

Supplementary materials

Title: Functional effect of grapevine 1-deoxy-D-xylulose 5-phosphate synthase substitution K284N on Muscat flavor formation

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Number of supplementary Figures: 6

Number of supplementary Tables: 5

Supporting methods

1. Monoterpenoids analysis

1.1 Preparation of volatile extracts from berries

Aroma-active components were extracted after crushing 100 g of berries. Both free and glycosidically bound monoterpenes were fractionated using Solid Phase Extraction (SPE) based on selective retention of each form on a mix of hydrophobic cross-linked polystyrene copolymer (XAD-2 resin). According to the procedures described by Gunata et al. (1985) and Versini et al. (1993), 200 ml of must were adsorbed on approximately 3.3 g of XAD-2 with particle size: 0.1-0.4 mm (SERVA Electrophoresis GmbH, Heidelberg).

The free monoterpene fraction was eluted with 100 mL of pentane-dichloromethane (2:1) and the eluate was dried over anhydrous sodium sulphate. The eluate was then concentrated to 0.5 µL by evaporation and stored at -20 °C before high-resolution gas chromatogram-mass spectrometry (HRGC-MS) analysis. The bound monoterpene fraction was eluted with 100 mL of methanol-ethyl acetate (1:9) and concentrated to dryness in Rotavapor evaporator (BÜCHI, Flawil, Switzerland), before dissolution in citrate-phosphate buffer (pH 5.0, 5mL). A 1.4 mg aliquot of a commercial enzyme product containing glucosidase enzymes from *Aspergillus niger* (AR-2000; Gist Brocades, France) was added and the mixture was incubated at 40 °C for 18 h to accomplish enzymatic hydrolysis of the glycosidically bound monoterpenes. 1-heptanol (25 µg) was added as internal standard to the hydrolysates which were subsequently extracted three times with pentane-dichloromethane (2:1,

6 mL). The extract was then concentrated at 40°C to ~500 µL by distillation through a Vigreux column before HRGC-MS analysis.

Volatile extracts were analysed using a PerkinElmer gas chromatograph with a Clarus 500 Mass Spectrometer (Perkin Elmer, Norwalk, CT) equipped with a HP-InnoWax fused silica column (30 m x 0.32 mm I.D., 0.5 µm film thickness, J and W Scientific, CA, USA). Helium was used as carrier gas with a constant flow of 2 ml min⁻¹. The GC oven program was as follows: 50 °C for 1 min, 60 °C at 10 °C min⁻¹, 60 °C for 30 sec., then 200 °C at 2.5 °C min⁻¹, 200 °C for 10 min, finally 250 °C at 10 °C min⁻¹ and holding for 10 min; injector temperature, 250 °C; detector temperature was set to 220 °C. The mass spectrometer operated in electron ionization mode (EI, internal ionization source; 70 eV) with a scan range of m/e 30-300 amu. Compound identification was based on mass spectra matching in the standard NIST-98/Wiley library and retention indices of authentic reference standards. All monoterpenes and benzyl derivatives, such as benzyl alcohol and 2-phenylethyl alcohol, were quantified in relation to the internal standard 1-heptanol.

1.1.1 Repeatability tests: In order to calculate the percentage of variance in the methods used for the monoterpene analysis, a pool of 1.2 kg berries (18 °Brix) was collected from the cultivar Moscato Bianco. To evaluate the variance due to the random sub sampling of berries, six samples were prepared by collecting 100 g of berries from the homogeneous pool and analysed immediately following the method described above. The remaining 600 g of berries were crushed under a N₂ atmosphere and maintained on ice to avoid the oxidation of terpenoids. The resulting grape juice was quickly distributed into 20 aliquots of approximately 30 g each and

stored at -20 °C in 50 mL tubes. This set of reference samples was used to assess the repeatability of the method during sample preparation.

1.2 Preparation of volatile extracts from Tobacco Leaves

The 25th leaf was taken from tobacco plants grown under controlled conditions in a greenhouse and stored at -80°C until analysis. Powder obtained by crushing with N₂ liquid was put into 10mL of distilled water, containing heptanol as internal standard (25 µg) and D-(+)-Gluconic acid δ-lactone (0.5 g). The mixture was vigorously homogenised (for 3 min with vortex) and cooled at 4°C over night. Both free and bound compounds were fractionated by Solid Phase Extraction (SPE) using hyper cross-linked hydroxylated polystyrene-divinylbenzene copolymer (INSOLUTE ENV+ with particles size of 90 µm, StepBio, Bologna). The free fraction was eluted with 30mL of dichloromethane and 60 mL n-pentane was immediately added. The extract was dried over anhydrous sodium sulphate and then concentrated at 40°C to 500 µL. Bound fraction was then eluted with 30mL of methanol and concentrated to dryness in Rotavapor evaporator, before dissolution in citrate-phosphate buffer. Enzymatic hydrolysis of bound forms and HRGC_MS analyses of extracts were performed as described above.

