Prioritization of vigor QTL-associated genes for future genome-directed *Vitis* breeding

Priorización de genes asociados a QTLs de vigor para futuros planes de mejoramiento dirigido en *Vitis*

Inés Hugalde [1, 2, *], Marcos Paolinelli[1, 3, *], Cecilia B. Agüero [2], Summaira Riaz [2], Sebastián Gómez Talquenca [1], M. Andrew Walker [2], Hernán Vila [1†]

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Abstract

Vigor control in grapevine may become especially important under climate change. A better understanding of gene-phenotype relationships is required in order to exploit plant genomics for breeding purposes. This research aims to use quantitative trait loci (QTLs) for vigor identified in the progeny from a cross of Ramsey (*Vitis champinii*) × Riparia Gloire (*V. riparia*). Genes located 700 kb up and downstream from each QTL position were interrogated for functional enrichment through ShinyGO online tool, based on the gene ontology annotation of *Vitis vinifera* PN40024. Key biological processes like phloem and xylem development, cell cycle, response to hormones, amino acid transport, tissue development, sugar metabolism, nitrogen transport, and stress/immune responses, showed functional enrichment. Integral response to light and auxin might be required for fine molecular tuning of vegetative growth in *Vitis*. Fifty out of 1318 candidate genes were prioritized, reducing their amount to a manageable number of candidates genes for further directed breeding strategies.

Keywords
biological processes • gene ontology • QTL map • vigor • gene prioritization

1 Instituto Nacional de Tecnología Agropecuaria (INTA). Estación Experimental Agropecuaria Mendoza. Luján de Cuyo (5507). Mendoza. Argentina. hugalde.ines@inta.gob.ar
2 University of California Davis. Department of Viticulture and Enology. Davis (CA 95616). California. USA.
3 Centro Científico Tecnológico-Consejo Nacional de Investigaciones Científicas y Técnicas de Argentina (CCT-CONICET). Mendoza (5500). Mendoza. Argentina.

* This authors contributed equally to this study.

Resumen

El control del vigor en vid es un factor de gran importancia en el contexto de cambio climático actual. Es necesario desarrollar una mejor comprensión de la relación gen-fenotipo para la asistencia del mejoramiento vegetal mediante genómica. En este trabajo se utilizó la información obtenida de un mapeo de QTLs para vigor realizado con la progenie de Ramsey (*Vitis champinii*) × Riparia Gloire (*V. riparia*). De acuerdo a la anotación ontológica de Vitis vinifera PN40024, los genes ubicados 700 kb por encima y por debajo de cada marcador fueron interrogados para determinar enriquecimiento funcional a través de la herramienta online ShinyGO. Distintos procesos clave en la definición de vigor mostraron enriquecimientos altamente significativos, tales como desarrollo tisular (floema y xilema), ciclo celular, transporte de aminoácidos (nitrógeno) metabolismo de azúcares y respuesta a hormonas, a estrés e inmune. La respuesta integral a la luz y la producción de auxinas parece modular molecularmente el crecimiento vegetativo en *Vitis*.

Palabras clave
proceso biológico • ontología génica • mapeo de QTL • vigor • priorización de genes

Introduction

The understanding of genotype-phenotype associations constitute a major challenge for plant scientists. The causal association between variation of a certain trait and genotypic differences is the foundation for developing targeted strategies to be used in molecular breeding (1).

Vigor is considered as the propensity to assimilate, store, and/or use non-structural carbohydrates to produce large canopies. It is associated with high metabolism and rapid shoot growth (17). The main processes that account for vigor are: 1- carbon assimilation or photosynthesis, 2- cell growth, by division and expansion, and 3- leaf area development, for the reception of CO_2 and light. All these complex processes contribute to what is known as a highly quantitative trait.

