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On-vine grape withering as a sustainable innovation to premium wine and maturity decoupling

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ABSTRACT

Withering, a traditional post-harvest practice for premium wines such as Amarone and Recioto, involves grape dehydration to enhance concentration and complexity. This study compared two techniques applied to Corvina grapes: dehydration in *fruttaio* (FD) and on-vine withering after peduncle twist (PD), evaluating their effects on grape composition, wine quality, and transcriptome dynamics. FD accelerated sugar accumulation and berry weight loss compared to PD, though both methods ultimately reached similar sugar levels. In grapes, no significant differences emerged between FD and PD for total anthocyanins (1–1.5 mg kg^{-1}), polyphenols (2.5–3 mg kg^{-1}), or antioxidant capacity (≈1.5 mM g^{-1}). These results indicate that changes were largely driven by water loss, with anthocyanins and polyphenols remaining stable while antioxidant activity declined under both treatments. In wines, differences became more evident. FD-derived wines contained higher anthocyanin levels (1000–1900 mg L^{-1}) and polyphenols (20–29 mg L^{-1}), compared with PD wines, which retained greater antioxidant activity. The enrichment in FD wines reflects both solute concentration due to dehydration and ethanol-enhanced extraction during fermentation. Differences in antioxidant capacity could be linked to tannin structure and polymerization, affecting wine smoothness and astringency. Transcriptomic analysis identified approximately 10,000 differentially expressed genes, with significant shifts in stilbene and pectin metabolism, highlighting roles in stress adaptation and cell wall remodeling. FD berries exhibited stronger transcriptional responses, with more pronounced activation of stress-related genes, reflecting the faster dehydration dynamics of *fruttaio* conditions. In conclusion, while both withering methods produced grapes with comparable phenolic content, they resulted in distinct wine compositions and transcriptomic signatures. On-vine withering emerges as a promising sustainable approach, reducing energy demand while maintaining wine quality, an important advantage in the context of climate change and low-impact viticulture.

1. Introduction

Grapevine (*Vitis vinifera* L.) is a perennial species that have been cultivated for centuries, firstly in the Northern and then also in the Southern hemisphere, for their fruits, used worldwide for wine production, fresh grape consumption, raisin production, and juice (Dong et al., 2023). During the ripening, grapes develop the key physical and chemical properties sought by growers and winemakers to produce wine. Among the countless styles of wine, sweet and dry wines are noteworthy made from dried grapes. These kind of wines are common in many countries, such as Croatia, France, Greek, Hungary, Italy and Spain,. Italy boasts a rich winemaking tradition of sweet wines, and

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several regions are renowned for their unique production methods and grape varieties. Among these, the northeastern Italian region Valpolicella (Province of Verona) focuses on the production of two iconic wines: Recioto della Valpolicella, a sweet wine, and Amarone della Valpolicella, a dry, full-bodied red wine. Recioto and Amarone wines are made from the blend of dried red grapes Corvina (45–95 %), Rondinella (5–30 %) and other autochthonous not aromatic red varieties, such as Molinara (up to 15 %). However, a critical step in the production of these highly sought-after wines involved the withering of the grapes. The partial drying of grapes to concentrate sugars and promoting the accumulation of overripening- and/or withering-related aroma compounds are the crucial steps, able to enhance the complexity and the intensity of the resulting wine (D'Onofrio et al., 2019; Paronetto and Dellaglio, 2011). There is a distinct difference in the dehydration kinetics of different grape varieties under identical controlled conditions, probably due to their morpho-anatomical characteristics, such as the size of the berries, the thickness of their skin, and the characteristics of their cuticles. Pinot noir grapes subjected to dehydration did not change the berry anthocyanin content and wine made from these dehydrated grapes contained a higher quantity of volatile compounds when compared to wine made from undried grapes (Moreno et al., 2008). The impact of short-term withering on grape volatile compounds resulted in contrast in previous studies. Indeed, Bellincontro et al. (2016) observed a decrease in volatile compounds, while a higher grape-derived and fermentation-produced volatile profiles by withering was more recently reported (Slaghenaufi et al., 2020). Additionaly, Corvina grapes were reported to undergo significant metabolic changes during the prolonged withering period, more pronounced by the accumulation of stress-related amino acids, sugars, organic acids, flavonols, stilbene compounds, and ethanolamine as well as the decrease of flavanols (Degu et al., 2021).

