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 3

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-fecha 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

Introducción

El entendimiento de las asociaciones genotipo-fenotipo constituye un desafío mayor para los científicos de las plantas. La asociación causal entre la variación de una cierta característica y diferencias genotípicas es la fundación para el desarrollo de estrategias dirigidas a ser utilizadas en la cría molecular (1).

El vigor se considera como la propensión para assimilar, almacenar, y/o usar no-estructurales hidratos de carbono para producir grandes canopies. Estas están asociadas con un alto metabolismo y rápido crecimiento y desarrollo (17). Los procesos principales que intervienen en el vigor son: 1- asimilación de carbono o fotosíntesis, 2- crecimiento celular, por división y expansión, y 3- desarrollo de la superficie foliar para la recepción de CO₂ y luz. Todos estos procesos complejos contribuyen a lo que se conoce como un trazo cuantitativo altamente hereditario.

Hay un creciente interés en entender el fundamento genético de vigor y producción de biomasa, dado su impacto sustancial en rendimiento, manejo del agua, salud de la planta, y calidad de los frutos. Avances en el entendimiento de las bases genéticas de caracteres complejos a través de la cartografía genética y análisis de trazos cuantitativos (QTL) han vinculado ciertos complejos fenotipos a regiones específicas de los cromosomas, y ayudado a identificar la acción, número, y localización precisa de estas regiones. El mapa de QTLs para vigor en esta progenie (Ramsey (Vitis champinii) × Riparia Gloire (V. riparia)) fue desarrollado por Lowe y Walker (2006) con el objetivo de cartografiar caracteres como resistencia a plagas, tolerancia al estrés hídrico y vigor. Recientemente, hemos reportado QTLs para vigor en esta progenie que están asociados con área foliar, área foliar específica, biomasa de la canopia y varios índices de particionamiento (12). Sin embargo, los genes detrás de estas características cuantitativas aún no han sido identificados. Aquí, analizamos la función de genes en el rango de QTLs basado en la ubicación del gen y la anotación Gen Ontology (GO) de V. vinifera. GO es una representación computacional de las funciones de proteínas y RNA no-codificantes generadas por genes de muchos diferentes organismos. Se puede utilizar para interpretar experimentos de biología molecular a gran escala para obtener见解 sobre el estructura, función, y dinámica del organismo. Este enfoque fue propuesto por Correa et al. (2014) para identificar QTLs y candidatos a genes asociados con la arquitectura de los clusters en vid, y más tarde por Ritcher et al. (2019), cuando se acercan arquitectura de los clusters y revelan genes enriquecidos por proyección física de los QTLs sobre el cromosoma de referencia PN40024.

En este artículo, comunicamos los resultados de una GO de enriquecimiento funcional para priorizar los genes candidatos que se interrogaron 1318 genes obtenidos desde el QTL de la cartografía del Ramsey x Riparia Gloire progeny. También intentamos elucidar el papel protáctivo de los genes en términos de procesos fisiológicos y bioquímicos y identificar genes de prioridad para futuros enfoques de cría molecular.
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 a hypergeometric test retrieving those GO terms with statistical significance (False Discovery Rate < 0.05) when comparing their percentage of occurrence with the percentage of each GO term 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, amino acid 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 amino acid 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.

| Chrom # Marker ID | Locus | Protein/Accession | Function |
|------------------|-------|-------------------|----------|
| 1 ssrVvUCH29     | LOC100240857 | Lysine histidine transporter 1 (LHT1) | Amino acid transmembrane transport |
|                  | LOC100247632 | LHT1 | 1d. |
|                  | LOC100247728 | LHT2 | 1d. |
|                  | LOC100257870 | LHT1 | 1d. |
|                  | LOC100264875 | LHT1 | 1d. |
|                  | 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 | 1d. |
|                  | 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. Immune 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-TCCCTACAAATCTCATGGGAA, RV-CATGGCTCAAGAGAGTGCA)  
*2 CTG1011026 (5′−3: FW-GAACAGACCCACGAGAAAGCA, RV-AAATGCGAAATCTCCCGGAC)