There is increasing interest in understanding the genetic basis of grapevine vigor and biomass production, given its substantial impact on yield, water management, plant health, and fruit quality. Advances in understanding the genetic bases of complex traits through genetic mapping and quantitative trait locus (QTL) analysis have linked certain complex phenotypes to specific regions of chromosomes, and helped to identify the action, number, and precise location of these regions. The Ramsey (*Vitis champinii*) × Riparia Gloire (*V. riparia*) linkage map was developed by Lowe and Walker (2006) with the goal of mapping traits like biotic resistance, drought tolerance and vigor. We recently reported QTLs for vigor in this progeny that are associated with leaf area, specific leaf area, field canopy biomass and several partitioning indices (12). However, the genes behind these quantitative traits have not been identified. Here, we analyze the function of genes surrounding the QTLs based on gene location and the Gene Ontology (GO) annotation of the *V. vinifera* genome. GO is a computational representation of the functions of protein and non-coding RNA molecules produced by genes from many different organisms. It can be used for interpreting large-scale molecular biology experiments to gain insight into the structure, function, and dynamics of an organism. This approach was proposed by Correa *et al.* (2014) when identifying QTLs and candidate genes associated with cluster architecture in grapevines, and later by Ritcher *et al.* (2019), when approaching cluster architecture and revealing genes enriched by physical projection of the QTLs onto the PN40024 reference genome sequence.

In this paper, we communicate the results of a GO functional enrichment to prioritize candidate genes responsible for vigor that interrogated 1318 genes obtained from the QTL mapping of the Ramsey x Riparia Gloire progeny. We also attempt to elucidate the putative role of genes in terms of physiological and biochemical processes and identify priority genes for further targeted breeding approaches.

Materials and Methods

Six highly significant QTLs for vigor components from the Ramsey x Riparia Gloire map were selected based on their LOD values above the genome wide significance level (12). The BLAST server against the *V. vinifera* PN40024 genome (https://urgi.versailles.inra.fr/blast/) was then used to locate the hybridization regions of PCR primers targeting the simple sequence repeat (SSR) markers linked to the selected QTLs (table 1, page 30-31). The coordinates 700 kb upstream and downstream of primer hybridization on the reference sequence were used to identify the genes within the region flanked by those limits. The 700 kb limit was established considering that candidate genes should be no farther than 3-4 cM from the marker, since the *V. vinifera* genome has been estimated at about 475 Mb and 1 cM equals 200-300 kb (3). For that purpose, a script was developed to search for annotated genes flanking the QTL regions in the annotation file of the *V. vinifera* PN40024 genome.

The annotated genes found with the script were then used to interrogate for functional enrichment through ShinyGO online tool version 0.61 (8). For the set of genes contained in the QTL regions associated with vigor, the occurrence of associated gene ontology terms (GO terms) was statistically evaluated for overrepresentation through an hypergeometric test retrieving those GOterms with statistical significance (False Discovery Rate < 0.05) when comparing their percentage of occurrence with the percentage of each GOterm in the whole annotated genome. Integral plots that associate gene locations in chromosomes with enriched GO categories were performed with shinyCircos (22).

Results

In this work, GO functional enrichment allowed the identification of genes involved in key processes related to vigor and prioritization reduced the number of candidate genes from 1318 to 50.

Our results show that associations between overrepresented GO terms and vigor helped to rank candidate genes, based on their putative function. Phloem and xylem development, cell cycle, response to hormones, tissue development, aminoacid and nitrogen transport, sugar metabolism and immune responses, all showed functional enrichment (figure 1 page 32; table 1, page 30-31). On chromosome 1, most identified genes encode for aminoacid transmembrane transporters (figure 1 page 32; table 1, page 30-31). In addition, the single gene found on chromosome 3 influences the enrichment for functions related to transport of organic acids and nitrogen compounds.

Two important transcription factors (TFs) related to photomorphogenesis (TF 104879018) and auxins (TF 104879021) were identified on chromosome 4 (figure 1 page 32; table 1, page 30-31). TFs are especially interesting because they control the expression of several genes. Genes in chromosome 10 are involved in biotic and abiotic stress responses (figure 1 page 32; table 1, page 30-31). Another transcription factor related to this processes, identified in table 1 (page 30-31) as 100243518, appears significant in this chromosome. On chromosomes 14 and 19, we found the most varied group of genes in terms of function (figure 1 page 32; table 1, page 30-31), including, once again, genes encoding for nitrogen transport, that are key for growth, cell cycle regulation and development (13, 19). Many genes encode for proteins involved in phloem and xylem development. This was the function with major enrichment (figure 1 page 32; table 1, page 30-31). It represents a key aspect for growth, as transport of water, nutrients, proteins and sugars is vital for the plant to develop.

Discussion

Although the analysis used the *V. vinifera* PN40024 genome, due to its superior characterization and annotation, sequence alignment with *V. riparia* Gloire (9) of chromosomes with significant QTLs averaged 96,77 % (data not shown). Also, Liang *et al.* (2019) determined a 97.34-97.65% identity through whole genome comparison of *V. vinifera* PN40024 with two *V. riparia* accessions.