Postharvest dehydration induced a higher expression of genes involved in hormones (e.g., ethylene) and sugars metabolism, defense mechanisms and stress protection mechanisms, such as dehydrin and osmolyte accumulation. Furthermore, genes involved in hexose metabolism and transport, cell wall composition, and secondary metabolism (pathways for phenolic and terpene compounds) were upregulated by grapes dehydration (Rizzini et al., 2009; Zamboni et al., 2008). The wilting process strongly induced the stilbene synthase (STS) in several cultivars, among which Corvina and Sangiovese (Versari et al., 2001; Zenoni et al., 2016). After grapes dehydration, transcriptomics highlighted a massive upregulation of genes related to phenylpropanoid, stilbene, and lignin metabolism, a significant expression level reduction of anthocyanin-related genes, the modulation of several genes related to ethylene and auxin metabolism, oxidative and osmotic stress responses, anaerobic respiration, defense responses, cell wall metabolism, and carbohydrate metabolism (Zenoni et al., 2016).

The current regulations for Amarone wine production mandate an average withering period ranging from weeks to months. Several traditional and modern withering methods were used. Among them, the following techniques are used for Corvina grapes: i) dehydration in "*fruttaio*" (closed facility), with a meticulous control of the environment (temperature, humidity, and air) and an accurate management of withering, resulting in grapes of consistent quality and potentially minimizing spoilage or uneven drying; ii) withering in *fruttaio* under natural environmental conditions, leading potentially to more variability in grape dehydration (Sanmartin et al., 2021); iii) on-vine techniques, based on late harvest, cane cutting or peduncle twist, inducing gradual and controlled dehydration of the grapes, concentrating sugars, aromas, and phenolic compounds as they soften and shrivel (Mencarelli and Bellincontro, 2013).

The metabolomic and transcriptomic comparison of Corvina grapes withered following natural or controlled dehydration conditions highlighted a significant dampens of transcriptomic and metabolomic reprogramming. Indeed, a slower dehydration process is required to induce genes expression and metabolites accumulation related to the quality traits, such as a higher production of stilbenoids (Zenoni et al., 2020). Additionally, the wines obtained from on-vine (peduncle twist) and *fruttaio* withering have significant differences in terms of aroma volatile profile. The concentration of esters and citronellol can be mostly associated with wines obtained from grapes withered using a *fruttaio*, while, linalool, β-damascenone, and α-ionol can be mostly associated with wines producing by on-vine withering grapes (Slaghenaufi et al., 2020).

Given the increasing interest in exploring the impact of postharvest dehydration techniques on grape behavior, in the present work, for the first time, the on-vine withering and dehydration in *fruttaio*, employed to wither Corvina grapes, were compared, analyzing their effects on grape composition, transcriptome, and the quality of resulting wines. Through a detailed investigation of the two withering techniques, we aimed to provide a comprehensive understanding of the influence of these practices on the transformation of Corvina grapes during withering. Moreover, our findings raise important questions about the role of on-vine withering in modern viticulture, particularly in the context of climate change, sustainability, and the optimization of grape phenolic and technological maturity. Understanding how on-vine withering can be effectively integrated into contemporary winemaking practices could offer new insights for both quality enhancement and resource efficiency.