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 y 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. Catalytic activity                        |
|                  | LOC100242213 | Sieve element occlusion B-like/                       | Phloem development                                                        |
|                  | LOC1002444128 | Sieve element occlusion B                            | Id.                                                                       |
|                  | LOC100245845 | Sieve element occlusion B                            | Id.                                                                       |
|                  | LOC100249294 | Sieve element occlusion B                            | Id.                                                                       |
|                  | LOC10025392  | Sieve element occlusion B-like/                       | Phloem development                                                        |
|                  | LOC100261056 | Sieve element occlusion B                            | Id.                                                                       |
|                  | LOC100264492 | Sieve element occlusion B                            | Id.                                                                       |
|                  | LOC100268167 | Sieve element occlusion B-like/                       | Phloem development                                                        |
|                  | LOC100245735 | Cystinosin homolog                                    | L-cystine transmembrane transport                                         |
|                  | LOC100250888 | Cystinosin homolog                                    | Id.                                                                       |
|                  | LOC100248125 | Mitochondrial import receptor subunit TOM20          | Protein insertion into mitochondrial outer membrane                      |
|                  | LOC10025496 | Transport prot Sec61 subunit gamma                    | Protein transmembrane transport                                           |
|                  | LOC100258382 | Transcription and mRNA export factor SUS1            | Nitrogen compound transport. Regulation of transcription                  |
|                  | LOC100260066 | Chloride channel CIC4.                                | 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. Photo morphogenesis. Regulation of transcription |
|                  | LOC100264036 | TOM1-like protein 2                                   | Intracellular protein transport                                           |
|                  | LOC100265880 | Ribokinase                                            | Phosphorylation of ribose, can then be used for synthesis of nucleotides and aminoacids, or in plants, phosphate pathway |
|                  | LOC100265921 | Ras-related protein Rab11D-like                      | GTPase activity. Hypersosmatic salinity response. Vesicle mediated transport |

* 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.
Prioritization of vigor genes in Vitis

Lines connect genes to enriched GO terms (ranging from FDR 0.034 in black, to FDR 1x10^-9 in gold).

Las líneas conectan 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 CO₂ 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.

REFERENCES

1. Bargsten, J. W.; Nap, P.; Sanchez-Perez, G. F.; Aalt, D. J. van Dijk. 2014. Prioritization of candidate genes in QTL regions based on associations between traits and biological processes. BMC Plant Biol 14: 330. https://doi.org/10.1186/s12870-014-0330-3
2. Calonnea, A.; Burie, J. B.; Langlais, M.; Güyader, S.; Saint-Jean, S.; Sache, I.; Tivoli, B. 2013. Impacts of plant growth and architecture on pathogen processes and their consequences for epidemic behaviour. Eur J Plant Pathol 135: 479-497. doi: 10.1007/s10658-012-0111-5
3. Cipriani, G.; Di Gaspero, G.; Canaguier, A.; Jusseaume, J.; Tassin, J.; Lemainque, A.; Thureau, V.; Adam-Blondon, A.; Testolino, F. 2011. Molecular linkage maps: strategies, resources and achievements. In: Adam-Blondon, A.F.; Martínez Zapater, J.M.; Cole, C. (eds). Genetics, genomics and breeding of grapes. Science Publishers and CRC Press. 111-136. doi: 10.1201/b10948
4. Correa, J.; Mamani, M.; Muñoz-Espinoza, C.; Laborie, D.; Muñoz, C.; Pinto, M.; Hinrichsen, P. 2014. Heritability and identification of QTLs and underlying candidate genes associated with the architecture of the grapevine cluster (Vitis vinifera L.). Theoretical and applied genetics. 127(5): 1143-1162. doi: https://doi.org/10.1007/s00122-014-2286-y
5. Dinis, L. T.; Bernardo, S.; Luizo, A.; Pinto, G.; Meijón, M.; Pintó-Marijuán, M.; Cotado, A.; Correia, C.; Moutinho Pereira, J. 2018. Koaln modulates ABA and IAA dynamics and physiology of grapevine under Mediterranean summer stress. J Plant Physiol. 220: 181-192. doi: 10.1016/j.jplph.2017.11.007
6. Fajstav, M.; Paschová, Z.; Giagli, K.; Vavrčík, H.; Gryc, V.; Urban, J. 2018. Auxin (IAA) and soluble carbohydrate seasonal dynamics monitored during xylemogenesis and phloemogenesis in Scots pine. Forest. 11: 553-562. doi: 10.3832/ifor2734-011
7. Gatti, M.; Squeri, C.; Garavani, A.; Frioni, T.; Dosi, P.; Diti, I.; Poni, S. 2019. Effect of variable nitrogen application on cv Barbera performance: yield and grape composition. Am J Enol Vitic. 70:188-200. doi: 10.5344/ajev.2019.18072
8. Ge, S. X.; Jung, D.; Yao, R. 2020. ShinyGO: a graphical gene-set enrichment tool for animals and plants. Bioinformatics. 36: 2628-2629. doi: 10.1093/bioinformatics/btz931
9. Girolet, N.; Rubio, B.; López-Roques, C.; Valière, S.; Ollat, N.; Bert, P. F. 2019. De novo phased assembly of the Vitis riparia grape genome. Sci Data. 6: 127. doi: 10.1038/s41597-019-0133-3
10. Halliday, K. J.; Martínez-Garcia, J. F.; Josse, E. M. 2009. Integration of light and auxin signalling. Cold Spring Harb Perspect Biol. 1:a001586. doi: 10.1101/cshperspect.a001586
11. Hornitschek, P.; Kohnen, M. V.; Lorrain, S.; Rougemont, J.; Ljung, K.; López-Vidriero, I.; Franco-Zorrilla, J. M.; Solano, R.; Trevisan, M.; Pradervand, S.; Xenarios, I.; Fankhauser, C. 2012. Phytochrome interacting factors 4 and 5 control seedling growth in changing light conditions by directly controlling auxin signaling. Plant J. 71: 699-711. doi: 1111/j.1365-313X.2012.05033.x
12. Hugalde, I. P.; Riaz, S.; Agüero, C. B.; Vila, H.; Gomez Talquenca, S.; Walker, M. A. 2019. Studying growth and vigor as quantitative traits in grapevine populations. In: Maia RT, de Araújo Campos M (eds). Integrated View of Population Genetics. IntechOpen. doi: 10.5772/intechopen.82537
13. Lang, C. P.; Merkt, N.; Zörb, C. 2018. Different nitrogen (N) forms affect responses to N form and N supply of rootstocks and grafted grapevines. Plant Sci. 277: 311-321. doi: 10.1016/j.plantsci.2018.10.004
14. Liang, Z.; Duan, S.; Sheng, J.; Zhu, S.; Ni, X.; Shao, J.; Liu, C.; Nick, P.; Du, F.; Fan, P.; Mao, R.; Zhu, Y.; Deng, W.; Yang, M.; Huang, H.; Liu, Y.; Ding, Y.; Liu, X.; Jiang, J.; Zhu, Y.; Li, S.; He, X.; Chen, W.; Dong, Y. 2019. Whole genome resequencing of 472 Vitis accessions for grapevine diversity and demographic history analyses. Nat Commun. 10: 1190. doi: 10.1038/s41467-019-01935-8
15. Lowe, K. M.; Walker, M. A. 2006. Genetic linkage map of the interspecific grape rootstock cross Ramsey (Vitis champinii) × Riparia Gloire (Vitis riparia). Theor Appl Genet. 112: 1582-1592. doi: 10.1007/s00122-006-0264-8
16. Murcia, G.; Pontin, M.; Reinoso, H.; Baraldi, R.; Bertazza, G.; Gómez-Talquenca, S.; Bottini, R.; Piccoli, P. N. 2016. ABA and GAs increase carbon allocation in different organs of grapevine plants by inducing accumulation of non-structural carbohydrates in leaves, enhancement of phloem area and expression of sugar transporters. Physiol Plant. 156: 323-337. doi: 10.1111/plp.12390
Prioritization of vigor genes in *Vitis*