1.3 Technical procedures:

HRGC-MS was performed using a PerkinElmer gas chromatograph with a Clarus 500 Mass Spectrometer (Perkin Elmer, Norwalk, CT) equipped with a HP-InnoWax fused silica column (30 m x 0.32 mm I.D., 0.5 µm film thickness, J and W Scientific, CA, USA). Helium was used as carrier gas with a constant flow of 2 ml min⁻¹. The

oven program was as follows: 50 °C for 1 min, 60 °C at 10 °C min⁻¹, 60 °C for 30 sec., then 200 °C at 2.5 °C min⁻¹, 200 °C for 10 min, finally 250 °C at 10 °C min⁻¹ and holding for 10 min; injector temperature, 250 °C; detector temperature was set to 220 °C and MS detector was employed and it was set as solvent delay for 5 min. Mass spectra were scanned in the range m/e 30-300 amu; total ion chromatograms (TIC) profiles were obtained. All monoterpenes and benzyl derivatives, such as benzyl alcohol and 2-phenylethyl alcohol, were quantified in relation to the internal standard 1-heptanol.

2. Tobacco transformation

Overnight cultures of *Agrobacterium* were grown in LB medium at 28° C in a shaking incubator. Tobacco leaf discs (1 cm) collected from young *in vitro* plants were co-cultured with *Agrobacterium* suspension (OD₆₀₀=0.3). After a 10 min incubation at room temperature the leaf discs were placed onto Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) containing 4.4 µM 6-benzylaminopurine (BAP) at 25°C with a 16 h photoperiod. After a 48 h co-cultivation, the leaf discs were transferred on MS medium with 4.4 µM BAP, 300 mg/l cefotaxime in order to inhibit further bacterial growth and 100mg/l kanamycin for the selection of transgenic cells. After 2 months of culture, the shoots (2–3 cm) were excised and then transferred to solid half-strength MS medium with 300mg/l cefotaxime, 100 mg/l kanamycin and without plant growth regulators for root generation. The plants were maintained under *in vitro* conditions at 25°C with a 16 h photoperiod and some of them were transferred to soil under growth room conditions for seed set.

The detection of integrated transgenes was performed by PCR and Southern hybridization. The PCR was performed by using the forward primer *npt II* 1 (5'-GAAGGGACTGGCTGCTATTG-3') and reverse primer *npt II* 2 (5'-AATATCACGGGTAGCCAACG-3') with the following PCR conditions: 95°C for 2 min, followed by 35 cycles of 95°C for 30 s , 58°C for 30 s and 72°C for 1 min, with a final extension of 10 min at 72°C.

Tobacco pK7WG2:VvDXS N284 and pK7WG2:VvDXS K284 lines were also verified by transgene PCR using primers pK7WG2S and pK7WG2AS followed by *StyI* digestion. Approximately 20 µg of genomic DNA was digested with the appropriate restriction endonuclease: *HindIII*, *EcoRI*, (50 units each; Promega, USA). The digestion mixture was further purified, precipitated, electrophoresed through a 0.9% agarose gel in 0.5 × TBE and capillary-blotted in 20×SSC (1×SSC is 0.15 M NaCl plus 15 mM sodium citrate) onto a positively-charged nylon membrane (Roche Diagnostics, Switzerland). A fragment of the *nptII* gene, used as probe, was amplified by PCR and digoxigenin-labeled (DIG-dUTP) with a PCR Dig Probe Synthesis Kit (Roche Diagnostics, Switzerland) according to the manufacturer's instructions. Pre-hybridization was carried out for 2 h at 42° C in DIG easy Hyb buffer (Roche) before labeled probe (20 ng/ml) was added and the hybridization carried out overnight at 42° C. Signals on the membranes were detected with CSPD following the Roche protocol and exposed to Kodak® BioMax™ light film (Sigma).

References

- Gunata Y, Bayonove C and Baumes R.** 1985. The aroma of grapes I. Extraction and determination of free and glycosidically bound fractions of some grape aroma components. *Journal of Chromatography A*, **331**, 83–90.
- Murashige T, and Skoog F.** 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiology Plant*, **15**, 473-497.
- Reid K, Olsson N, Schlosser J, Peng F and Lund S.** 2006. An optimized grapevine RNA isolation procedure and statistical determination of reference genes for real-time RT-PCR during berry development. *BMC Plant Biology*, **6**, 27.
- Versini G, Dalla Serra A, Monetti A, De Micheli L, Mattivi F.** 1993. Free and bound grape aroma profiles variability within the family of muscat-called varieties. In: Bayonove C, Crouzet J, Flanzy C, Martin JC, Sapis JC, eds. *Proceedings of the International Symposium “Connaissance aromatique des cépages et qualité des vins”*, Montpellier, France, 9–10 February, 1993. *Revue Française d’Oenologie*, Lattes, 12–21.