2. Methods

2.1. Experimental design and sampling

The experimental vineyard, owned by Masi winery, is located in Lazise (Verona, Italy), at an altitude of 70 m above sea level (45°29′22.9″ N 10°46′20.5″ E). Planted with Corvina vines grafted onto SO4 rootstock in 2007, the vineyard is trained using the Guyot system with a planting density of 2.8 m between rows and 0.8 m within rows. The trial, conducted in 2017 growing season, utilized nine rows split into three blocks, totaling 2538 vines. Each row within a block was assigned to one of three different conditions: *i*) vinification without withering (FF); *ii*) on-vine dehydration after peduncle twist (PD); *iii*) dehydration in *fruttaio*, closed facility (FD). Sampling times were defined following the sugar content (using °Babo as unit). All conditions were monitored starting from 18 °Babo (T0; control samples). At 18 °Babo, grapes for FD were harvested and stored for dehydration in *fruttaio* at Gargagnago di Valpolicella (Veneto, Italy), in three different racks, one for each replicate. For the FD condition, grapes were stored in a non-conditioned withering facility (*fruttaio*) with natural ventilation, where the temperature gradually decreased from approximately 16 °C to 7 °C, while the relative humidity progressively increased from about 55 % to 80 %. Concurrently, PD grapes were subjected to peduncle twist. Both drying processes were carried out until a 30 % reduction in berry weight was achieved, which was after 55 and 49 days under PD and FD conditions, respectively. Samples were collected at full commercial maturity, corresponding to around 19 °Babo (Fig. 1A). Approximately 800 g in total of berries from each condition were harvested at four different time points (Fig. 1A). Sugar concentration was monitored by mechanically crushing a 100-berry sample and determining total soluble solids using a refractometer (Global Water Instruments, Sacramento, CA, USA). The same plant material was used for quality evalutation (total anthocyanin and polyphenol content, and antioxidant capacity) and molecular analysis (RNA-Seq), considering at least three biological replicates for each application. Only healthy undamaged bunches were used. Berries for the whole transcriptomic analysis belonging to each thesis and time (Fig. 1A) were immediately frozen with liquid nitrogen and stored at −80 °C until use.

