Integration Of Meta-QTL Analysis With Transcriptomic Data Identifies Candidate-Genes Controlling Veraison Time In Grapevine

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Introduction

In grapevine, the genetic and molecular knowledge of phenology-related processes like flowering, veraison and ripening is a key step towards the breeding of varieties that are adapted to a world affected by severe climate change. Different quantitative trait loci (QTL) studies are available in literature attempting the dissection of the genetic control of phenology-related traits. However, the identified genomic regions often span very large portions of the genome where thousands of candidate-genes are present. Transcriptomic studies have identified different sets of genes modulated along ripening, including

direct regulators (switch) and indirectly modulated genes (Palumbo et al., 2014). Also in this case, the number of genes is still too large for a functional characterization.



cultivar during veraison

Aims

With the aim of identifying among this big number the most promising candidate-genes

involved in the regulation grapevine phenology, we developed a candidate-gene prioritization strategy based on two steps. In the first step we performed a meta-QTL analysis in order to narrow down the QTL intervals available in literature obtained from studies investigating the traits flowering, veraison and ripening. In the second step we checked the co-localization of genes identified from transcriptomic studies (Zenoni et al., 2010 and Fasoli et al., 2012) with the genes underlying the newly generated mQTLs. In this way, the number of candidate genes putatively modulating grapevine phenology was reduced by several orders of magnitude.

Experimental procedure

Step 1 – Meta-QTL analysis

- Grapevine genetic linkage maps were collected from literature
- The individual maps were integrated into a consensus map using Biomercator v4.2 (Arcade et al., 2004)
- Phenology-related QTLs were projected onto the consensus map
- Meta-QTL analysis was performed

Step 2 – Transcriptomic data integration

- The grapevine consensus map anchored to the grapevine genome
- Genes below the meta-QTLs intervals were identified
- Co-localization of the positional meta-QTL with transcriptional genes candidates was assessed

Results

Venuti et al. 2013

Viana et al. 2013

Duchêne et al. 2012 Guo et al. 2015 1,256.4 1,270 (CS); 1,410 Riaz et al. 2006

■ Unique Markers
■ Shared Markers Figure 3. Bar-plot showing the occurrence of markers composing the consensus map. In

Figure 2. The nineteen linkage groups of the grapevine consensus map. Distances are in centiMorgan.

dark green the number of markers in common between the original linkage

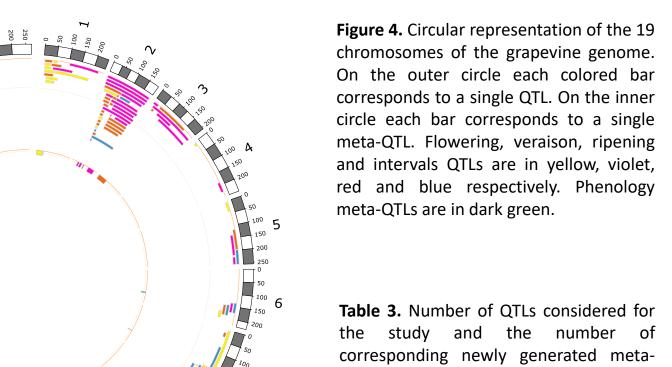
Table 2. List of all the phenology-related QTL studies and QTL traits description used in the meta-QTL analysis.

Consensus map

3130 markers (164/LG)

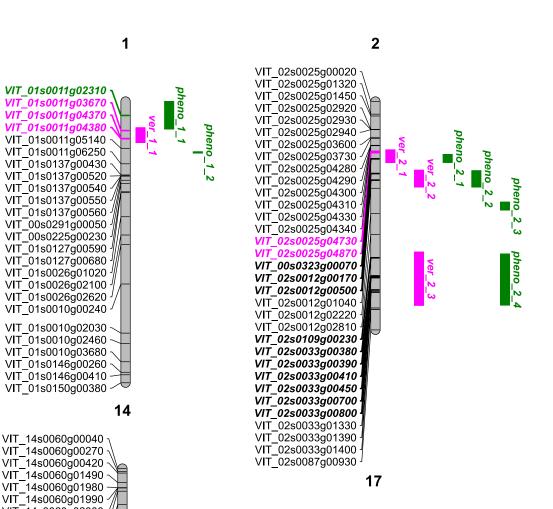
1209 shared markers (63/LG)