Supplementary Tables

Table S1 List of primers used for semi-quantitative Real-Time RT-PCR analysis.

Gene name	Primer name	Primer sequence (5'-3')
	RT-VvDXSF	CCAAGGGCGTTACCAAACAG
1-deoxi-D-xilulose 5-phosphate synthase ¹	RT-VvDXSR	TCAACTTTTGCAGCCAATTCA
	RT-EF1 α _F	GAACTGGGTGCTTGATAGGC
Elongation factor 1-alpha ‡	RT-EF1 α _R	AACCAAAATATCCGGAGTAAAAGA
Glyceraldehyde 3-phosphate	RT-GAPDH_F	TTCTCGTTGAGGGCTATTCCA
dehydrogenase ‡	RT-GAPDH_R	CCACAGACTTCATCGGTGACA

‡ Reid et al., (2006);¹ Nucleotide sequence corresponding to NC_012011 locus

Table S2: List of primers and FRET Hybridization probes used in Real-Time PCR and melting curve analysis.

Primer / FRET probe name	Sequence (5'-3')	Tm°
VvDXS_RT_fw	AAGTGTGATCCAGAACCACTGAT	65
VvDXS_RT_rw	TGCTCTTAGTAGGTTACAATCAAACAGAC	65
Anchor probe VvDXS	TCATGCATCGGTCCGCCAATCT--FL	75
Sensor probe VvDXS K284	LC640-TTTGGTAACGCC <u>CT</u> TGGCAAC--PH	70

Underlined nucleotide corresponds to the mismatch

Table S3 Normalized DNA melting curves used to calculate the fractional value of each allele in plasmids mixtures of pENTR/D-TOPO:VvDXS N284 pENTR/D-TOPO:VvDXS K284 with different molar ratios.

	pENTR/D-TOPO:VvDXS N284	plasmides mixture (pENTR/D-TOPO:VvDXS N284 : pENTR/D-TOPO:VvDXS K284)									pENTR/D-TOPO:VvDXS K284
molar mixture	10:0	9:1	8:2	7:3	6:4	5:5	4:6	3:7	2:8	1:9	0:10
γ VvDXS K284 _(exp)	0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1
γ VvDXS K284 _(Ob)	0	0.2187	0.4037	0.5467	0.6448	0.7127	0.7881	0.8625	0.9020	0.9589	1
γ VvDXS K284 _(c)	NA	0.1014	0.2144	0.3270	0.4225	NA	0.5998	0.7166	0.7878	0.9040	NA
SD	NA	0.0007	0.0010	0.0013	0.0019	NA	0.0017	0.0033	0.0027	0.0027	NA

γ VvDXS K284s (exp) = expected fraction value of K284 allele, γ VvDXS K284s (Ob) = observed fraction value of K284 allele, γ VvDXS K284s (c) = calculated fraction value of K284 allele, NA = not available, SD = standard deviation

Table S4 Concentration (micrograms per Kg of leaves, dry weight) of free aromatic compounds from T₀ tobacco plants transformed with *VvDXS N284* and *VvDXS K284* alleles.

	VvDXS N284						VvDXS K284			
	WT		B3-2 line		B3-12 line		B6-14 line		B6-13 line	
Monoterpenoids	Mean (µg/Kg)	SD	Mean (µg/Kg)	SD	Mean (µg/Kg)	SD	Mean (µg/Kg)	SD	Mean (µg/Kg)	SD
Geraniol	nd		7811***	+/- 347	15272***	+/- 681	118***	+/- 6	nd	
Geranic acid	nd		1241***	+/- 115	2265***	+/- 268	46***	+/- 5	nd	
C ₁₃ nor-isoprenoids										
3-hydroxy-β-Damascone	186	+/- 30	312	+/- 26	353**	+/- 55	201	+/- 29	294	+/- 59
2-hydroxy-β-ionone	186	+/- 11	449***	+/- 2	341**	+/- 1	125	+/- 1	154	+/- 6
3-hydroxy-5,6-epoxy-β-ionone	900	+/- 4	1305	+/- 11	1880***	+/- 22	812	+/- 10	1029	+/- 35
Fatty acid derivated										
3-methyl-1-butanol	269	+/- 22	388	+/- 18	387	+/- 17	310	+/- 13	332	+/- 9
1-pentanol	154	+/- 4	263	+/- 1	176	+/- 6	164	+/- 4	192	+/- 1
2-methyl-2-buten-1-ol	nd		2158***	+/- 8	4181***	+/- 51	nd		nd	
2-methyl-butanoic-acid	62	+/- 7	79	+/- 4	51	+/- 2	98	+/- 3	137***	+/- 9
C ₆ compounds										
2-hexenal	1022	+/- 2	1957**	+/- 59	3102***	+/- 106	964	+/- 24	765	+/- 24
Hexanoic acid	498	+/- 10	955**	+/- 34	984**	+/- 9	365	+/- 5	345	+/- 0
1-hexanol	237	+/- 1	420	+/- 2	452**	+/- 0	308	+/- 6	238	+/- 3
<i>cis</i> 3-hexenol	153	+/- 4	212	+/- 1	489***	+/- 4	399***	+/- 3	247	+/- 1
2-hexen-1-ol	78	+/- 1	1619***	+/- 2	188***	+/- 3	149**	+/- 3	85	+/- 2
4-methyl-1-hexanol	111	+/- 4	199**	+/- 2	262***	+/- 1	151	+/- 2	122	+/- 2