Table 1. Prioritized genes for vigor based on Gene Ontology enrichment analysis and hypothetical function of predicted proteins. SSR markers associated with selected QTLs are shown with chromosome number.

Tabla 1. Genes priorizados para vigor basados en enriquecimiento por ontología génica y sus respectivas funciones proteicas hipotéticas. Los marcadores SSR asociados con los QTLs elegidos se identifican por número de cromosoma.

Chrom # Marker ID	Locus	Protein/Accession	Function
1 ssrVvUCH29	LOC100240857	Lysine histidine transporter 1 (LHT1)	Amino acid transmembrane transport
	LOC100247632	LHT1	Id.
	LOC100247728	LHT2	Id.
	LOC100257870	LHT1	Id.
	LOC100264875	LHT1	Id.
	LOC100244217	Signal recognition particle subunit SRP72	Targeting secretory proteins to rough endoplasmic reticulum membrane. SRP-dependent cotranslational protein targeting to membrane
	LOC100254597	Protein kish-like	Intracellular transport. Protein secretion
	LOC100256355	ATP-dependent DNA helicase Q-like 5	DNA recombination, repair, replication
3 CTG1030395*[1]	LOC100265163	Vacuolar-sorting receptor 3	Protein targeting to vacuole
4 CTG1011026*[2]	LOC100242237	GTP-binding nuclear prot. Ran-3-like	Nucleocytoplasmic transport. Import prot. into the nucleus and RNA export
	LOC100247365	GTP-binding nuclear prot. Ran-3	Id.
	LOC100243952	Stromal cell-derived factor 2-like prot.	Innate immune response. Defense response to bacteria and fungi
	LOC100249173	Ammonium transporter 3 member 1	Ammonium transmembrane transport
	LOC100254226	Overexpressor of cationic peroxidase 3	1. Innate immune response. Response to bacteria, fungi, ABA, jasmonic acid and water deprivation
	LOC100262771	RNA-dependent RNA polymerase 6	RNA silencing pathway and generation of small interfering RNAs
	LOC100268069	Uncharacterized prot.	Protein import into chloroplast stroma
	LOC104879018	Transcription factor HY5	Red/far red signalling pathway. Regulation of photomorphogenesis
	LOC104879021	Auxin-responsive prot. IAA28	Repression of early auxin response genes at low auxin concentrations
10 VrZAG64	LOC100243518	Transcription factor VOZ1	Response to heat, cold, salt, drought, and light. Defense to bacteria, incompatible interaction
	LOC100243637	MACPF domain-containing prot. NSL1	Hypersensitive response. Inmune response
	LOC100245146	Uncharacterized prot.	Regulation of systemic acquired resistance (SAR) and transcription. Histone modification
	LOC100247033	Homeobox-DDT domain protein RLT1	Regulation of transcription. Regulation of transition from vegetative to reproductive phase
	LOC100255452	Mitochondrial arginine transporter BAC1	Nitrogen compound transport. Mitochondrial transmembrane transport

*[1] CTG1030395 (5′–3: FW-TCCCTACAATCTCATCGCAA, RV-CATGGCTCAAGAGAGTGCAA)

*[2] CTG1011026 (5′–3: FW-GAAGAACACCACAGCAAGCA, RV-AAAATGCACAATCTCCCACC)

Additional information is available at http://www.ncbi.nlm.nih.gov/gene and www.uniprot.org. Locus with lowest FDR values and the highest QTL LOD scores have been shaded.

Información adicional se encuentra disponible en http://www.ncbi.nlm.nih.gov/gene and www.uniprot.org. Los locus con menor FDR y valores mayores de LOD score en el mapa de QTLs, se ven sombreados.

Table 1 (cont.). Prioritized genes for vigor based on Gene Ontology enrichment analysis and hypothetical function of predicted proteins. SSR markers associated with selected QTLs are shown with chromosome number.

Tabla 1(cont.). Genes priorizados para vigor basados en enroqiecimiento por ontología génica y sus respectivas funciones proteicas hipotéticas. Los marcadores SSR asociados con los QTLs elegidos se identifican por número de cromosoma.