Markers Occurrence



corresponds to a single QTL. On the inner		Rp	Ripening	Ripening, Phenolog
circle each bar corresponds to a single		Vr-Rp	Veraison - Ripening Period	Phenology
meta-QTL. Flowering, veraison, ripening	Carreño Ruiz, PhD thesis 2012	BClr	Berry Color	Ripening, Phenolog
	, , , , , , , , , , , , , , , , , , , ,	FT	Flowering Time	Flowering, Phenolog
and intervals QTLs are in yellow, violet,		VT	Veraison Time	Veraison
red and blue respectively. Phenology		RT	Ripening Time	Phenology
meta-QTLs are in dark green.	Costantini et al. 2008	FT	Flowering Time	Flowering, Phenolog
		VT	Veraison Time	Veraison, Phenolog
		VP	Veraison Period	Veraison, Phenolog
		R	Ripening	Phenology
		F-V	Flowering - Veraison Interval	Phenology
		F-R	Flowering - Ripening Interval	Phenology
		V-R	Veraison - Ripening Interval	Phenology
Table 3. Number of QTLs considered for	Duchêne et al. 2012	B-F	Budbreak - Flowering Interval	Flowering, Phenolog
the study and the number of		F-V	Flowering - Veraison Interval	Phenology
corresponding newly generated meta-	Fechter et al. 2014	FBL	Time Of Full Bloom	Flowering, Phenolog
		FS	Flowering Start	Flowering, Phenolog
QTLs, divided by trait. The number of		VT	Veraison Time	Veraison, Phenolog
positional candidate-genes is also	Grzeskowiak et al. 2013	FB	Flowering Beginning	Flowering
reported.		VB	Veraison Beginning	Veraison, Phenolog
reported.		VE	Veraison End	Veraison, Phenolog
Category QTL QTL Meta-QTL Const	Mejía et al. 2007	RDA	Ripening Date	Ripening, Phenolog
Genes Genes	Viana et al. 2013	At	Tartaric Acid	Ripening
	viana et al. 2013	Bpc	Brix Per Cluster	Ripening
Flowering 28 5584 7 1220		БРС	DIIX FEI CIUSIEI	Whening
	Zhao et al. 2015	SSC	Soluble Solids Content	Ripening, Phenolog
Versison 19 1783 / //86				

Bayo Canha, PhD thesis 2015



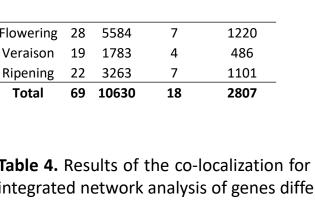
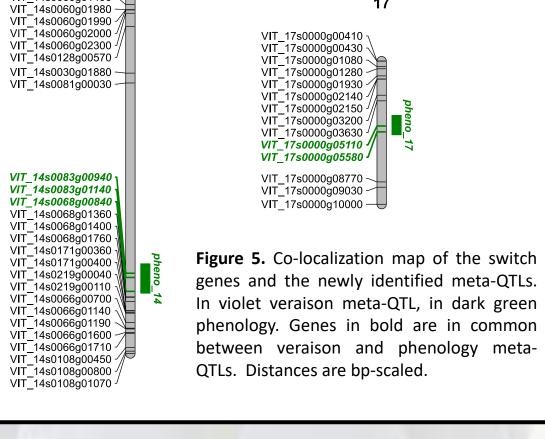