*** P < 0.001, ** P < 0.01 as compared with wild type (WT); SD = Standard Deviation; nd = not detected.

Table S5. Concentration (micrograms per Kg of leaves, dry weight) of bound aromatic compounds from T₀ tobacco plants transformed with *VvDXS N284* and *VvDXS K284* alleles.

Monoterpenoids	VvDXS N284				VvDXS K284					
	WT		B3-2 line		B3-12 line		B6-14 line		B6-13 line	
	Mean (µg/Kg)	SD	Mean (µg/Kg)	SD	Mean (µg/Kg)	SD	Mean (µg/Kg)	SD	Mean (µg/Kg)	SD
Linalool	3 +/- 0		259*** +/- 12		894*** +/- 55		48*** +/- 0		27*** +/- 1	
<i>trans</i> 8-OH linalool	35 +/- 3		84** +/- 9		107*** +/- 4		41 +/- 6		47 +/- 3	
<i>cis</i> 8-OH linalool	82 +/- 8		517*** +/- 7		678*** +/- 32		260*** +/- 36		212** +/- 2	
Geraniol	271 +/- 27		11746*** +/- 223		26885*** +/- 680		1000*** +/- 118		506 +/- 22	
Geranic acid	9 +/- 0		1786*** +/- 75		4380*** +/- 135		68*** +/- 10		15 +/- 0	
Nerol	13 +/- 1		202*** +/- 7		470*** +/- 5		24 +/- 3		25 +/- 1	
Neryl acetate	8 +/- 0		161*** +/- 1		271*** +/- 18		18 +/- 2		18 +/- 1	
Citronellol	4 +/- 0		72*** +/- 4		179*** +/- 5		6 +/- 1		8 +/- 2	
C ₁₃ nor-isoprenoids										
β Damascenone	22 +/- 4		13 +/- 0		22 +/- 0		27 +/- 1		34 +/- 1	
3-oxo-α-ionolo	2271 +/- 54		2163 +/- 75		1876 +/- 85		2131 +/- 123		3967 +/- 98	
3-hydroxy-β-Damascene	481 +/- 69		321 +/- 14		421 +/- 7		425 +/- 46		672 +/- 32	
3-oxo-7,8-Dihydro-α-ionol	1247 +/- 33		1495 +/- 68		1467 +/- 122		927 +/- 82		1338 +/- 17	
Fatty acid derivated										
3-methyl-1-butanol	124 +/- 14		460*** +/- 37		356*** +/- 60		169 +/- 17		172 +/- 20	

1-pentanol	42 +/- 3	23 +/- 7	4 +/- 0	43 +/- 1	71 +/- 4
2-methyl-2-buten-1-olo	42 +/- 3	1637*** +/- 39	2540*** +/- 25	63 +/- 0	85 +/- 4
2-methyl-butanoic-acid	55 +/- 2	216*** +/- 9	65 +/- 10	50 +/- 13	124 +/- 13
1-octanol	13 +/- 1	43*** +/- 15	41*** +/- 2	17 +/- 1	20 +/- 2
3,6-octadien-1-olo	8 +/- 1	11 +/- 2	10 +/- 0	13 +/- 2	19 +/- 1
C ₆ compounds					
Hexanoic acid	13 +/- 3	65*** +/- 2	26 +/- 3	20 +/- 7	27 +/- 3
1-hexanol	31 +/- 3	79** +/- 1	111*** +/- 1	51 +/- 2	67 +/- 3
<i>cis</i> 3-hexenol	45 +/- 4	99 +/- 5	294*** +/- 4	123** +/- 5	121** +/- 2
Octanoic acid	21 +/- 6	66*** +/- 2	45 +/- 9	17 +/- 5	26 +/- 2

