agronomy

MDPI

Brief Report

Transcriptome Data Analysis Applied to Grapevine Growth Stage Identification

Francisco Altimiras [1,2,3,*], Leonardo Pavéz [2], Alireza Pourreza [4], Osvaldo Yañez [2], Lisdelys González-Rodríguez [2,5], José García [6], Claudio Galaz [7], Andrés Leiva-Araos [8,*] and Héctor Allende-Cid [1,9,10]

1 School of Informatics Engineering, Pontificia Universidad Católica de Valparaíso, Valparaíso 2340025, Chile; hector.allende@pucv.cl
2 Núcleo de Investigación en Data Science (NIDS), Facultad de Ingeniería y Negocios, Universidad de Las Américas, Santiago 7500000, Chile; lpavez@udla.cl (L.P.); lgonzalezr@udla.cl (L.G.-R.); oyanez@udla.cl (O.Y.)
3 Inria Chile Research Center, Santiago 7550312, Chile
4 Digital Agriculture Laboratory, Department of Biological and Agricultural Engineering, University of California, Davis, CA 94143, USA; apourreza@ucdavis.edu
5 Facultad de Ingeniería y Negocios, Universidad de Las Américas, Sede Concepción, Concepción 4030000, Chile
6 School of Construction and Transportation Engineering, Pontificia Universidad Católica de Valparaíso, Valparaíso 2362804, Chile; jose.garcia@pucv.cl
7 Department of Computer Science, Universidad Técnica Federico Santa María, Santiago 8940897, Chile; claudio.galaz@alumnos.usm.cl
8 Instituto de Data Science, Facultad de Ingeniería, Universidad del Desarrollo, Av. La Plaza 680 Las Condes, Santiago 7610658, Chile
9 Knowledge Discovery, Fraunhofer-Institute of Intelligent Analysis and Information Systems (IAIS), 53757 Sankt Augustin, Germany
10 Lamarr Institute for Machine Learning and Artificial Intelligence, 53115 Dortmund, Germany
* Correspondence: faltimiras@udla.cl (F.A.); andresleiva@udd.cl (A.L.-A.)

Abstract: In agricultural production, it is fundamental to characterize the phenological stage of plants to ensure a good evaluation of the development, growth and health of crops. Phenological characterization allows for the early detection of nutritional deficiencies in plants that diminish the growth and productive yield and drastically affect the quality of their fruits. Currently, the phenological estimation of development in grapevine (*Vitis vinifera*) is carried out using four different schemes: Baillod and Baggiolini, Extended BBCH, Eichhorn and Lorenz, and Modified E-L. Phenological estimation requires the exhaustive evaluation of crops, which makes it intensive in terms of labor, personnel, and the time required for its application. In this work, we propose a new phenological classification based on transcriptional measures of certain genes to accurately estimate the stage of development of grapevine. There are several genomic information databases for *Vitis vinifera*, and the function of thousands of their genes has been widely characterized. The application of advanced molecular biology, including the massive parallel sequencing of RNA (RNA-seq), and the handling of large volumes of data provide state-of-the-art tools for the determination of phenological stages, on a global scale, of the molecular functions and processes of plants. With this aim, we applied a bioinformatic pipeline for the high-throughput quantification of RNA-seq datasets and further analysis of gene ontology terms. We identified differentially expressed genes in several datasets, and then, we associated them with the corresponding phenological stage of development. Differentially expressed genes were classified using count-based expression analysis and clustering and annotated using gene ontology data. This work contributes to the use of transcriptome data and gene expression analysis for the classification of development in plants, with a wide range of industrial applications in agriculture.

Keywords: phenology; gene expression; *Vitis vinifera*; RNA sequencing

Citation: Altimiras, F.; Pavéz, L.; Pourreza, A.; Yañez, O.; González-Rodríguez, L.; García, J.; Galaz, C.; Leiva-Araos, A.; Allende-Cid, H. Transcriptome Data Analysis Applied to Grapevine Growth Stage Identification. *Agronomy* **2024**, *14*, 613. https://doi.org/10.3390/agronomy14030613

Academic Editor: Concetta Licciardello

Received: 19 February 2024
Revised: 29 February 2024
Accepted: 11 March 2024
Published: 19 March 2024

1. Introduction

In viticulture, the cultivation of grapevine (*Vitis vinifera*), key phenological and metabolic events play a crucial role in determining the size and organoleptic composition of the fruits [1]. Phenological characterization enables the early detection of diseases or nutritional deficiencies in plants, which can significantly reduce the productive yield and adversely affect the quality of fruits. Phenology is a vital component in evaluating crop productivity and crop management [2–4].

At present, the estimation of phenological stages in grapevine is carried out using various established systems, including Baillod and Baggiolini, Extended BBCH, Eichhorn and Lorenz, and Modified E-L [5,6]. In these systems, the phenological stage of a plant is determined by observing specific structures or events (such as budburst, shoot growth, the beginning of flowering, full bloom, setting, veraison, and harvest) and helps in characterizing its development and growth. While these systems have been employed for phenological estimation for over two decades, they necessitate a thorough assessment of crops, which can be labor-intensive and time-consuming. To address this issue, various approaches have been adopted for estimating phenological stages, including the analysis of time-series data from MODIS and NOAA satellite images [7,8]. Satellite and drone remote sensing imagery have emerged as valuable tools for monitoring multiple facets of viticulture, such as drought surveillance, nitrogen status assessment, and evapotranspiration measurement [9,10]. Furthermore, the National Snow and Ice Data Center has been engaged in the construction of a time series utilizing data from both the MODIS and VIIRS missions. This effort can offer valuable insights for the research community, particularly in the context of climate change [11,12].

Current challenges in determining grape berry phenology depend on different variables, including specific genotypic response, water availability, climate, agronomic practices, and crop load [13]. The introduction of next-generation sequencing offers the opportunity to use the dynamic expression patterns of broad gene sets to track fruit development and improve classification systems with molecular information [14–16]. Some examples of studies that have successfully applied this strategy in plants were used to reconstruct the transcriptional development of individual organs in maize and sorghum, linking the appearance of morphological traits to precisely defined molecular developmental stages [17]. This method successfully created a transcriptomic-aging clock, accurately determining the biological age of organisms such as *Caenorhabditis elegans* [18]. Recent advances in gene expression quantification methods could be used to improve the accuracy of phenological-stage classification [19].

Grapevine (*Vitis vinifera*) has been extensively characterized at the genome, transcriptome, and metabolome levels [20–24]. For instance, Licausi et al., 2010, investigated the genome of *V. vinifera* by analyzing the expression dynamics of two gene groups associated with grape development and growth [25]. Additionally, Zhu et al., 2017, studied the Sucrose Synthase Gene Family in the grape genome [26]. Several genomic information databases exist for *Vitis vinifera*, and the functions of its genes have been comprehensively characterized. Moreover, numerous protocols and tools have been developed for the molecular analysis of the grape. The adoption of advanced molecular biology techniques, including RNA parallel massive sequencing (RNA-seq), and the management of large-scale, highly complex data sets, offer cutting-edge tools for studying the precision of phenological and metabolic dynamics on a global scale. These tools encompass all integrated processes and molecular functions of plants [27].