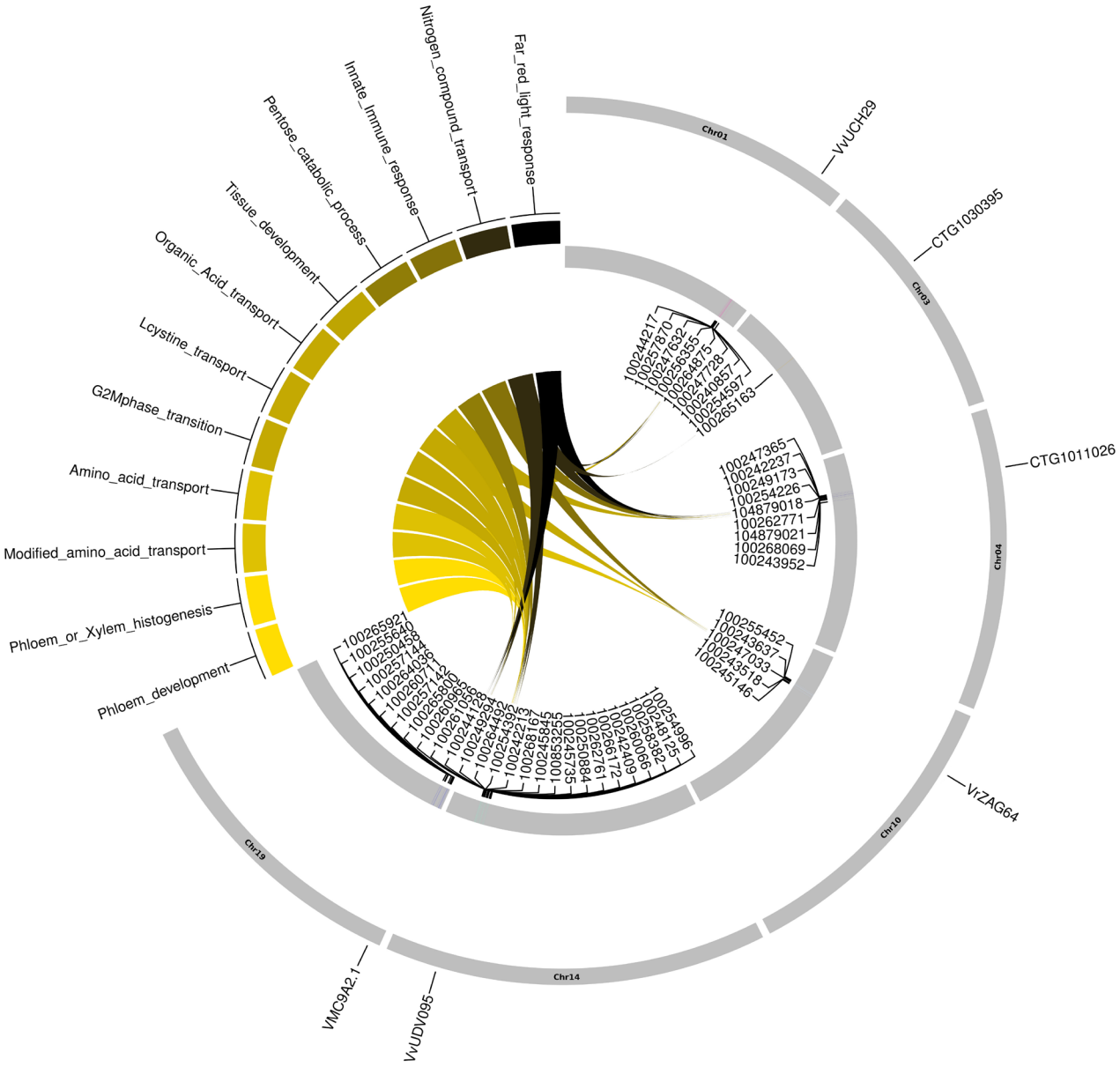
Chrom # Marker ID	Locus	Protein/Accession	Function
14 UDV-095	LOC100242409	Ribulose-phosphate 3-epimerase, cytoplasmic isoform	Carbohydrate metabolic process. Catalitic activity
	LOC100242213	Sieve element occlusion B-like/	Phloem development
	LOC100244128	Sieve element occlusion B	Id.
	LOC100245845	Sieve element occlusion B	Id.
	LOC100249294	Sieve element occlusion B	Id.
	LOC100254392	Sieve element occlusion B-like	Id.
	LOC100261056	Sieve element occlusion B	Id.
	LOC100264492	Sieve element occlusion B	Id.
	LOC100268167	Sieve element occlusion B-like	Id.
	LOC100245735	Cystinosin homolog	L-cystine transmembrane transport
	LOC100250884	Cystinosin homolog	Id.
	LOC100248125	Mitochondrial import receptor subunit TOM20	Protein insertion into mitochondrial outer membrane
	LOC100254996	Transport prot Sec61 subunit gamma	Protein transmembrane transport
	LOC100258382	Transcription and mRNA export factor SUS1	Nitrogen compound transport. Regulation of transcription
	LOC100260066	Chloride channel ClC4.	Nitrate transmembrane transport. Chloride transport
	LOC100260965	Ras-related protein RABB1b	Nitrogen compound transport. Intracellular protein transport
	LOC100262761	LHT1	Amino acid transmembrane transport
	LOC100266172	COP9 signalosome complex subunit 3	Positive regulation of G2/M transition of mitotic cell cycle.Protein catabolic process
	LOC100853255	RPM1-interacting prot. 4	Defense response signaling pathway. AvrRpt-cleavage domain-containing prot
19 VMC9A2.1	LOC100250458	Transport prot Sec61 subunit alpha	Protein transmembrane transport
	LOC100255640	IRK-interacting protein	Negative gravitropism. Response to light
	LOC100257142	Probable sodium/metabolite cotransporter BASS1 chloroplastic	Nitrogen compound transport. Panthotenate import across plasmamembrane
	LOC100257144	Septum-promoting GTP-binding protein 1	GTPase activity. Intracellular protein transport
	LOC100260711	B-box zinc finger protein 22	Anthocyanin-and chlorophyll biosynthetic process. Chloroplast organization. Photomorphogenesis. Regulation of transcription
	LOC100264036	TOM1-like protein 2	Intracelullar protein transport
	LOC100265800	Ribokinase	Phosphorylation of ribose, can then be used for sythesis of nucleotides and aminoacids, or in pentose phosphate pathway
	LOC100265921	Ras-related protein Rab11D-like	GTPase activity. Hyperosmotic salinity response. Vesicle mediated transport