*** P < 0.001, ** P < 0.01 as compare with wild type (WT); SD = Standard Deviation.

Supplementary Figures

Figure S1

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VvDXS_N284 MALCTLSFPAHF*SQAAASNPQRLTPQC*SHLFLGVDLQCQSQQRSKARKRPNGVCASLSDREEYHSQRPPPTLLD*TINYPI [ 80]
VvDXS_K284 MALCTLSFPAYF*SQAAASNPQRLTPQC*SHLFLGVDLQCQSQQRSKARKRPNGVCASLSDREEYHSQRPPPTLLD*TINYPI [ 80]

VvDXS_N284 HMK*NLSVKELKQLADELRS*DVVFNVSKTGGHLGSSSLGVVELTVALHYVFNAPQDRILWDVGHQSYPHKILTGRRDQMHTM [160]
VvDXS_K284 HMK*NLSVKELKQLADELRS*DVVFNVSKTGGHLGSSSLGVVELTVALHYVFNAPQDRILWDVGHQSYPHKILTGRRDQMHTM [160]

VvDXS_N284 RQTDGLAGFTKRSESEYDCFGTGHSS*TTISAGLGMVGRDLK*GKNNNVI*AVIGDGAMTAGQAYEAMNAGYLSDSMIVIL [240]
VvDXS_K284 RQTDGLAGFTKRSESEYDCFGTGHSS*TTISAGLGMVGRDLK*GKNNNVI*AVIGDGAMTAGQAYEAMNAGYLSDSMIVIL [240]

VvDXS_N284 NDNKQVSLPTATLDGPIPPV*GALSSALSRLQSNRPLRELRE*VANGVTKQIGGPMHELA*AAKVDEYARGMISGSGSTLFEEL [320]
VvDXS_K284 NDNKQVSLPTATLDGPIPPV*GALSSALSRLQSNRPLRELRE*VANGVTKQIGGPMHELA*AAKVDEYARGMISGSGSTLFEEL [320]

VvDXS_N284 GLYYIGPVDGHNIDDLVAILKEVKSTKTTGPVLIHVVTEKGRGYPYAEKAADKYHGVT*KFDPATGKQFKSSAPTQSYTTY [400]
VvDXS_K284 GLYYIGPVDGHNIDDLVAILKEVKSTKTTGPVLIHVVTEKGRGYPYAEKAADKYHGVT*KFDPATGKQFKSSAPTQSYTTY [400]

VvDXS_N284 FAEALIAEAEV*DKDIVAIHAAMGGGTGLNLFHRRFPTRCFDVGIAEQH*AVTFAAGLACEGIKPFCAIYSSFMQRAYDQVV [480]
VvDXS_K284 FAEALIAEAEV*DKDIVAIHAAMGGGTGLNLFHRRFPTRCFDVGIAEQH*AVTFAAGLACEGIKPFCAIYSSFMQRAYDQVV [480]

VvDXS_N284 HDVDLQKLPVKFAMDRAGLVGADGPTHCGAFD*VAFMA*CLPNMVVMA*PADEAE*LFHMVATAAAIDDRPSCFRYPRNGVGI [560]
VvDXS_K284 HDVDLQKLPVKFAMDRAGLVGADGPTHCGAFD*VAFMA*CLPNMVVMA*PADEAE*LFHMVATAAAIDDRPSCFRYPRNGVGV [560]

VvDXS_N284 ELPPGNGGPIEVGRGRILIEGERVALLGYGTAVQ*SCLVASSLLEQHGLRITVADARFCKPLDHALIRSLAKSHEVLITV [640]
VvDXS_K284 ELPPGNGGPIEVGRGRILIEGERVALLGYGTAVQ*SCLVASSLLEQHGLRITVADARFCKPLDHALIRSLAKSHEVLITV [640]

VvDXS_N284 EEGSIGGFGSHVAQFLALNGLLDGTTK*WSPMVL*PD*RYIDHGAPADQLAMAGLTPSHIAATVFNILGQTREALEIMS [716]
VvDXS_K284 EEGSIGGFGSHVAQFLALNGLLDGTTK*WSPMVL*PD*RYIDHGAPADQLAMAGLTPSHIAATVFNILGQTREALEIMS [716]
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Figure S1 Alignment of the amino acid sequence of VvDXS alleles. The alleles of Moscato Bianco differ for three amino acid substitutions (H11Y, K284N, V560I) in the predicted protein sequence. These non-synonymous mutations are indicated by asterisk (*).