The primary aim of this study was to perform a phenological analysis utilizing transcriptome data to pinpoint differentially expressed genes (DEG) throughout the growth stages of grapevine. This work contributes significantly to the advancement of intelligent systems in molecular biology that enhance productivity and facilitate informed decision making to yield high-quality fruits. By applying a bioinformatic pipeline to the analysis of RNA-seq datasets [28] and conducting gene expression analysis for DEG identification [16,29,30], we were able to characterize the gene ontology processes enriched at

various phenological stages [31]. Our work holds substantial potential to revolutionize the understanding and management of grapevine growth stages, leading to improved agricultural practices and better fruit quality. Moreover, this research offers valuable insights into the molecular processes governing grapevine development, which could be instrumental in optimizing crop production and mitigating potential challenges.

In this study, we conducted an exhaustive analysis of the global transcriptional profile of grape berries, enabling us to accurately describe the functional roles of most genes in *Vitis vinifera*. Our investigation centered on table grape seedless segregants exhibiting contrasting phenotypes for berry weight, thereby enhancing our understanding of the mechanisms governing berry weight determination. To facilitate cross-species comparisons of plant developmental stages, we utilized Plant Ontology (PO). Through our comparative transcriptome profiling, we identified candidate genes associated with berry weight. These genes hold potential as selection tools in table grape breeding programs. We employed the Grape RNA-Seq Analysis Pipeline Environment (Grape) for the processing and analysis of RNA-Seq data. This pipeline supports raw sequencing reads produced by various technologies, in FASTA or FASTQ formats, as well as pre-aligned reads in SAM/BAM formats. It conducts quality control measures, aligns the reads to the genome, and performs gene expression analysis. Our approach and results contribute significantly to the field of viticulture, offering new insights into the genetic basis of berry weight variation. The identification of candidate genes associated with berry weight will inform future breeding programs, helping to optimize grape production and enhance fruit quality.

2. Materials and Methods

2.1. Phenological Classification

For the phenological stage classification in this work, we used the Modified E-L system proposed in [5,6] (Table 1). The growth stage of each sample, which was retrieved from the ArrayExpress metadata, has a different format and classification system for each dataset. To overcome this, we used the equivalences between different systems for grapevine growth stage identification, and manually assigned each sample to its respective Modified E-L stage.

2.2. RNA-Seq Data Processing and Quantification

Transcriptome data for grapevine was searched using ArrayExpress and GEO databases [32,33]. Details of the datasets used for the phenological classification are available in the Supplementary Materials. With the aim of designing a rapid and accurate workflow for RNA-seq data analysis, we implemented the bioinformatic pipeline described in Figure 1. We started with preprocessing of the transcriptome data using FastQC for quality control [34], and Trimmomatic for sequence trimming [35]. For quantifying transcript abundance from RNA-seq reads, we used Salmon, which is an accurate and fast pseudo-aligner that can be used on standard computers [36]. For each tool, it was necessary to create an index from the transcriptome, for which the IGGP 12X genome assembly with V1 annotation of the *Vitis vinifera* genome was used (http://plants.ensembl.org/Vitis_vinifera/Info/Index, accessed on 18 February 2024).

2.3. Differential Gene Expression Analysis

To identify DEGs in a variety of plant physiology stages, we implemented edgeR according to [37]. EdgeR is a Bioconductor package for gene expression analyses of read counts arising from RNA-seq or similar technologies [38]. The first step to identifying DEGs is to read counts from the RNA-seq quantification output. We used the Bioconductor package tximport to create a read-count matrix, and then, used this to create a DGEList data object with edgeR [39,40]. The second step was to carry out the filtering and normalization of the RNA-seq quantification libraries, according to the following criteria: counts-per-million (CPM) > 1, log-fold change (logFC) > 5, and adjust *p*-value (FDR) < 0.01. Volcano,

biological coefficient of variation (BCV), and multidimensional scaling (MDS) plots were created using R version 3.5.2.

2.4. Gene Ontology

We identified gene ontology (GO) terms for the DEGs in grapevine reproductive stages. For functional annotation and GO enrichment analysis, we used BioMart, Ensembl Plant, Panther, and PlantRegMap [41–44]. Different GO IDs were associated with the DEGs selected; molecular functions and enzyme codes associated with the DEGs were analyzed.

We used a Microsoft Azure virtual machine with an Intel Xeon CPU E5-2673 v3 @ 2.40 GHz processor and 32 GB of RAM.