*[1] CTG1030395 (5′–3: FW-TCCCTACAATCTCATCGCAA, RV-CATGGCTCAAGAGAGTGCAA)

*[2] CTG1011026 (5′–3: FW-GAAGAACACCACAGCAAGCA, RV-AAAATGCACAATCTCCCACC)

Additional information is available at http://www.ncbi.nlm.nih.gov/gene and www.uniprot.org. Locus with lowest FDR values and the highest QTL LOD scores have been shaded.

Información adicional se encuentra disponible en http://www.ncbi.nlm.nih.gov/gene and www.uniprot.org. Los locus con menor FDR y valores mayores de LOD score en el mapa de QTLs, se ven sombreados.



Lines connect genes to enriched GO terms (ranging from FDR 0.034 in black, to FDR 1x10^{-9} in gold).
Las líneas conetan los genes con los términos de enriquecimiento desde FDR 0,034 en negro hasta FDR 1x10^{-9} en dorado).

Figure 1. Chromosome location of vigor-associated genes identified through GO functional enrichment. Genes and SSR markers showing GO categories enrichments (FDR<0.05) are indicated on chromosomes of *V vinifera.*

Figura 1. Ubicación cromosómica de genes asociados a vigor identificados por enriquecimiento funcional GO (FDR<0,05) y marcadores SSR en los cromosomas de *V vinifera.*

As mentioned, on chromosome 1, most identified genes encode for aminoacid transmembrane transporters (figure 1; table 1, page 30-31). Transmembrane aminoacid transport is associated with enhanced growth and high rates of protein synthesis (20). Nitrogen can be taken up from soil in various forms, being one of them amino acids, and is of considerable importance in vigor control, yield and berry quality (7). The fact that these genes are closely located, suggests some kind of structural regulation. It has been observed that root elongation and enlargement in the rootstock 110R, partly depend on transcriptomic regulations of sugar and protein transporter genes *SWEET* and *NRT1/PTR* in roots. This was found to facilitate carbohydrate and nitrogen accumulation, providing essential energy to 110R roots under drought (21).