2.2. Total anthocyanin content, total polyphenolic index and antioxidant capacity of grapes

Total anthocyanin (TAC) and polyphenolic (TPC) content, and

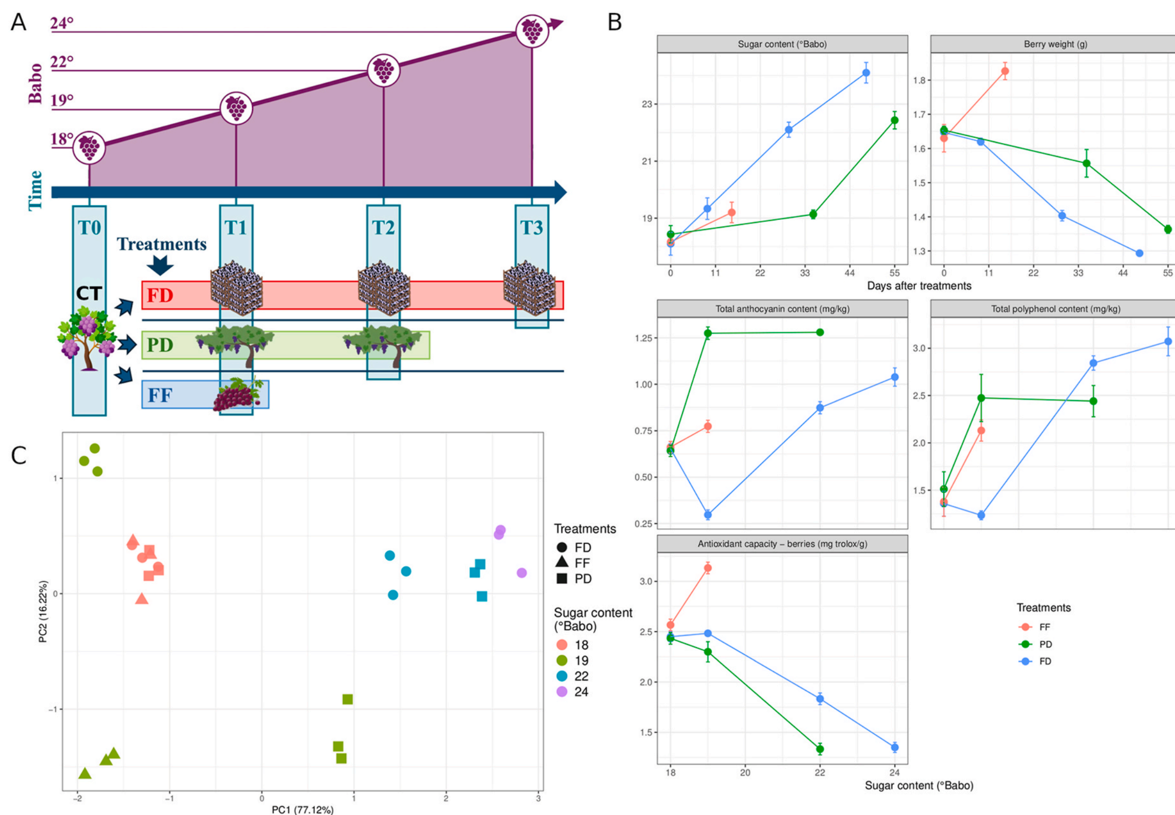

Fig. 1. **A.** Graphical representation of experimental plan used to investigate the impact of dehydration techniques on Corvina berries. **B.** Sugar content, berry weight, total anthocyanin and polyphenol content, antioxidant capacity of Corvina grapes under dehydration techniques. Statistical analysis results are reported in the main text. **C.** Principal Component Analysis using phenotypical data. CT: control samples; FF: traditional vinification without withering; FD: dehydration in *fruttaio*; PD: on-vine dehydration after peduncle twist. Error bars represent standard deviations ($n = 3$). Samples for each condition were collected following the °Babo, as unit of sugar content: T0 - 18 °Babo (August 31) for FF, FD, and PD; T1 - 19 °Babo for FF (September 14), FD (September 8), and PD (October 4); T2 - 22 °Babo for FD (September 28), and PD (October 24); T3 - 24 °Babo for FD (October 17).

antioxidant capacity (AC) were determined spectrophotometrically, using a GENESYS 180 scan UV–Vis spectrometer (Thermo Fisher Scientific, Waltham, Massachusetts). Three biological replicates (20 berries) of each condition and sampling date were used for the analythical determinations. Two-steps extractions were performed for TAC on fresh grape skins in 50 + 50 mL of ethanol:water:hydrochloric acid solution (70:29:1, v/v/v), for a total of 48 h at room temperature. The absorbance of the extract was measured at 540 nm. Data were expressed as milligrams of malvidin-3-O-glucoside per kilogram of grapes, using a molar extinction coefficient (ε) of 16.17 L mol^{-1}. The same extract was used to evaluate the TPC, using the Folin–Ciocalteu assay as reported in Rustioni et al. (2020). The absorbance of the extract was measured at 700 nm. Data are presented as milligrams of catechin per kilogram of grapes. Finally, AC was assessed by DPPH (2,2-difenil-1-picrilidrazile) assay, following the method described in Rustioni et al. (2020), starting from grape skin extracts, diluted in methanol at 70 % (v/v). The absorbance of the extract was measured at 515 nm. Data were expressed as millimoles of Trolox equivalents per kilogram of grapes.

2.3. Total anthocyanin content, total polyphenolic index and antioxidant capacity of wines

The grapes collected at the final stage of each condition (Fig. 1A) were vinified by colleagues from the Department of Biotechnology, University of Verona (Verona, Italy). The vinification protocol have been previously reported in Slaghenaufi et al. (2020). Briefly, all vinifications were performed in triplicate using 100 kg of destemmed and crushed grapes per replicate. Musts were treated with 100 mg/L potassium metabisulfite and fermented in 75 L steel tanks inoculated with the proprietary *S. cerevisiae* strain MASY03 (Microbion, Castel d'Azzano, Italy). After fermentation, potassium metabisulfite was added to achieve 30 mg L^{-}1 free SO_2. Wines were filtered (1 μm), bottled, and stored at 16 °C until analysis. In this study, TAC, TPC, and AC of wines were evaluated using the same methods adopted for grape skin extracts. The values obtained were expressed as milligrams of malvidin-3-glucoside, milligrams of catechin, and millimoles of Trolox equivalents per liter of wine, respectively.