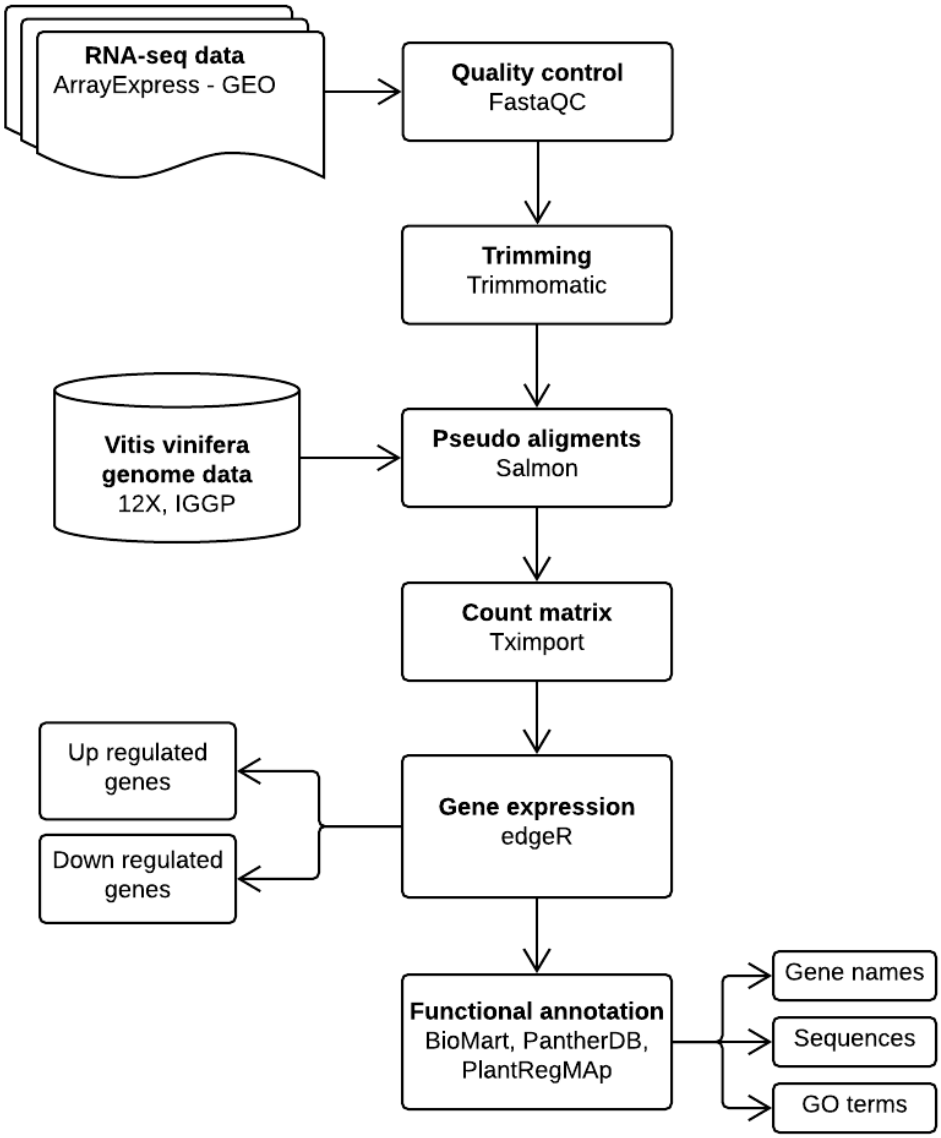

Figure 1. Proposed bioinformatic pipeline for RNA-sequencing data processing. This figure illustrates a step-by-step workflow for processing RNA-sequencing data using bioinformatics tools and software. The pipeline begins with the acquisition of raw sequencing data, typically in the form of FASTQ files, and progresses through several key stages, including quality control, read alignment, transcript assembly, differential expression analysis, and functional annotation. Each stage involves specific bioinformatics tools and algorithms, which are applied to the raw data to generate meaningful biological insights.

Table 1. Phenological growth stage schemes for grapevine. This table outlines the stages of growth that grapevine goes through, from dormancy to ripeness. The stages are adapted from the work of Lorenz et al. (1995) and Coombe et al. (1995). These stages are crucial for grape growers and winemakers, as they determine when certain tasks need to be performed, such as pruning, watering, and harvesting. Understanding the phenological growth stages helps in managing grapevine effectively, which, in turn, leads to better grape quality and wine production. These schemes provide a standard framework that can be used across different grape varieties and regions, allowing for consistent terminology and management practices in the grape and wine industry [5,6].

Major Stage	Detailed Stage	Baillod and Baggiolini	Extended BBCH	Eichhorn and Lorenz	Modified E-L
Shoot and inflorescence development		A	0	1	1
			1	2	2
		B	3		
			5	3	3
	Budburst	C	7	5	4
		D	9		5
		E	11	7	7
			12	9	9
			13		
			14		11
	Shoots 10 cm	F	15, 53	12	12
			16		13
					14
		G	55	15	15
			19		16
		H	57	17	17
					18
Flowering	Flowering begins		60	19	19
			61		20
			63	21	21
	Full bloom	I	65	23	23
			68	25	25
			69	26	26
Berry development	Setting	J	71	27	27
			73	29	29
	Berries pea size	K	75	31	31
			77	33	32
		L	79		33

Table 1. *Cont.*

Major Stage	Detailed Stage	Baillod and Baggiolini	Extended BBCH	Eichhorn and Lorenz	Modified E-L
Ripening					34
	Veraison	M	81	35	35
					36
					37
	Harvest	N	89	38	38
					39
Senescence		O	91	41	41
			92		
		P	93	43	43
			95		
			97	47	47

3. Results and Discussion

In this article, we report on a transcriptome data analysis for phenological stage identification in grapevine. The implementation of a bioinformatic pipeline for RNA-seq data processing allowed us to quantify multiple datasets from gene expression databases. Different stages of phenology were found in the available information from *Vitis vinifera* transcriptomic studies. This global analysis of transcriptomes enabled a comprehensive study for the identification of phenological stages in *Vitis vinifera* through molecular examination. To evaluate the development of grapevine, we evaluated a phenological classification that compares the four schemes used currently in the viticulture industry, including "Baillod and Baggiolini", "Extended BBCH", "Eichhorn and Lorenz", and "Modified E-L". We used, for further analysis, the Modified E-L system for identifying major and intermediate grapevine growth stages. The different associated events of plant development in the Modified E-L system are "Shoot and inflorescence development", "Flowering", "Berry development", "Ripening", and "Senescence".

The transcriptome data used in this study were obtained from ArrayExpress and GEO. The datasets were quantified according to the proposed bioinformatic pipeline (Figure 1). In total, 1340 million reads (89.1 GB of data) were quantified and used for further analysis. In the major stage of "Shoot and inflorescence development", a total of 479 million reads were quantified, equal to 26.136 GB of data. In the "Berry development" major stage, 214 million reads were quantified, equivalent to 16.794 GB of data. In the "Ripening" major stage, 577 million reads were quantified, equivalent to 43.102 GB of data. In the "Senescence" major stage, 65.9 million reads were quantified, equivalent to 3.099 GB of data. After the processing of the reads, including quality control and trimming, pseudo-alignments against the *Vitis vinifera* genome data (12X, IGGP) were performed. A count matrix of 29,958 observations (gene counts) and 68 variables (biological replicates) was created. This count matrix was used for gene expression analysis and gene ontology enrichment. The raw library sizes were scaled with the calculation of the normalization factors. As is shown in Figure 2, a multidimensional scaling (MDS) plot of distances between gene expression profiles was made to represent the relationships between the groups of samples, as a visualization of the distances between replicates. The biological coefficient of variation (BCV) was calculated as the relative variability value of the true abundances of the gene between biological replicates. As is shown in Figure 2, the BCV distances correspond to the dispersion estimates among samples, in which each color corresponds to a phenological stage according to the Modified E-L system. It represents

the coefficient of variation that would remain between biological replicates if sequencing depth could be increased indefinitely [45].