17. Rebolledo, M.C.; Dingkuhn, M.; Courtois, B.; Gibon, Y.; Clément-Vidal, A.; Cruz, D.F.; Duitama, J.; Lorieux, M.; Luquet, D. 2015. Phenotypic and genetic dissection of component traits for early vigour in rice using plant growth modelling, sugar content analyses and association mapping. *J Exp Bot.* 66: 5555-5566. doi: 10.1093/jxb/erv258

18. Richter, R.; Gabriel, D.; Rist, E.; Töpfer, R.; Zyprian, E. 2019. Identification of co-located QTLs and genomic regions affecting grapevine cluster architecture. *Theor Appl Genet.* 132: 1159-1177. https://doi.org/10.1007/s00122-018-3269-1

19. Tegeder, M.; Masciaux-Daubresse, C. 2018. Source and sink mechanisms of nitrogen transport and use. *New Phytol.* 217: 35-53. doi: 10.1111/nph.14876

20. Wipf, D.; Ludewig, U.; Tegeder, M.; Rentsch, D.; Koch, W.; Frommer, W.B. 2002. Conservation of amino acid transporters in fungi, plants and animals. *Trends Biochem Sci.* 27: 139-147. doi: 10.1016/S0968-0004(01)02054-0

21. Yıldırım, K.; Yağcı, A.; Sucu, S.; Tunç, S. 2018. Responses of grapevine rootstocks to drought through altered root system architecture and root transcriptomic regulations. *Plant Physiol Bioch.* 127: 256-268. doi: 10.1016/j.plaphy.2018.03.034

22. Yu, Y.; Ouyang, Y.; Yao, W. 2018. ShinyCircos: an R/Shiny application for interactive creation of circos plot. *Bioinformatics.* 34: 1229-1231. doi: 10.1093/bioinformatics/btx763

**ACKNOWLEDGMENTS**

Funding from the Instituto Nacional de Tecnología Agropecuaria (INTA, Argentina) and the California Grape Rootstock Improvement Commission is gratefully acknowledged. We also thank Noelia Carrasquilla and Alberto Iandolino for their valuable help. MP acknowledges a CONICET postdoctoral fellowship.

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