2.4. RNA extraction, library preparation, and sequencing

Four-hundred (400) mg of ground berry tissue was used for total RNA extraction, using the Spectrum Plant Total RNA kit (Sigma-Aldrich, Darmstadt, Germany), as reported in De Lorenzis et al. (2016). RNA integrity was assessed using an Agilent Bioanalyzer RNA nanochip (Agilent, Wilmington, DE). Sequence libraries were prepared as previously reported (Puccio et al., 2022). Quality, insert size distribution and abundance of libraries were assessed using the Agilent Bioanalyzer DNA 1000 chip. Sequence libraries were pooled in equimolar concentration and analyzed on an Illumina NextSeq500 generating 2 × 75 nt paired end (PE) reads.

2.5. RNA-seq analysis

Raw reads were first assessed for quality using FastQC v0.11.8 (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) and then quality trimmed using Trimmomatic v0.38.0 (Bolger et al., 2014) with the following parameters: LEADING:3 TRAILING:3 SLIDINGWINDOW:4:15 MINLEN:36. Reads were filtered by length and only

those longer than 36 bp were selected. The clean reads were used for quantification through SALMON (Patro et al., 2017) using the latest *Vitis vinifera* genome available on ensembl plants (PN40024. v4) (Bolser et al., 2016). Differentially Expressed Genes (DEGs) were obtained using DESeq2 (Love et al., 2014) with pairwise contrasts between control and treatments and between time-points (log2FoldChange $\geq \pm 1.0$ and Padj <0.05). Principal Component Analysis (PCA), and heatmap visualization were carried out by DESeq2 and pheatmap (Kolde and Kolde, 2015) R (R Core Team, 2021) packages. Gene Ontology (GO) enrichment analysis was performed using the g:profiler R package (Reimand et al., 2016). Clustering of significantly enriched GO terms was performed through the enrichment map plugin (Merico et al., 2010), using Cytoscape V.3.10.1 (Smoot et al., 2011) and the Mcode clustering method. Cluster labels were selected using the WordCloud cytoscape app on each cluster with a minimum word occurrence of three. DEGs were further clustered using the hclust function of R with the ward. D2 method.

Reverse transcription quantitative PCR (RT-qPCR) was performed to validate the transcript abundance of randomly selected genes in the comparison CT *versus* FD (Supplementary Table S1). Total RNA reverse transcription was performed using 500 ng of RNA, SuperScript® IV Reverse Transcriptase (Thermo Fisher Scientific, Waltham, MA USA), and a 1:1 mix of random primers and 50 μM oligo(dT)20 primers (Thermo Fisher Scientific), according to the manufacturer's instructions. RT-qPCR was carried out on a Quant Studio 3 Real-Time PCR System (Thermo Fisher Scientific). Each reaction contained 500 nM of each primer, 4 μL of cDNA (1:10 dilution of the synthesis reaction), 10 μL of PowerUpTM SYBRTM Green Master Mix (Thermo Fisher Scientific), and water up to 20 μL. Each reaction was performed in triplicate. The applied thermal cycling conditions amounted to a hold stage at 50 °C for 2 min and 95 °C for 10 min, followed by 50 cycles: 95 °C for 20 s, 57 °C for 45 s, and 72 °C for 30 s. Finally, a melting curve stage at 95 °C for 15 s, 57 °C for 1 min, and 95 °C for 1 s to detect non-specific amplification in cDNA samples has been introduced. *Actin* (VIT_06s0004g04570) was used as reference gene. Expression levels were calculated using the delta-delta Ct (ΔΔCt) method (Livak and Schmittgen, 2001).

2.6. Co-expression analysis and phenotypic correlation

Weighted Gene Co-expression Network Analysis (WGCNA) was performed on the entire RNA-Seq variance stabilized transformed (VST) data using the *WGCNA* R package (Langfelder and Horvath, 2008). Briefly, the soft threshold was selected using the scale-free topology method. The VST data were then used to generate a weighted adjacency matrix that was then converted into the Topological Overlap Matrix (TOM), which was employed to identify co-expression modules. These modules were subsequently merged based on a 0.3 similarity threshold using the merge module function. To highlight the specific response of each module under different conditions, both linear regression and correlation matrix approaches were utilized. To fit a linear regression between the eigengenes of each module and each experimental condition and then ranked the gene clusters through the resulting coefficients, the *glm* R function was used. The Pearson correlation coefficient between each module eigengene and the experimental conditions was also evaluated using a binary matrix.

