscatele Galego Tinto' (dR) (Table 1) were sampled from the same experimental vineyard located in Vila Real (Campus of University of Trás-os-Montes e Alto Douro, 41°17'N, 7°44'W, 500 m above mean sea level, Baixo Corgo sub-region of the demarcated Douro Region, northern Portugal). All vines were grafted on 'SO4' and planted in a schistous soil. Plants were managed without irrigation and grown according to a commercial protocol, as applied in commercial farms.

Table 1. List of the studied accessions and allele sizes in base pairs at each of six microsatellite loci analysed.

Cultivar	VVS2		VVMD5		VVMD7		VVMD27		VrZag62		VrZag79	
	Allele 1	Allele 2										
Malvasia Fina	140	142	222	236	237	255	175	191	187	187	245	249
Malvasia Fina Roxa	140	142	222	236	237	255	175	191	187	187	245	249
Moscatele Galego Branco	130	130	224	232	231	247	175	191	185	195	249	253
Moscatele Galego Roxo	130	130	224	232	231	247	175	191	185	195	249	253
Moscatele Galego Tinto	130	148	222	224	237	247	175	185	185	187	249	253
Pinot Blanc	134	148	224	234	237	241	181	185	187	193	237	243
Pinot Gris	134	148	224	234	237	241	181	185	187	193	237	243
Pinot Noir	134	148	224	234	237	241	181	185	187	193	237	243

The ampelographic characterisation was based on the main descriptors that correspond to the 'Primary descriptor priority list' suggested by the Organisation Internationale de la Vigne et du Vin (Table 2) (OIV, 2009).

Young leaves from each accession were collected in the experimental vineyard. Leaves were kept at -80°C and macerated in liquid nitrogen at the start of the extraction protocol. Genomic DNA was extracted using the DNeasy® Plant Mini Kit (QIAGEN) purification kit, according to the manufacturer's instructions. Extracted DNA was quantified by UV spectrophotometry (Nanodrop® ND-1000, Fisher Scientific) followed by quality check in a 1.0% agarose gel electrophoresis, and a working solution of 10 ng μ L⁻¹ was made.

Table 2. Primary descriptor priority list suggested by the OIV (OIV, 2009).

Code	Descriptor
OIV 001	Young shoot: aperture of tip
OIV 004	Young shoot: density of prostrate hairs on tip
OIV 016	Shoot: number of consecutive tendrils
OIV 051	Young leaf: colour of upper side of blade (4th leaf)
OIV 067	Mature leaf: shape of blade
OIV 068	Mature leaf: number of lobes
OIV 070	Mature leaf: area of anthocyanin colouration of main veins on upper side of blade
OIV 076	Mature leaf: shape of teeth
OIV 079	Mature leaf: degree of opening/overlapping of petiole sinus
OIV 081-2	Mature leaf: petiole sinus base limited by veins
OIV 084	Mature leaf: density of prostrate hairs between main veins on lower side of blade
OIV 087	Mature leaf: density of erect hairs on main veins on lower side of blade
OIV 223	Berry: shape
OIV 225	Berry: colour of skin

A total of six microsatellite loci were amplified (Table 1), corresponding to the OIV core set: 'VVS2', 'VVMD5', 'VVMD7', 'VVMD27', 'VrZAG62', 'VrZAG79', that correspond to OIV801-806 descriptors (OIV, 2009). One primer of each pair was fluorescently labeled with 6-FAM (blue), TET (green) or HEX (yellow).

Each 20 µL PCR reaction contained 2.5 mM of dNTP, 25 mM of MgCl₂, 10 ng of template DNA, different concentrations of primers and 5 U µL⁻¹ of Taq DNA polymerase in the manufacturer's buffer. PCR amplifications were performed in a T-100™ Thermal Cycler (Bio-Rad, München, Germany), a basic thermocycler. The programme comprised an initial denaturation step (95°C/5 min), followed by 40 cycles of 94°C/45 s, 50°C/60 s and 72°C/90 s.

Two multiplex PCRs were carried out with the OIV SSR core set, the first one involving 'VVS2', 'VVMD5' and 'VVMD7' (*set A*), and the second 'VVMD27', 'VrZAG62' and 'VrZAG79' (*set B*).

The *set A* multiplex reactions contained 0.2 µM of each VVS2 primer, 0.5 µM of each VVMD5 primer, and 0.25 µM of the VVMD7 primers; and the *set B* reactions contained 0.5 µM of each VVMD27 and VrZAG79 primers, and 0.1 µM of each VrZAG62 primer.

The amplicons were separated in a 3% (w/v) agarose gel electrophoresis in TBE buffer, for 2 h at a constant voltage of 120 V, followed by ethidium bromide staining and by capillary electrophoresis (ABI PRISM model 310, PE Applied Biosystems, CA, USA). GENESCAN-350 TAMRA (PE Applied Biosystems, CA, USA.) was included as an internal sizing standard and labeled products were analysed and sized using Peak Scanner V1.0 software (PE Applied Biosystems, CA, USA).