Table 4. Results of the co-localization for the veraison and phenology meta-QTLs with candidate-genes identified by an integrated network analysis of genes differentially expressed along development and berry ripening (switch genes). Category Meta-QTL Start bp End bp Positional **Functional Annotation** Bifunctional nuclease Phosphatidylserine synthase 2 Acyl-(acyl carrier protein) thioesterase VIT 01s0011g02310 Phenology *pheno_1_1* 944396 3194933 Unknown protein *ver_2_1* 3976090 5018212 VIT_00s0323g00070 Pectin methylesterase inhibitor **Glyoxylate reductase** VIT_02s0025g04870 VIT_02s0012g00170 1,4-alpha-D-glucan maltohydrolase VIT 02s0012g00500 Invertase/pectin methylesterase inhibitor *ver_2_3* 12323095 16685865 137 VIT_02s0033g00380 VvMybA2 (C-term) VIT_02s0033g00410 VvMybA1 VIT 02s0033g00450 VvMybA3 VIT 02s0033g00700 Nitrilase Nitrilase 4 (NIT4) Early-responsive to dehydration protein / ERD VIT_02s0109g00230 Phenology *pheno_2_1* 4351615 5007569 VIT 00s0323g00070 Pectin methylesterase inhibitor VIT_02s0012g00170 *pheno_2_2* 5681425 7020161 1,4-alpha-D-glucan maltohydrolase VIT_02s0012g00500 Invertase/pectin methylesterase inhibitor VIT 02s0033g00380 VvMybA2 (C-term) VIT 02s0033g00390 VvMybA2 VIT_02s0033g00410 VvMybA1 VIT_02s0033g00450 VvMybA3



pheno 2 3 8246035 8904947 pheno 2 4 12475143 16681501 Phenology *pheno_6* 15093493 15862725 23 Phenology *pheno_14* 22368340 24770364 Phenology *pheno_16* 14943687 15373367 Phenology *pheno_17* 4856064 6453219 Phenology *pheno_18* 32821037 33053258 15

Nitrilase 4 (NIT4) Early-responsive to dehydration protein / ERD VIT_02s0109g00230 VIT 14s0068g00840 RBR1 (retinoblastoma-related 1) VIT_14s0083g01140 VIT_17s0000g05110 VIT_17s0000g05580 Isopiperitenol dehydrogenase

VIT_02s0033g00700

Nitrilase

Conclusions

Through this two-step prioritization strategy we were able to reduce the number of potential candidate genes by several fold. Interestingly, we found 361 genes previously identified as modulated along grapevine development (Fasoli et al. 2012) to colocalize with the newly identified flowering, veraison or ripening related meta-QTLs. We further checked the colocalization of the switch genes (Palumbo et al., 2014) with the veraison meta-QTLs. We found 25 switch genes co-localizing with the veraison meta-QTLs suggesting a key functional involvement for these

genes during the transition from immature to the mature phase. Among these we found different transcription factors such as MYBA1 and MYBA2 proteins which have been shown to be associated with berry development and ripening. To further assess the involvement of the selected candidate genes in the grapevine phenology process, their nucleotide diversity will be tested for statistical association with phenology-related phenotypic traits in a collection of grapevine germplasm.

Literature cited

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Fasoli M. et al. (2012) The Grapevine Expression Atlas Reveals a Deep Transcriptome Shift Driving the Entire Plant into a Maturation Program. Plant Cell 24, 3489–3505

Palumbo M.C. et al. (2014)

Integrated network analysis identifies fight-club nodes as a class of hubs encompassing key putative switch genes that induce major transcriptome reprogramming during grapevine development. Plant Cell 26, 4617–4635

Zenoni S. et al. (2010) Characterization of Transcriptional Complexity during Berry Development in Vitis vinifera Using RNA-Seq. Plant Phys. 152, 1787–1795

Key message

We have identified a small number candidate-genes for veraison grapevine using meta-QTL analysis integration of transcriptomic data



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