In relation to the two important TFs related to photomorphogenesis and auxins identified on chromosome 4, they are particularly intriguing given that they control the expression of several genes. The particularity of having both TFs in the same chromosomal region increases the probability of a synergistic response through coregulation. Indeed, the coordination between TFs in response to light and auxins was well established in *Arabidopsis thaliana* by Halliday, *et al.* (2009), who showed that light regulates growth of distant tissues from the site of light exposure through auxin production. Something similar was observed in *A. thaliana* by Hornitschek, *et al.* (2012), where phytochrome interacting factors 4 and 5 (*PIF4* and *PIF5*) regulated elongation growth by controlling the expression of genes that encode for auxin biosynthesis and signaling. Interestingly, TF 104879018 in our study is homologous to *HY5* of *A. thaliana*, which is found downstream in the signaling cascade of *PIF1*/*PIF3* (10). *HY5* promotes growth, especially through photosynthesis induction and higher nutrient uptake by roots. The other prioritized TF 104879021, is a homolog to the auxin-responsive protein IAA28 of *A. thaliana*, which plays a role in regulation of lateral root growth. In grapevines, kaolin, a mineral that reflects radiation from the leaf surface, produced an increase in IAA content. This treatment also caused higher values of stomatal conductance, net CO_2 assimilation rate, intrinsic water use efficiency, and a slight decrease in ABA (5). These results might be supported by the same mechanism connecting growth hormones and light interception. Therefore, vigor in grapevines may partly depend on promotion of photosynthesis, lateral root development and nitrate uptake, and these processes may be associated through the expression of genes downstream-regulated by TF 104879018 and TF 104879021.

Regarding the genes and TF found in chromosome 10, involved in biotic and abiotic stress responses, some authors have shown significant correlation between vigor and tolerance/susceptibility to diseases that could induce different defense responses in the host plant (2). Vigorous plants may have developed stronger immune responses to defend themselves.

Considering our findings in chromosomes 14 and 19, many genes encode for proteins involved in phloem and xylem development. This function showed the major enrichment (figure 1 page 32; table 1, page 30-31), representing a key aspect for growth, as transport of water, nutrients, proteins and sugars is vital for the plant to develop. These functions are tightly correlated to auxins and soluble carbohydrates seasonal dynamics, since cambium activity and xylem/phloem development respond to this signaling in woody species. It has been observed that IAA and soluble carbohydrate dynamics directly affect xylem and phloem formation in trees (6). In addition, gibberellins increase carbon allocation to different organs by inducing accumulation of non-structural carbohydrates in leaves, enhancement of phloem area and expression of sugar transporters (16).

Our results may lead to deeper gene selection strategies, aiming at choosing a smaller number of genes. Candidates with the smallest enrichment FDR values that are associated with QTLs that explain the highest percentages of variation, constitute interesting targets. For quantitative characters, where positive feedbacks cause large effects, significant explanatory effects from 10% to 20%, may result in impressive effects. This strategy considers that both approaches, QTL mapping and GO enrichment, work at different levels. While QTLs indentify regions on chromosomes containing genes encoding for a certain trait, the enrichment process takes into account all the genes involved in a pathway related to that trait. In processes where numerous genes are involved, a meaningful change should include many of them. Consequently, transcription factors could produce phenotype differences even at higher FDR values. In our work, this last effort selected 16 genes and 4 TRFs as plausible candidates for further breeding studies (table 1, page 30-31). Further functional genomic studies should weigh the importance of these selected genes on the final phenotype.

Conclusions

In conclusion, this analysis allowed the detection of plausible candidate genes encoding for the components of key processes governing vegetative growth in *Vitis*. The analysis allowed the reduction of candidate gene number based on marker proximity and functional enrichment, clearly demonstrating a suitable shortcut for target-directed genome-guided breeding strategies. This approach is particularly useful when a map is not densely saturated.

Two TFs, which potentially enhance growth by relating light response to hormone activation, and then to photosynthesis and morphogenesis, are strong candidates for targeted breeding. The nitrogen transport encoding genes would allow this light/hormone promoted growth by facilitating amino acid/protein synthesis and transport. Phloem and xylem related genes would also be part of this process by enabling water and nutrient transport. All these functions need to be tightly correlated, since auxins and soluble carbohydrates seasonal dynamics play key roles in tissue growth, cambium activity and xylem/phloem development. Gene characterization in individuals of the Ramsey × Riparia Gloire progeny will be the topic for future research.

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"We respectfully remember Dr. Hernán Vila, who lost his battle against COVID-19 in October 2020 and dedicate this article to his memory"