Figure S2

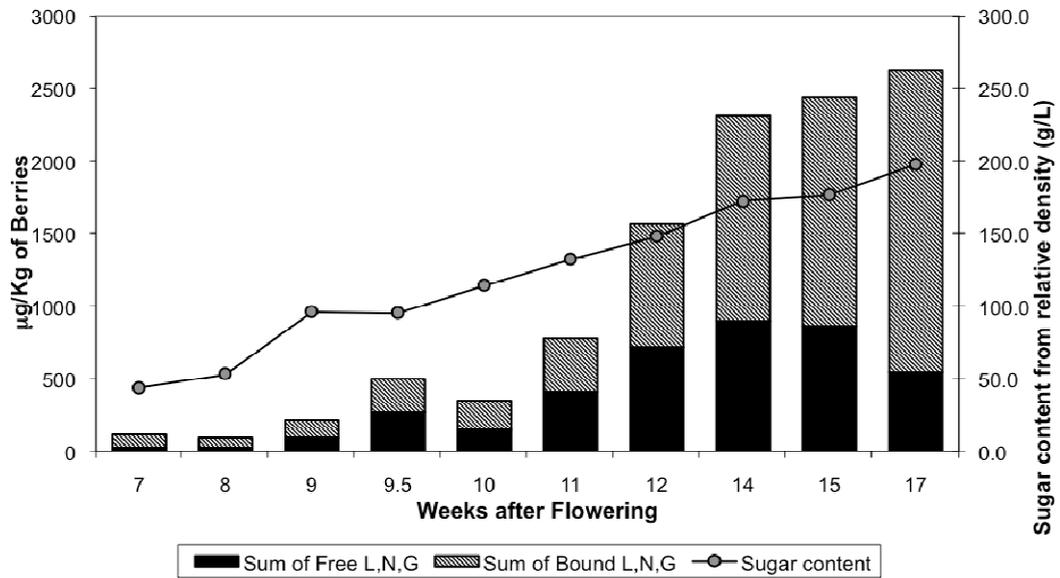


Figure S2 Evolution of sugar and monoterpoid contents during berry maturation.

Dots show the sugar concentration inferred from relative density (g/L).

L = linalool; N = nerol; G = geraniol.

Figure S3

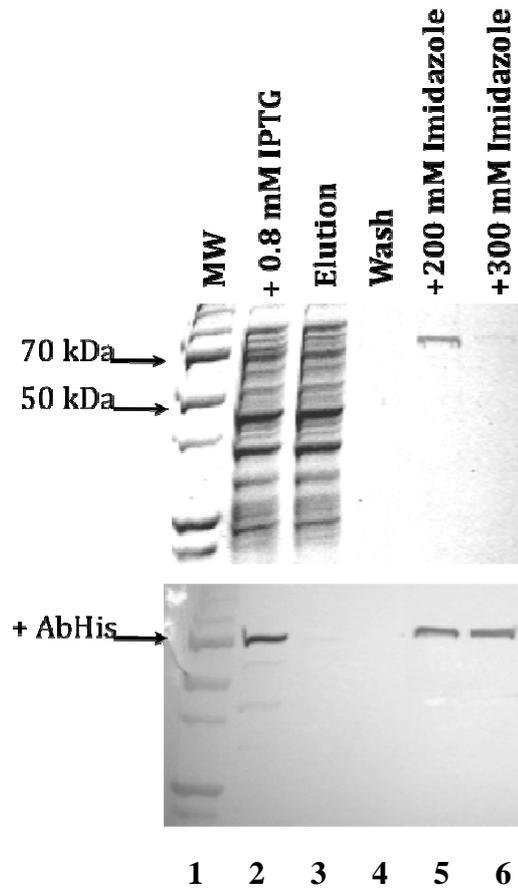


Figure S3: His-tagged protein purification using Ni-NTA resin and immunoblot test. Lane 1 = Molecular weight standard (MW); Lane 2 = Lysate of *Escherichia coli* after induction with 0.8 mM Isopropil β -D-1-thiogalattopiranoside (IPTG); Lane 3 and Lane 4 = Elution and Washing of *E.coli* lysate after purification on Ni-NTA resin. Lane 5 and Lane 6 = VvDXS protein purified on Ni-NTA resin by using two different imidazole concentrations. AbHis = Anti-Histidine Tag antibody