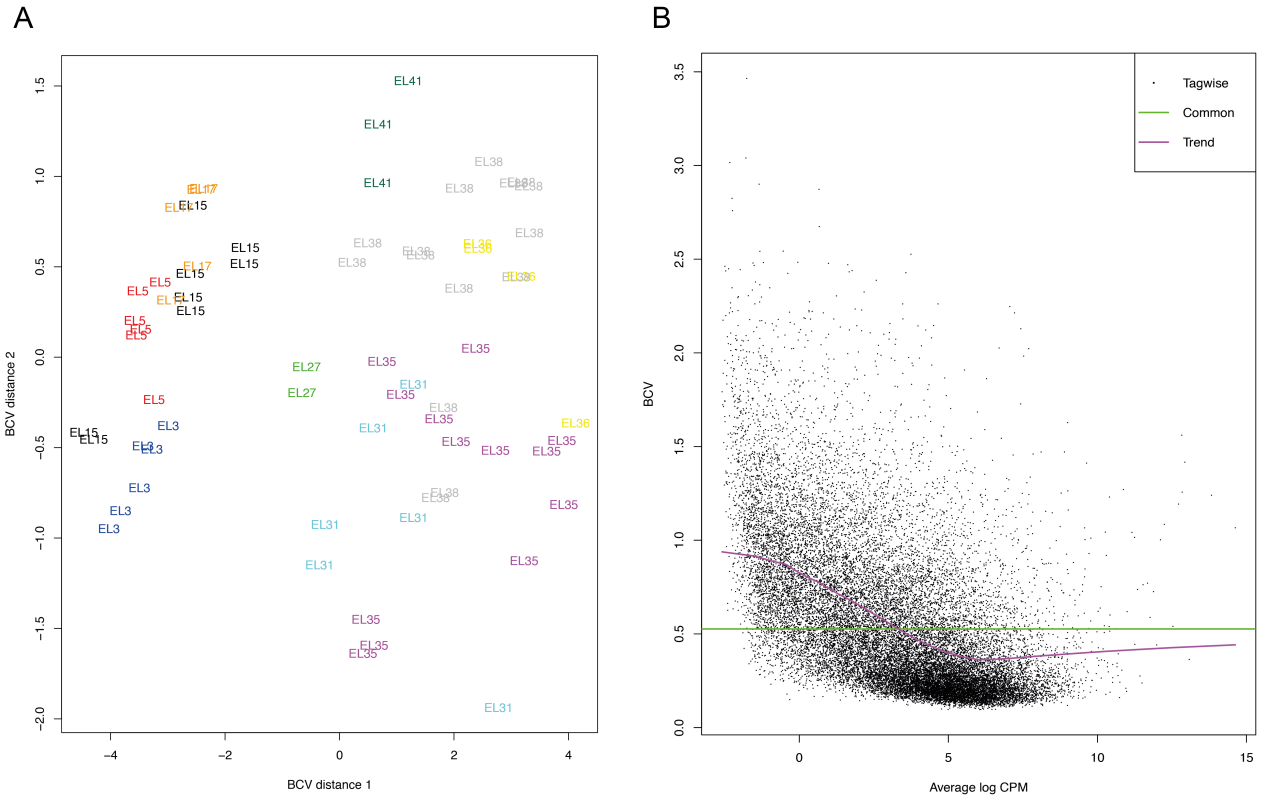

Figure 2. Multidimensional scaling (MDS) and biological coefficient of variation (BCV) plots generated using edgeR showing segregation of distances between gene expression profiles. This figure illustrates the use of MDS and BCV plots to assess the distances between gene expression profiles, which can provide insights into the reliability and variability of experimental data. (**A**) Multidimensional scaling plot for the biological replicates. (**B**) Biological coefficient of variation.

We report the DEGs for the classification of grapevine using the reproductive phenological stages according to the Modified E-L system. We applied a bioinformatic pipeline for the processing of large amounts of data derived from RNA-sequencing experiments. The DEGs were calculated using available transcriptome data from the four major stages mentioned above and ten detailed stages according to the Modified E-L system. This included the representation of four stages from "Shoot and inflorescence development" (EL-3, EL-5, EL-15, EL-17), two stages from "Berry development" (EL-27, EL-31), three stages from "Ripening" (EL-35, EL-36, EL-38), and one stage from "Senescence" (EL-41). All the comparisons were made using the stage EL-3 as a control. For the representation of the transcriptional dynamics across phenological stages, volcano plots were made showing the observed DEGs in each phenological stage (Figure 3).

The DEGs found in this analysis were used for a gene classification according to their molecular functions for functional categorization. The general transcriptional dynamics observed show general downregulation of gene expression in the last stages of development, ripening, and senescense, with the highest number of DEGs (Figure 3). This reflects a very dynamic and active process in the late stages of fruit ripening maturity and senescence [46]. Several processes could be involved in the transcriptional downregulation observed in the last stage of development. One of theses could be attributed to mitochondrial activity, due to the gradual degradation of chlorophyll in the senescence stage [47].