RESULTS AND DISCUSSION

Analysing the SSR loci amplified using multiplex PCR, it was possible to identify seven true berry colour mutant cultivars, out of the eight analysed, belonging to three distinct varieties (Table 1). The cultivars of each variety identified as true berry colour mutants showed the same SSR pattern, i.e., the same alleles in all SSR loci, being distinguished by the colour of the berry skin. Thus, despite the fact that these cultivars vary in relation to the colour of the berry, they were considered true berry colour mutant cultivars with the same SSR profile and origin in one single variety. This was the case of the groups Pinot ('P. Blanc', 'P. Gris' and 'P. Noir') and Malvasia ('Malvasia Fina' and 'Malvasia Fina Roxo'). However, the three 'Moscatele Galego' cultivars revealed two different SSR profiles, one of the mutant cultivars 'Moscatele Galego Branco' and 'Moscatele Galego Roxo' and another of 'Moscatele Galego Tinto' – the last one showing a different genotype.

Apart from berry colour, the cultivars with the same SSR profile presented similar ampelographic characteristics (Figure 1).

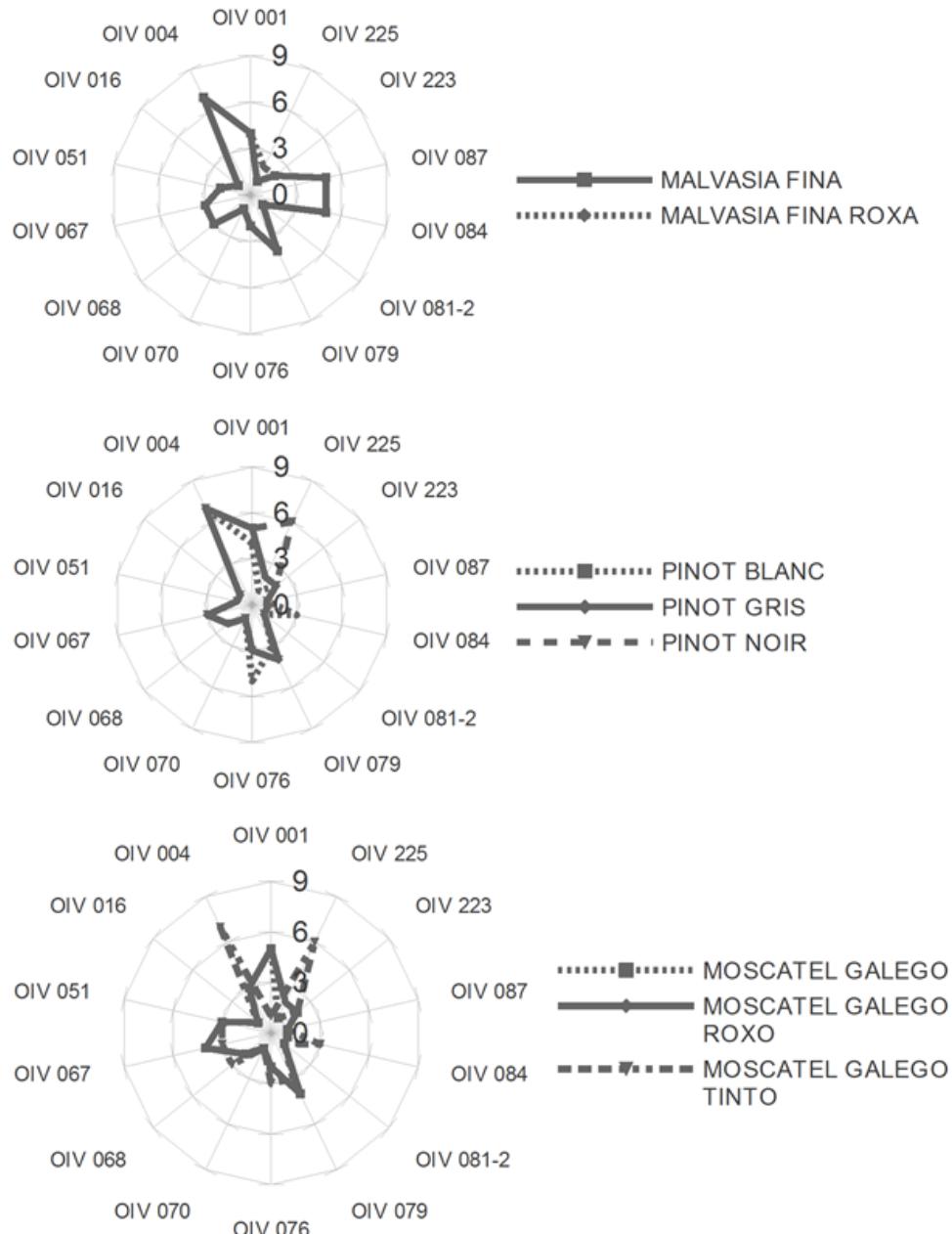


Figure 1. Ampelographic characterisation based on the main descriptors that correspond to the 'Primary descriptor priority list' suggested by the OIV (see Table 2).

CONCLUSIONS

Through the amplification of the six OIV SSR core set and the 14 OIV primary descriptors, this study allowed the identification of three varieties that by mutation originated seven cultivars, with berry colour skin somatic variants.

In the future, varieties and respective cultivars with different berry skin will be analysed using other molecular marker techniques to assess cultivar specific markers, eventually linked to the berry skin colour.

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