Figure S4

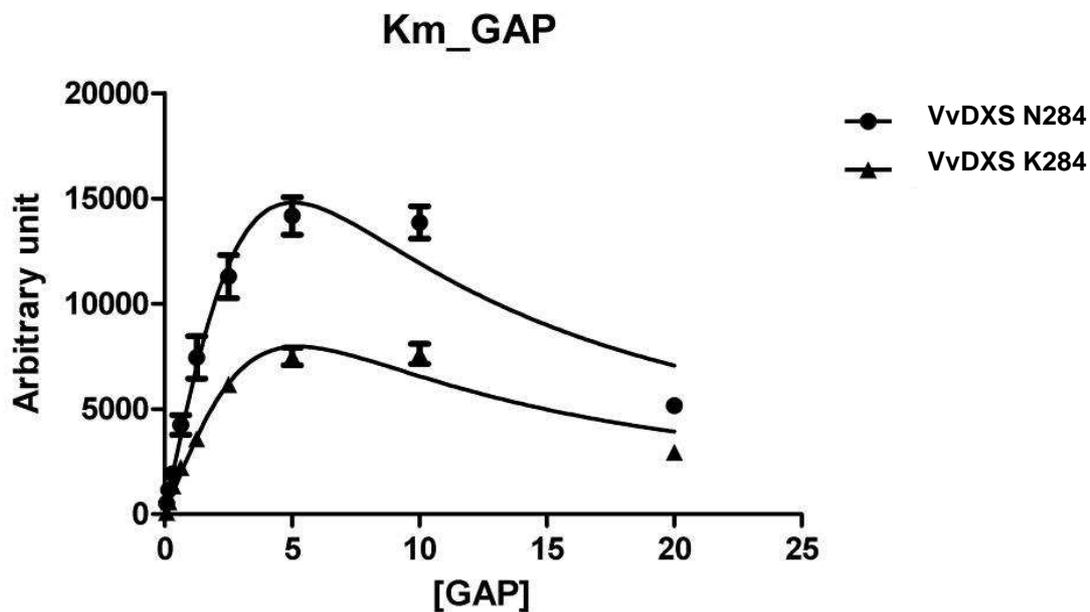


Figure S4: Effects of substrate concentration on the activities of purified VvDXS N284 and VvDXS K284. [GAP] is the DL-glyceraldehyde 3-phosphate (mM) concentration. Initial velocity is expressed as an arbitrary unit and calculated by measuring the amount of DXP. Michaelis constant (K_M) is experimentally defined as the concentration at which the rate of the enzyme reaction is half the maximum velocity.

Figure S5

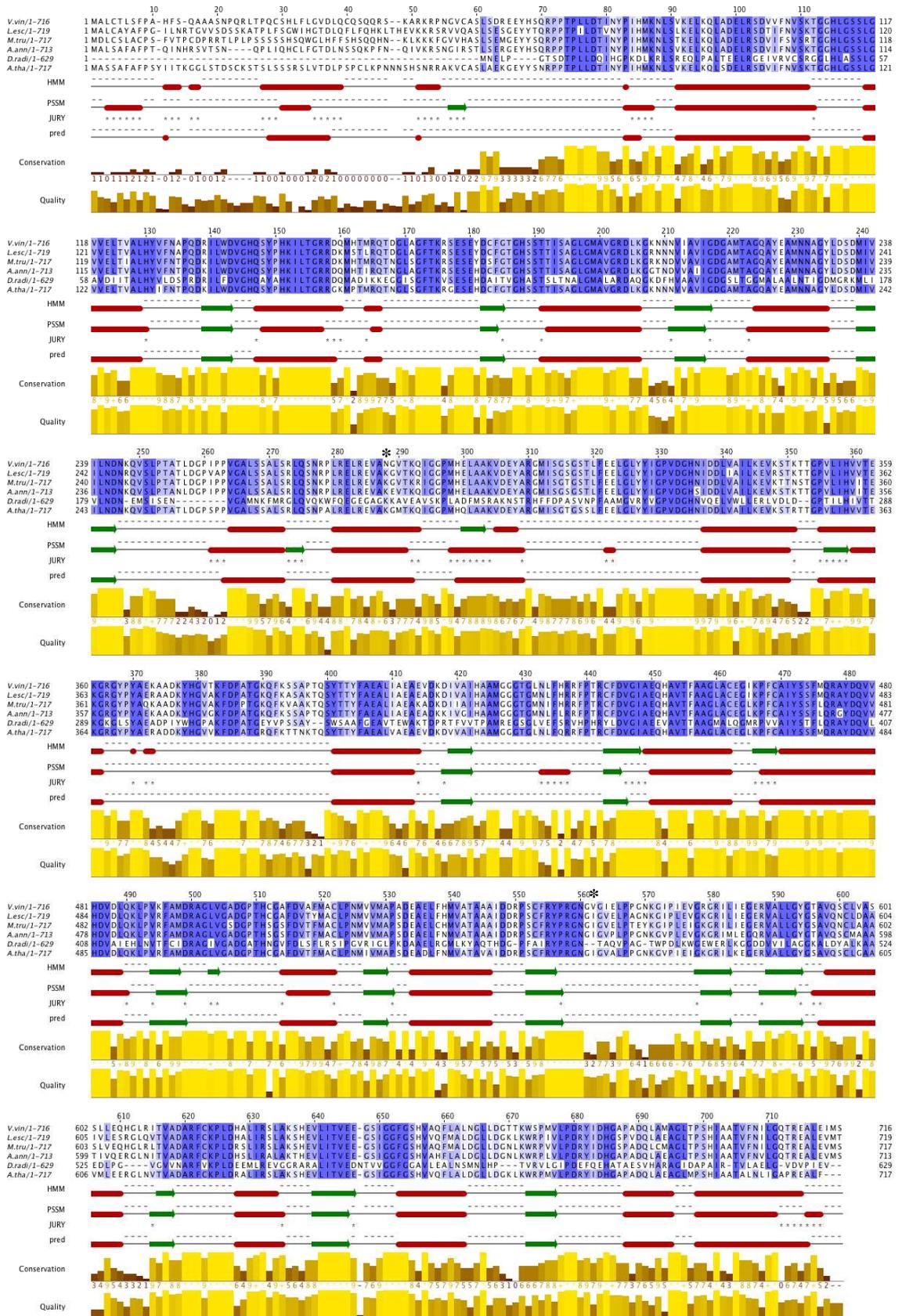
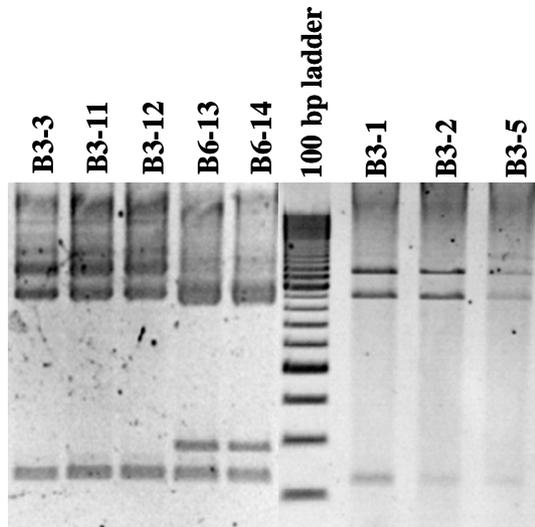