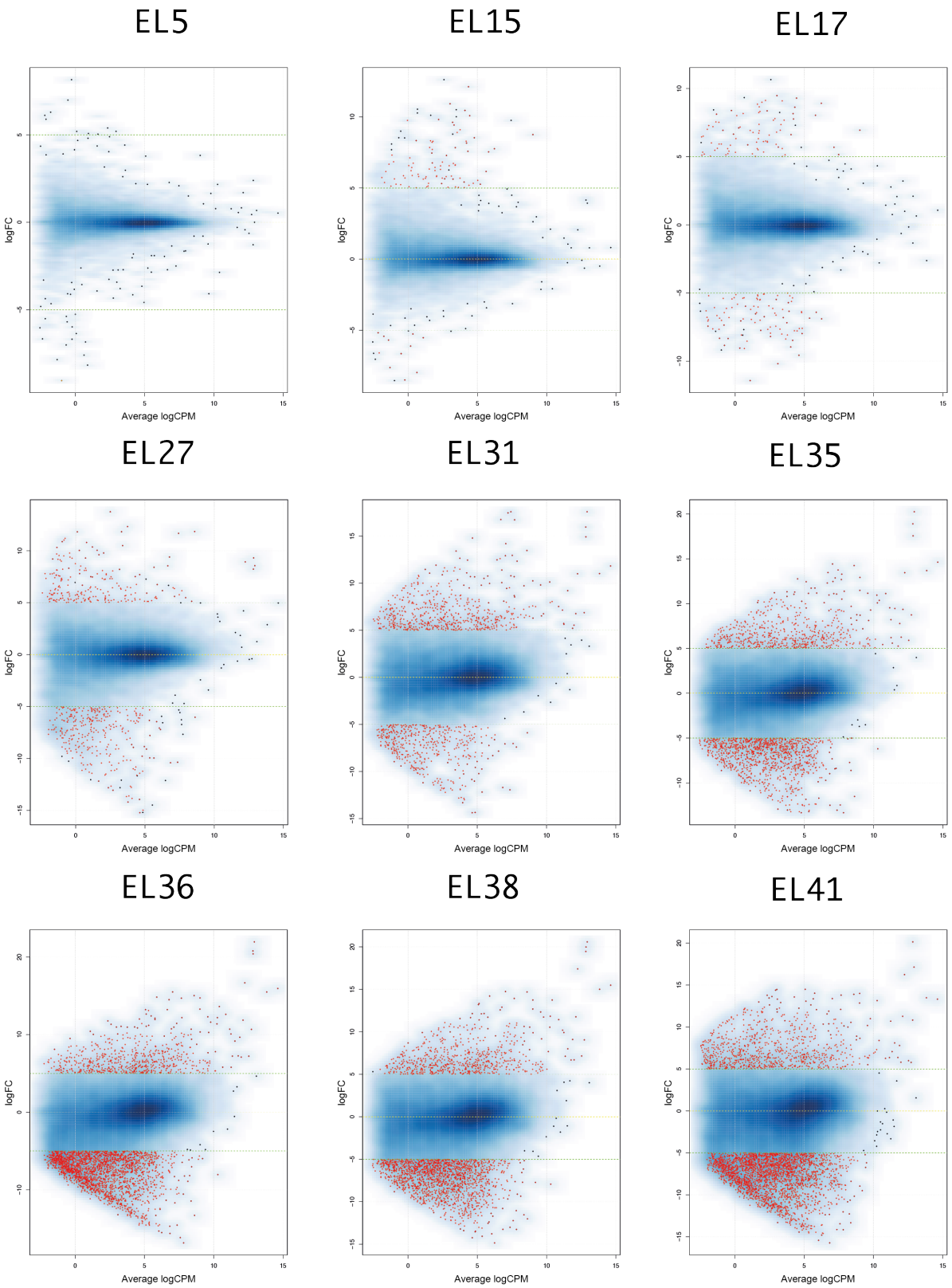

Figure 3. Volcano plots of differentially expressed genes in multiple phenological stages. The volcano plots depict the results of differential gene expression analysis across multiple phenological stages of grapevine growth. Each point in the plot represents a gene, and its position is determined by both the statistical significance of its expression change (x-axis) and the magnitude of that change (y-axis).

According to our results, multiple DEGs found in the last stages of development were attributed to oxidoreductase activity (GO:0016722); some of them are associated with phenylpropanoid biosynthetic (GO:0009699) and metabolic (GO:0009698) processes (Figure 4), such as the genes that code for phenylalanine ammonia-lyase, flavanone-3-hydroxylases, and anthocyanin acyltransferase. These biochemical pathways underlie the synthesis of a wide range of important phenolic and flavonoid compounds that could provide beneficial effects on health, including anthocyanins, which lead to a change in the color of the fruit [48]. The concentration of these secondary metabolites is a relevant indicator of fruit quality and obviously has an important economic impact [49]. On the other hand, phenylpropanoid metabolism can also be regulated to determine the action of members of the MYB transcription factor family. We detected four DE MYB genes, indicating that they play a very relevant role in anthocyanin synthesis. In the last stages of ripening, the anthocyanin accumulation ceased [50]; this is correlated with the downregulation of MYB genes, and indicates that the precursors of anthocyanin are channeled to the production of other phenolic compounds, such as stilbenes.

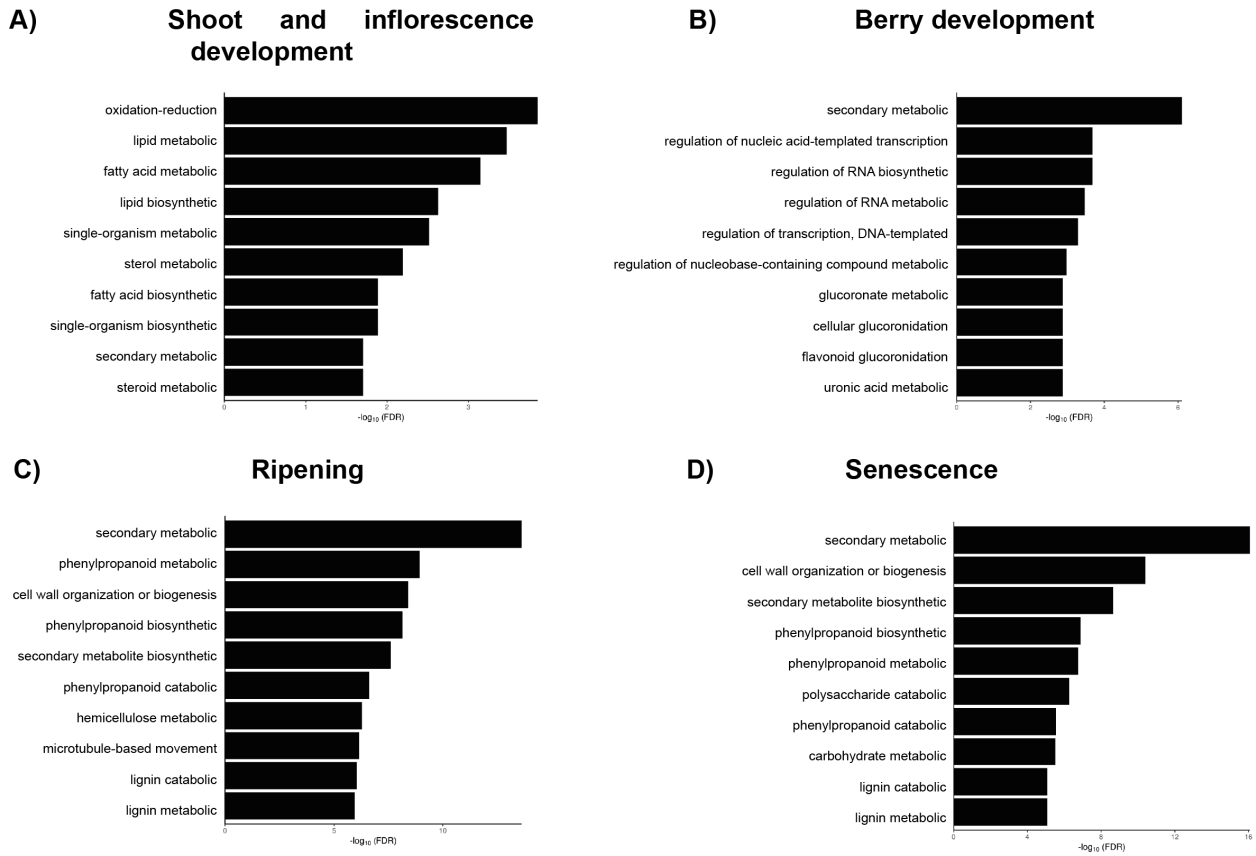

Figure 4. Gene ontology enrichment. (**A**) Shoot and inflorescence development, (**B**) berry development, (**C**) ripening, and (**D**) senescence. This figure illustrates the results of gene ontology (GO) enrichment analysis for different phenological stages of grapevine growth. The enrichment analysis identifies GO terms that are significantly over-represented in the set of differentially expressed genes (DEGs) for each phenological stage. Each bar represents a GO term, and its height indicates the statistical significance of enrichment, typically measured using adjusted *p*-values or enrichment scores.

Serine-type carboxypeptidase activity (GO:0004185) was also found to be enriched in the last stage of senescence. This process of protein degradation is involved in efficient protein remobilization during leaf senescence. Serine-type carboxypeptidase proteins may be vital for normal plant growth and development, for the synthesis of compounds that protect plants against pathogens and xenobiotic agents [51].

