

## Research Note

# Marker assisted selection for berry colour in a ‘Nektár’ x ‘Jacquez’ grape progeny

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**Introduction:** Genetic pool of the grapevine cultivars in viticulture is very narrow and the majority of the varieties of *Vitis vinifera* origin are susceptible to diseases and pests. Recently the main efforts of grape breeders are to combine excellent quality and high level of fungal resistance in one genotype. Therefore we have given rise to a progeny by crossing an aromatic, but pathogen susceptible white wine cultivar ‘Nektár’ (*Vitis vinifera* L.) with a fungal resistant, black colour, but neutral taste variety ‘Jacquez’ (*V. bourquina* Munson ex Viala/*V. aestivalis* Michx. x *V. vinifera*) for breeding and multipurpose genetic study. Since berry colour is an important qualitative trait in viticulture, as a first step we analyzed 49 individuals from the ‘Nektár’ x ‘Jacquez’ cross together with the parents, three reference varieties and one *Vitis aestivalis* accession to identify and select the white/black berried offspring. Our marker assisted selection is based on the discovery that the white berried varieties arose from the ancient black/red fruited grapes by bud mutations resulting in inhibition of anthocyanin biosynthesis. KOBAYASHI *et al.* (2004) identified the insertion of the *Gret1* retrotransposon in the promoter of the *VvMybA1* transcription factor gene as one of the possible reasons for colourless phenotype. Absence of this retroelement was observed in North American and East Asian *Vitis* species (MITANI *et al.* 2009), independently of their berry colour (CADLE-DAVIDSON and OWENS 2008). Back mutation, excision of the retroelement in *V. vinifera* varieties, also occurred resulting in reappearance of colour grape berries. Our aim was to genotype the individuals of the ‘Nektár’ x ‘Jacquez’ progeny with a *VvMybA1*-linked marker to predict the berry colour.

**Material and Methods:** Parental varieties (‘Nektár’ and ‘Jacquez’), their progeny of 49 individuals, three reference cultivars (‘Barbera’, ‘Chardonnay’, ‘Pinot noir’) and one *V. aestivalis* accession (*V. aestivalis*, Pécs) were analyzed with the *VvMybA1*-linked CAPS marker

20D18CB9 (WALKER *et al.* 2007). DNA was isolated from young leaves by the CTAB method (DOYLE and DOYLE 1990). PCRs were carried out in Bio-Rad iCycler. Twenty µL reaction mixture contained 20 ng DNA template, 6 pM primers, 75 µM-t dNTP, 2 mM MgCl<sub>2</sub>, 1x PCR buffer and 1 u *Taq*-polymerase (West-Team Biotech, Pécs, Hungary). Reaction conditions were the following: 2 min precycle at 94 °C; 10 cycles: denaturation at 94 °C for 30 s, annealing at 65 °C for 30 s, polymerization at 72 °C for 1 min; annealing temperature decreased by 1 °C in each cycle. Then 24 cycles denaturation at 94 °C for 30 s, annealing at 56 °C for 30 s, polymerization at 72 °C for 1 min; post cycle at 72 °C for 5 min. Primers were applied according to WALKER *et al.* (2007): forward: 5’-GATGACCAAACCTGCCACTGA-3’, reverse: 5’-ATCACCTGTCCCACCAA-3’. Ten µL amplification products were run on 1.5% agarose gel stained with ethidium bromide. The remaining 10 µL PCR product was digested with *DdeI* enzyme (Promega, Madison, USA) (as it was described by WALKER *et al.* (2007). Restricted fragments were separated in an ethidium bromide dyed 3% agarose gel. In order to be able to determine the accurate sizes of the digestion products the PCR products were sequenced. For sequencing undigested PCR fragments were ligated into pGemT-Easy vector (Promega, Madison, USA) and sequenced with M13 universal primers in an ABI PRISM 310 Genetic Analyser (Applied Biosystem, Foster City, USA). BioEdit and DNASTAR (Megalign és Editseq) softwares were applied for sequence analysis.

**Results and Discussion:** The 20D18CB9 primer pair amplified a 577 bp fragment in the reference varieties ‘Barbera’ [homozygous for the *Gret1*-less wild type *VvMybA1c* allele (BODOR *et al.* 2014)], ‘Chardonnay’ (homozygous for the *Gret1* insertion), ‘Pinot noir’ (heterozygous genotype) (WALKER *et al.* 2007; SZÖKE *et al.* 2012) and ‘Nektár’ (Fig. 1). A different PCR product was obtained in the *V. aestivalis* accession, easily distinguishable from the *V. vinifera*-specific fragment (577 bp). Sequencing showed that there is a 34 bp deletion in the PCR product defined by the 20D18CB9 primers in *V. aestivalis* resulting in a shorter amplicon (543 bp). Since black berried ‘Jacquez’ is an interspecific hybrid (*V. bourquina* Munson ex Viala/*V. aestivalis* Michx. x *V. vinifera*) it contains both a 577 (*V. vinifera* origin) and a 543 bp (*V. aestivalis* origin) DNA fragment (Fig. 1).

In 22 offsprings, the 20D18CB9 primer pair resulted in only one fragment (577 bp) deriving from *V. vinifera*, while 27 hybrids contained two (577 and 543 bp) bands indicating their interspecific character (Fig. 1). Although 20D18CB9 marker showed polymorphism between the two *Vitis* species it did not allow to conclude the expectable berry colour.

To predict the berry colour of offsprings, PCR products were digested with *DdeI* restriction endonuclease, resulting in different patterns dependently of the genotypes. In the *Gret1*-less black berried reference variety ‘Barbera’ the 577 bp DNA was cut into two fragments (248 and 329 bp). The *DdeI* digestion in the homozygous white reference ‘Chardonnay’ and ‘Nektár’ resulted in three fragments: 35 (not visible in agarose gel), 213 and 329 bp. As a consequence

of heterozygosity of ‘Pinot noir’ all three the 213, 248 and 329 bp bands were obtained. The 543 bp *V. aestivalis* PCR product digested differently, beside the 248 a 295 bp long cleavage product appeared. Being ‘Jacquez’ an interspecific hybrid, it shows both *V. aestivalis*- and ‘Chardonnay’-like digestion pattern (213, 248, 295, 329 bp) (Fig. 2). Twenty-two out of 49 seedlings shared the same fragments after *DdeI* treatment as the parent ‘Nektár’ 22, referring to the white berry colour. The other 27 offsprings were identical with the black berried parent ‘Jacquez’ (Fig. 2).

Our results with the 20D18CB9 CAPS marker linked to the *VvMybA1* gene prove that this marker - due to the presence of the *V. aestivalis* component in the parental variety ‘Jacquez’ - can be applied as a simple locus-specific PCR marker without *DdeI* restriction endonuclease digestion. The black berried ‘Jacquez’ originates from a *V. bourquina* /*aestivalis* x *vinifera*/ cross, therefore the presence of the 543 bp is expected in its offspring, and at the same time it predicts the colour fruits of the individuals. Comparing our results with the ones of WALKER *et al.* (2007), the 20D18CB9 primer pair beside the *VvMybA1*-allele-specific markers can be applied as a diagnostic tool for MAS of berry colour in the other individuals of the ‘Nektár’ x ‘Jacquez’ progeny.

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