Figure S5: Sequence comparison of the predicted amino acid sequence of DXS. *Vitis vinifera* - VvDXS_N284, *Deinococcus radiodurans* - Q9RUB5, *Lycopersicon. esculentum* - Q9XH50, *Artemisia annua* - Q9SP65, *Medicago. truncatula* - Q8L693 and *Arabidopsis thaliana* - Q681K3. The first 70 amino acids of each sequence represent the signal peptide of transition to chloroplast. Amino acid identity is marked in blue scale and * indicates non-synonymous mutations that change the amino acid sequence found in VvDXS.

Secondary structures are predicted by using Hidden Markov Model (HMM), Position-specific scoring matrix (PSSM), JURY and pred algorithms. Below the sequences are reported the alpha helix (red bars) and β -strand (green arrows) regions. Yellow histograms represent the alignment quality and the conservation level among sequences. Identical amino acids are marked by yellow asterisk and similar amino acid are marked by cross (+)

Figure S6

A)



B)

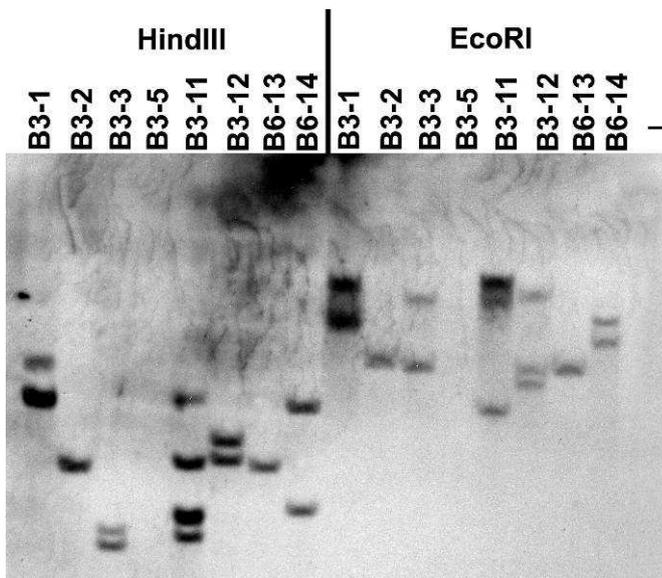


Figure S6: Evaluation of T-DNA copy number in transgenic tobacco lines **A)** Digested transgene PCR products of transformed T₀ tobacco plants. **B)** Southern blot with an *nptII* gene probe of genomic DNA digested with *HindIII* and with *EcoRI*. (-) = wild type