Fruit softening is one of the key features of this stage, and is associated with the disassembly of the primary cell wall and dissolution of the middle lamella [52], and this, in our analysis, is confirmed by changes in gene expression for a set of genes that encode for enzymes important for this process, such as pectinesterase, xyloglucan endotransglucosylase/hydrolase (XET), glycosyltransferase, and pectate lyase. Specifically, the role of XET is uncertain, since there is inconsistency between studies that show opposite expression patterns [53,54], indicating that they could be involved in cell wall extensibility and growth [55]. Our transcriptomic classification approach could be a great tool for identifying specific isoforms of XET that are up- and downregulated in this stage and contribute to applications in industrial agriculture.

Another important process in this stage corresponds to lipid metabolism. We detected changes in transcripts described like lipoxygenases that could be involved in fatty acid degradation and the synthesis of lipid-derived aroma volatiles, resulting in an increase in flavor [53]. Finally, we believe these genes represent interesting targets in the modulation of complete maturation and the ripening process. A certain combination of these genes could be applied for the classification of plants according to the Modified E-L scale. This system could be applied for the monitoring of the health of crops, including pathogens and pests such us the grapevine moth *Lobesia botrana* [56,57].

4. Conclusions

In this study, we aimed to contribute to the transcriptome analysis and characterization of the phenological stages of development in grapevine. We utilized several datasets of RNA-seq data obtained from public databases, including ArrayExpress and GEO, resulting in the quantification of 1340 million reads and the analysis of 89.1 GB of data. Transcriptome analysis techniques, such as microarrays and RNA sequencing (RNA-Seq), were employed to study the organism's transcriptome.

We observed that the number of differentially expressed genes (DEGs) increased as phenological development progressed, from shoot and inflorescence development until the senescence stage. The most enriched gene ontology (GO) categories were identified for each stage of development: oxidation-reduction processes during shoot and inflorescence development, the regulation of transcription during berry development, phenylpropanoid processes during ripening, and cell wall organization during senescence.

Our work contributes to the utilization of transcriptome data for the analysis of plant development, with potential applications in various types of crops. By understanding the gene expression patterns and biological processes associated with different phenological stages in grapevine, researchers and growers can make informed decisions regarding vineyard management, cultivar selection, and the timing of various interventions, ultimately leading to improved crop yield and quality.

The identification of specific genes involved in crucial stages of grapevine maturation and senescence opens up avenues for the development of targeted strategies aimed at enhancing fruit quality and bolstering disease resistance. By focusing on genes related to the biosynthesis of phenylpropanoids, for example, it becomes possible to direct the accumulation of beneficial phenolic compounds, thereby elevating the organoleptic qualities and antioxidant stability of the grapes. This knowledge also underpins more informed strategies for post-harvest handling, aiming to prolong shelf life and maintain the grapes' sensory attributes. Thus, the insights gained from our analysis offer a roadmap for selecting varieties and refining cultivation practices that align with the sustainable production of high-quality grapes, satisfying both consumer preferences and the wine industry's standards. While this study has laid a promising foundation, enriching it with further detailed examinations and expanding on the practical applications of these findings could significantly amplify its impact on this field.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agronomy14030613/s1, Table S1: Phenological schemes, Table S2: RNA-sequencing datasets, Table S3: Differentially expressed genes per stage, Table S4: Differentially expressed genes (Ensembl IDs), Table S5: Differentially expressed genes with gene names, logFC, logCPM, PValue, FDR and according growth stages.

Author Contributions: Conceptualization, F.A., A.L.-A. and L.P.; methodology, F.A., C.G., J.G. and L.P.; software, C.G. and F.A.; validation, L.G.-R., O.Y., H.A.-C. and A.L.-A.; formal analysis, F.A. and L.P.; writing—original draft preparation, F.A.; writing—review and editing, A.P. and H.A.-C.; visualization, F.A. and C.G.; supervision, A.P., A.L.-A. and H.A.-C. All authors have read and agreed to the published version of the manuscript.

Funding: Jose Garcia was partially supported by Grant CONICYT/FONDECYT/INICIACION/11180056. Francisco Altimiras was partially supported by the INF-PUCV Scholarship, the ANID Strengthening R&D capabilities Program CTI220002 Inria Chile, and the Inria Challenge OcéanIA.

Data Availability Statement: The original contributions presented in the study are included in the article and supplementary material.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Alturki, S.M. Phenology and production of Hassaoui grapevines as affected by climate anomalies in Al Ahsa region. *Saudi J. Biol. Sci.* **2022**, *29*, 1175–1184. [CrossRef]
2. Schwartz, M.D. *Phenology: An Integrative Environmental Science*; Springer Science & Business Media: Berlin, Germany, 2003.
3. Fenner, M. The phenology of growth and reproduction in plants. *Perspect. Plant Ecol. Evol. Syst.* **1998**, *1*, 78–91. [CrossRef]
4. Hudson, I.L.; Keatley, M.R. *Phenological Research: Methods for Environmental and Climate Change Analysis*; Springer Science & Business Media: Berlin, Germany, 2009.
5. Lorenz, D.; Eichhorn, K.; Bleiholder, H.; Klose, R.; Meier, U.; Weber, E. Growth Stages of the Grapevine: Phenological growth stages of the grapevine (*Vitis vinifera* L. ssp. vinifera)—Codes and descriptions according to the extended BBCH scale. *Aust. J. Grape Wine Res.* **1995**, *1*, 100–103. [CrossRef]
6. Coombe, B. Growth stages of the grapevine: Adoption of a system for identifying grapevine growth stages. *Aust. J. Grape Wine Res.* **1995**, *1*, 104–110. [CrossRef]
7. Sakamoto, T.; Yokozawa, M.; Toritani, H.; Shibayama, M.; Ishitsuka, N.; Ohno, H. A crop phenology detection method using time-series MODIS data. *Remote Sens. Environ.* **2005**, *96*, 366–374. [CrossRef]
8. Xin, J.; Yu, Z.; van Leeuwen, L.; Driessen, P.M. Mapping crop key phenological stages in the North China Plain using NOAA time series images. *Int. J. Appl. Earth Obs. Geoinf.* **2002**, *4*, 109–117. [CrossRef]
9. Dorjsuren, M.; Liou, Y.A.; Cheng, C.H. Time series MODIS and in situ data analysis for Mongolia drought. *Remote Sens.* **2016**, *8*, 509. [CrossRef]
10. Moghimi, A.; Pourreza, A.; Zuniga-Ramirez, G.; Williams, L.E.; Fidelibus, M.W. A novel machine learning approach to estimate grapevine leaf nitrogen concentration using aerial multispectral imagery. *Remote Sens.* **2020**, *12*, 3515. [CrossRef]
11. Albanai, J.A. Building a bridge between MODIS and VIIRS in measuring chlorophyll-a through linear regression: A case study in the Arabian Gulf. *Arab. J. Geosci.* **2023**, *16*, 1–9. [CrossRef]
12. Xu, W.; Wooster, M.J. Sentinel-3 SLSTR active fire (AF) detection and FRP daytime product-Algorithm description and global intercomparison to MODIS, VIIRS and landsat AF data. *Sci. Remote Sens.* **2023**, *7*, 100087. [CrossRef]
13. Parker, A.K.; Hofmann, R.W.; Van Leeuwen, C.; McLachlan, A.R.; Trought, M.C. Manipulating the leaf area to fruit mass ratio alters the synchrony of total soluble solids accumulation and titratable acidity of grape berries. *Aust. J. Grape Wine Res.* **2015**, *21*, 266–276. [CrossRef]
14. Chuine, I.; Régnière, J. Process-based models of phenology for plants and animals. *Annu. Rev. Ecol. Evol. Syst.* **2017**, *48*, 159–182. [CrossRef]
15. Gildor, T.; Smadar, B.T.D.L. Comparative studies of gene expression kinetics: Methodologies and insights on development and evolution. *Front. Genet.* **2018**, *9*, 339. [CrossRef]
16. Tornielli, G.B.; Sandri, M.; Fasoli, M.; Amato, A.; Pezzotti, M.; Zuccolotto, P.; Zenoni, S. A molecular phenology scale of grape berry development. *Hortic. Res.* **2023**, *10*, uhad048. [CrossRef]
17. Leiboff, S.; Hake, S. Reconstructing the transcriptional ontogeny of maize and sorghum supports an inverse hourglass model of inflorescence development. *Curr. Biol.* **2019**, *29*, 3410–3419. [CrossRef]
18. Meyer, D.H.; Schumacher, B. BiT age: A transcriptome-based aging clock near the theoretical limit of accuracy. *Aging Cell* **2021**, *20*, e13320. [CrossRef]
19. Kudoh, H. Molecular phenology in plants: In natura systems biology for the comprehensive understanding of seasonal responses under natural environments. *New Phytol.* **2016**, *210*, 399–412. [CrossRef]

