INVESTIGATION OF *VvDXS* FUNCTION AND ITS EFFECTS ON MUSCAT FLAVOR LEVELS[1]

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Key words: candidate gene, expression trend, association study, enzyme activity.

1. INTRODUCTION

Aroma plays an essential role in the perceived quality of grapes and wines so there is a tremendous interest to understanding how the accumulation of sensory compounds is regulated at the molecular level. In particular, the floral flavor typical of 'Muscat' cultivars, due to high levels of monoterpenoids (geraniol, linalool and nerol) (Ribéreau-Gayon *et al*., 1975, Günata *et al*., 1985), is highly distinct and has been greatly appreciated since ancient times. Muscat flavor determination in grapevine (*Vitis vinifera* L.) has up to now been studied by evaluating monoterpenoid quantity through QTL analysis (Doligez *et al*., 2006; Battilana *et al*., 2009; Duchêne *et al*., 2009). These studies have revealed colocalization of 1-deoxyxylulose-5-phosphate synthase (DXS) with the major QTL positioned on chromosome 5 (Battilana *et al*., 2009; Duchêne *et al*., 2009).

DXS catalyzes the first reaction of the plastidial pathway that produces 1-deoxyxylulose-5-phosphate (DXP) from the central metabolic intermediates glyceraldehyde-3-phosphate and pyruvate. A regulatory role in terpene biosynthesis has been suggested for DXS in bacteria and in several plant species (Estevéz *et al*., 2001). Accordingly, DXS was described as one of the main regulators of monoterpenoid biosynthesis in grapevine by Luan and Wüst (2002).

In the present study the connection between the positional candidate gene *VvDXS* and muscat flavor was evaluated by investigating the nucleotide diversity of full ORFs on grapevine accessions and its expression profiles in the berries from a Muscat-type cultivar ('Moscato Bianco') and a neutral cultivar ('Chardonnay').

[1] QUAD. VITIC. ENOL. UNIV. TORINO, 31, 2009-2010

2. MATERIALS AND METHODS

2.1 Association study

The *VvDXS* gene was amplified and directly sequenced in 148 accessions of *Vitis vinifera* subsp *sativa*. Nucleotide diversity and neutrality tests were evaluated by DnaSP. Linkage disequilibrium measures were calculated using DnaSP and TASSEL ver. 2.1 software (Thornsberry *et al*., 2001).

2.2 Gene expression and allelic pattern analysis

Berries of cultivar 'Moscato bianco' were sampled at 1-2 week intervals from pre-véraison to over-ripe stages, for a total of 13-14 collecting dates. Total RNA was extracted from pericarp tissue in triplicate for each sample. First strand cDNA was synthesized using Superscript™ III Reverse Transcriptase (Invitrogen, Carisbad, CA, USA) according to the manufacturer's instructions. Primers were designed for LightCycler® 480 SYBR Green I analysis (Roche, Mannheim, D).

Primers and FRET-Hybridization probes used in Real-Time PCR reactions were designed according to the manufacturer's instructions (LightCycler Probe Design Software 2.0, Roche Applied Science, Mannheim, Germany).

2.3 Kinetic proprieties and tobacco transformation

Full cDNA *VvDXS* was cloned into the *BamHI* and *XhoI* restriction site of pET30a (KanR and CamR), *Escherichia coli* BL21-DE3 plus pLysS was employed as host strain in protein synthesis and 0.8 mM IPTG was used to overexpress His6-DXS protein. DXS allele activities were determined by using a coupled assay method as reported by Han *et al*. (2003).

Gateway technology (Invitrogen, Carisbad, CA, USA) was employed to transfer the full ORF cDNA *VvDXS* alleles into a plant expression vector (Karimi *et al*., 2002). The constructs generated were transformed into *Agrobacterium tumefaciens* EHA 105 (Hood *et al*., 1993) by electroporation, and tobacco lines were then transformed following the protocol of Gallois and Marinho (1995) with modifications.

3. RESULTS AND DISCUSSION

Some SNPs in moderate LD resulted significantly associated with muscat-flavored varieties (Emanuelli *et al*., in press). Berries were sampled in the field throughout development and the expression of *VvDXS* compared to the accumulation patterns of the major monoterpenes produced in the berries. The relative expression ratios of *VvDXS* revealed no strong differences between the varieties, nonetheless temporal patterns of *VvDXS* expression through development stages were different suggesting cultivar dependent regulation.

Moreover the expression level of the two allele forms of 'Moscato Bianco' did not show any significant variation among sampling points (fig 1).

Functional assays suggest that one putative causal SNP could raise monoterpenoid accumulation by changing the 3D protein structure and by increasing the VvDXS activity (*kcat*) of about 2-fold in Muscat enzyme form.

In transgenic tobacco lines, volatiles and glycosidically bound aromatic compounds isolated by solid-phase extraction and quantified by HRGC-MS analysis clearly proved a different effect of two alleles of *VvDXS* from Moscato Bianco on monoterpenoids production. These results open new prospectives in terms of the role of DXS in grape and its metabolic function.

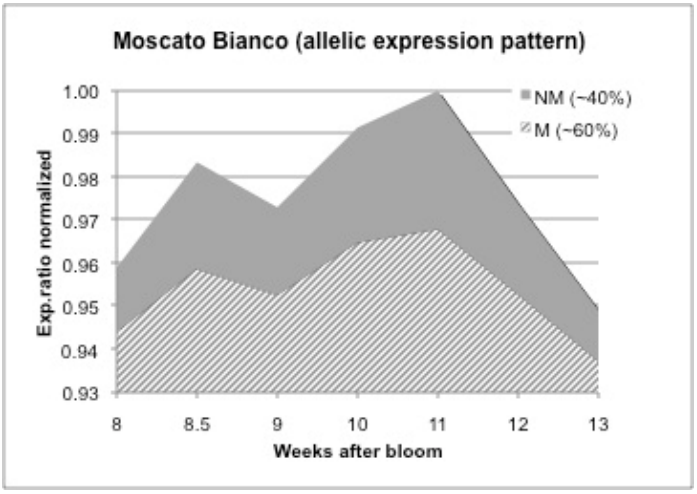

Fig. 1 - Expression trend of *VvDXS* allele **non Muscat (NM) and Muscat (M)** reported as maximum value normalized to 1 with respect to EF1 and GAPDH housekeeping genes. Between brackets the percentage of each allele expression during the ripening curve is shown.

Abstract

In the present study the connection between the positional candidate gene *VvDXS* and muscat flavor was evaluated by investigating the expression profiles in the berries from a Muscat-type cultivar and a neutral cultivar and its nucleotide diversity of full ORF on grapevine accessions. The relationship between the transcription profile of *VvDXS* alleles and monoterpenoid content suggests that a particular trend in gene expression rather than the level of expression ratio affects monoterpenoid accumulation in 'Moscato Bianco'. However a putative causal SNP responsible for a predicted non-neutral substitution was found to be significantly associated with muscat-flavored cultivars. Functional assays suggest that the putative causal SNP raises monoterpenoid accumulation by changing the 3D protein structure and by increasing VvDXS activity in Muscat enzyme form.

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