20. Jaillon, O.; Aury, J.M.; Noel, B.; Policriti, A.; Clepet, C.; Casagrande, A.; Choisne, N.; Aubourg, S.; Vitulo, N.; Jubin, C.; et al. The grapevine genome sequence suggests ancestral hexaploidization in major angiosperm phyla. *Nature* **2007**, *449*, 463–467.
21. Zenoni, S.; Ferrarini, A.; Giacomelli, E.; Xumerle, L.; Fasoli, M.; Malerba, G.; Bellin, D.; Pezzotti, M.; Delledonne, M. Characterization of transcriptional complexity during berry development in Vitis vinifera using RNA-Seq. *Plant Physiol.* **2010**, *152*, 1787–1795. [CrossRef]
22. Lijavetzky, D.; Cabezas, J.A.; Ibáñez, A.; Rodríguez, V.; Martínez-Zapater, J.M. High throughput SNP discovery and genotyping in grapevine (*Vitis vinifera* L.) by combining a re-sequencing approach and SNPlex technology. *BMC Genom.* **2007**, *8*, 424. [CrossRef]
23. Salmaso, M.; Faes, G.; Segala, C.; Stefanini, M.; Salakhutdinov, I.; Zyprian, E.; Toepfer, R.; Grando, M.S.; Velasco, R. Genome diversity and gene haplotypes in the grapevine (*Vitis vinifera* L.), as revealed by single nucleotide polymorphisms. *Mol. Breed.* **2005**, *14*, 385–395. [CrossRef]
24. Velasco, R.; Zharkikh, A.; Troggio, M.; Cartwright, D.A.; Cestaro, A.; Pruss, D.; Pindo, M.; FitzGerald, L.M.; Vezzulli, S.; Reid, J.; et al. A high quality draft consensus sequence of the genome of a heterozygous grapevine variety. *PLoS ONE* **2007**, *2*, e1326. [CrossRef]
25. Licausi, F.; Giorgi, F.M.; Zenoni, S.; Osti, F.; Pezzotti, M.; Perata, P. Genomic and transcriptomic analysis of the AP2/ERF superfamily in Vitis vinifera. *BMC Genom.* **2010**, *11*, 719. [CrossRef]
26. Zhu, X.; Wang, M.; Li, X.; Jiu, S.; Wang, C.; Fang, J. Genome-Wide Analysis of the Sucrose Synthase Gene Family in Grape (*Vitis vinifera*): Structure, Evolution, and Expression Profiles. *Genes* **2017**, *8*, 111. [CrossRef] [PubMed]
27. Hirai, M.Y.; Yano, M.; Goodenowe, D.B.; Kanaya, S.; Kimura, T.; Awazuhara, M.; Arita, M.; Fujiwara, T.; Saito, K. Integration of transcriptomics and metabolomics for understanding of global responses to nutritional stresses in Arabidopsis thaliana. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 10205–10210. [CrossRef]
28. Knowles, D.G.; Röder, M.; Merkel, A.; Guigó, R. Grape RNA-Seq analysis pipeline environment. *Bioinformatics* **2013**, *29*, 614–621. [CrossRef]
29. Wang, L.; Li, H.; Li, J.; Li, G.; Zahid, M.S.; Li, D.; Ma, C.; Xu, W.; Song, S.; Li, X.; et al. Transcriptome analysis revealed the expression levels of genes related to abscisic acid and auxin biosynthesis in grapevine (*Vitis vinifera* L.) under root restriction. *Front. Plant Sci.* **2022**, *13*, 959693. [CrossRef]
30. Sweetman, C.; Wong, D.C.; Ford, C.M.; Drew, D.P. Transcriptome analysis at four developmental stages of grape berry (*Vitis vinifera* cv. Shiraz) provides insights into regulated and coordinated gene expression. *BMC Genom.* **2012**, *13*, 691. [CrossRef] [PubMed]
31. Walls, R.L.; Cooper, L.; Elser, J.; Gandolfo, M.A.; Mungall, C.J.; Smith, B.; Stevenson, D.W.; Jaiswal, P. The plant ontology facilitates comparisons of plant development stages across species. *Front. Plant Sci.* **2019**, *10*, 631. [CrossRef]
32. Kolesnikov, N.; Hastings, E.; Keays, M.; Melnichuk, O.; Tang, Y.A.; Williams, E.; Dylag, M.; Kurbatova, N.; Brandizi, M.; Burdett, T.; et al. ArrayExpress update—Simplifying data submissions. *Nucleic Acids Res.* **2014**, *43*, D1113–D1116. [CrossRef]
33. Barrett, T.; Wilhite, S.E.; Ledoux, P.; Evangelista, C.; Kim, I.F.; Tomashevsky, M.; Marshall, K.A.; Phillippy, K.H.; Sherman, P.M.; Holko, M.; et al. NCBI GEO: Archive for functional genomics data sets—Update. *Nucleic Acids Res.* **2012**, *41*, D991–D995. [CrossRef] [PubMed]
34. Andrews, S. FastQC: A quality control tool for high throughput sequence data. *Babraham Bioinform.* **2010**. Available online: http://www.bioinformatics.babraham.ac.uk/projects/fastqc (accessed on 18 February 2024).
35. Bolger, A.M.; Lohse, M.; Usadel, B. Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics* **2014**, *30*, 2114–2120. [CrossRef] [PubMed]
36. Patro, R.; Duggal, G.; Love, M.I.; Irizarry, R.A.; Kingsford, C. Salmon provides fast and bias-aware quantification of transcript expression. *Nat. Methods* **2017**, *14*, 417. [CrossRef] [PubMed]
37. Altimiras, F.; Uszczynska-Ratajczak, B.; Camara, F.; Vlasova, A.; Palumbo, E.; Newhouse, S.; Deacon, R.M.; Farias, L.A.; Hurley, M.J.; Loyola, D.E.; et al. Brain transcriptome sequencing of a natural model of Alzheimer's disease. *Front. Aging Neurosci.* **2017**, *9*, 64. [CrossRef] [PubMed]
38. Robinson, M.D.; McCarthy, D.J.; Smyth, G.K. edgeR: A Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* **2010**, *26*, 139–140. [CrossRef] [PubMed]
39. Soneson, C.; Love, M.I.; Robinson, M.D. Differential analyses for RNA-seq: Transcript-level estimates improve gene-level inferences. *F1000Research* **2015**, *4*, 1521. [CrossRef] [PubMed]
40. Love, M.I.; Soneson, C.; Robinson, M.D. Importing transcript abundance datasets with tximport. *F1000Research* **2005**, *1*, 5.
41. Durinck, S.; Moreau, Y.; Kasprzyk, A.; Davis, S.; De Moor, B.; Brazma, A.; Huber, W. BioMart and Bioconductor: A powerful link between biological databases and microarray data analysis. *Bioinformatics* **2005**, *21*, 3439–3440. [CrossRef]
42. Bolser, D.; Staines, D.M.; Pritchard, E.; Kersey, P. Ensembl plants: Integrating tools for visualizing, mining, and analyzing plant genomics data. In *Plant Bioinformatics*; Springer: Berlin/Heidelberg, Germany, 2016; pp. 115–140.
43. Mi, H.; Muruganujan, A.; Casagrande, J.T.; Thomas, P.D. Large-scale gene function analysis with the PANTHER classification system. *Nat. Protoc.* **2013**, *8*, 1551. [CrossRef]
44. Jin, J.; Tian, F.; Yang, D.C.; Meng, Y.Q.; Kong, L.; Luo, J.; Gao, G. PlantTFDB 4.0: Toward a central hub for transcription factors and regulatory interactions in plants. *Nucleic Acids Res.* **2016**, *45*, D1040–D1045. [CrossRef]
45. McCarthy, D.J.; Chen, Y.; Smyth, G.K. Differential expression analysis of multifactor RNA-Seq experiments with respect to biological variation. *Nucleic Acids Res.* **2012**, *40*, 4288–4297. [CrossRef]

46. Cramer, G.R.; Ghan, R.; Schlauch, K.A.; Tillett, R.L.; Heymann, H.; Ferrarini, A.; Delledonne, M.; Zenoni, S.; Fasoli, M.; Pezzotti, M. Transcriptomic analysis of the late stages of grapevine (*Vitis vinifera* cv. *Cabernet sauvignon*) berry ripening reveals significant induction of ethylene signaling and flavor pathways in the skin. *BMC Plant Biol.* **2014**, *14*, 370. [CrossRef]
47. Ruberti, C.; Barizza, E.; Bodner, M.; La Rocca, N.; De Michele, R.; Carimi, F.; Schiavo, F.L.; Zottini, M. Mitochondria change dynamics and morphology during grapevine leaf senescence. *PLoS ONE* **2014**, *9*, e102012. [CrossRef] [PubMed]
48. Castellarin, S.; Bavaresco, L.; Falginella, L.; Gonçalves, M.V.Z.; Di Gaspero, G. Phenolics in grape berry and key antioxidants. In *The Biochemistry of the Grape Berry*; Bentham Science Publishers: Sharjah, United Arab Emirates, 2012.
49. Savoi, S.; Wong, D.C.; Arapitsas, P.; Miculan, M.; Bucchetti, B.; Peterlunger, E.; Fait, A.; Mattivi, F.; Castellarin, S.D. Transcriptome and metabolite profiling reveals that prolonged drought modulates the phenylpropanoid and terpenoid pathway in white grapes (*Vitis vinifera* L.). *BMC Plant Biol.* **2016**, *16*, 67. [CrossRef] [PubMed]
50. Wong, D.; Gutierrez, R.L.; Dimopoulos, N.; Gambetta, G.; Castellarin, S. Combined physiological, transcriptome, and cis-regulatory element analyses indicate that key aspects of ripening, metabolism, and transcriptional program in grapes (*Vitis vinifera* L.) are differentially modulated accordingly to fruit size. *BMC Genom.* **2016**, *17*, 416. [CrossRef] [PubMed]
51. Poret, M.; Chandrasekar, B.; van der Hoorn, R.A.; Avice, J.C. Characterization of senescence-associated protease activities involved in the efficient protein remobilization during leaf senescence of winter oilseed rape. *Plant Sci.* **2016**, *246*, 139–153. [CrossRef] [PubMed]
52. Jackman, R.L.; Stanley, D.W. Perspectives in the textural evaluation of plant foods. *Trends Food Sci. Technol.* **1995**, *6*, 187–194. [CrossRef]
53. Lijavetzky, D.; Carbonell-Bejerano, P.; Grimplet, J.; Bravo, G.; Flores, P.; Fenoll, J.; Hellín, P.; Oliveros, J.C.; Martínez-Zapater, J.M. Berry flesh and skin ripening features in Vitis vinifera as assessed by transcriptional profiling. *PLoS ONE* **2012**, *7*, e39547. [CrossRef]
54. Shangguan, L.; Mu, Q.; Fang, X.; Zhang, K.; Jia, H.; Li, X.; Bao, Y.; Fang, J. RNA-Sequencing Reveals Biological Networks during Table Grapevine ('Fujiminori') Fruit Development. *PLoS ONE* **2017**, *12*, e0170571. [CrossRef]
55. Miedes, E.; Zarra, I.; Hoson, T.; Herbers, K.; Sonnewald, U.; Lorences, E. Xyloglucan endotransglucosylase and cell wall extensibility. *J. Plant Physiol.* **2011**, *168*, 196–203. [CrossRef]
56. García, J.; Pope, C.; Altimiras, F. A Distributed-Means Segmentation Algorithm Applied to Lobesia botrana Recognition. *Complexity* **2017**, *2017*, 5137317. [CrossRef]
57. Thiéry, D.; Monceau, K.; Moreau, J. Different emergence phenology of European grapevine moth (*Lobesia botrana*, Lepidoptera: Tortricidae) on six varieties of grapes. *Bull. Entomol. Res.* **2014**, *104*, 277–287. [CrossRef] [PubMed]