For each module, significantly enriched metabolic pathways using the *pathview* R package (Luo and Brouwer, 2013) and the iPath online tool (Letunic et al., 2008) were detected.

2.7. Statistical analysis

TAC, TPC a AC of grapes and wines were analyzed for homogeneity of variance using Levene's test. To account for heteroscedasticity, a Generalized Least Squares (GLS) model was employed using the *nlme* R package (Pinheiro et al., 2020). *Post-hoc* comparisons were performed with the *multcomp* R package to determine statistical significance between treatments (Hothorn et al., 2008). The phenotypic data were analyzed using Principal Component Analysis (PCA) implemented in *ggplot2* R package (Wickham, 2010). RT-qPCR values were analyzed using Pearson's correlation implemented in R (R Core Team, 2021).

3. Results

3.1. Impact of the two withering methods on grapes

The effects of the withering process were assessed on Corvina grapes under two different dehydration conditions (FD and PD), with FF serving as the non-withered control. The FD and PD drying processes were applied to grapes at 18 °Babo, while for the samples without withering (FF), the grapes were harvested at 19 °Babo. The grape maturity indices were assessed at 19 (FF), 22 (PD and FD), and 24 (FD) °Babo, respectively, overlapping with the sampling times. FD condition speeded the total soluble solids concentration, reaching a maximum level at approximately 50 days. By contrast, the berries belonging to PD required more time (around 55 days) to attain a sugar content of 22 °Babo. A similar profile was observed for berry weight loss. The berries from FD showed a faster weight reduction compared to PD, reaching approximately 80 % of their initial weight after 48 days, compared to 55 days (Fig. 1B). The grapes from FF condition, after the first sampling (T0) remained on the grapevines for additional 15 days, reaching a sugar level of 19 °Babo and a weight of 1.83 g (Fig. 1B). Berry weight at 22 °Babo was significantly affected by the withering treatments ($F(1,4) = 13.1$, $p = 0.022$).

The TAC increased in the FF samples during ripening (18–19 °Babo), as well as both PD and FD samples throughout the process. However, significant differences between the two drying processes were observed at 19 °Babo (Fig. 1B; $F(2,6) = 795.4$, $p < 0.001$).

The skin TPC rose in all conditions. In the FF and PD berries, TPC resulted significantly higher than FD at 19 °Babo. However significant differences were not found between withering methods at 22 °Babo (Fig. 1B; $F(2,6) = 110.2$, $p < 0.001$).

The AC decreased in FD and PD samples throughout the time course, by contrast, increased in the FF samples. Interestingly, the FD antioxidant capacity declined more slowly than PD, with significant differences between drying methods at 22 °Babo ($F(1,4) = 112.5$, $p < 0.001$). At 24 °Babo, the FD berries showed similar AC values to those of PD recorded at 22 °Babo (Fig. 1B).

PCA plot generated using the aforementioned phenotypical data displayed a sample separation based on sugar content and applied condition (Fig. 1C). The plot highlighted a distinct clustering pattern, with samples primarily grouped by sugar content along Principal Component 1 (PC1), explaining 77 % of the variance. Samples collected at 22 and 24 °Babo are distinctly separated from those collected at 18 and 19 °Babo, except for the PD berries collected at 19 °Babo. Along PC2, which explains 16 % of the variance, the plot mainly separated FF and PD samples, collected at 19 °Babo, from the others.

3.2. Impact of the two withering methods on wines

At the end of each treatment, the grapes were vinified and TAC, TPC, and AC were measured in the resulting wines. FD wines showed the

Table 1
Total anthocyanin content (TAC), total polyphenol content (TPC) and antioxidant capacity (AC) of Corvina wine obtained by grapes subjected to different dehydration techniques (FF: traditional vinification without withering; FD: dehydration in *fruttaio*; PD: on-vine dehydration after peduncle twist). Statistical analysis results are reported in the main text.

Treatments	TAC (mg L^{-1})	TPC (mg L^{-1})	AC (mg L^{-1})
FF	684 ± 45	14.49 ± 0.33	1.53 ± 0.01
PD	1015 ± 18	20.77 ± 0.28	1.03 ± 0.01
FD	1912 ± 76	29.07 ± 0.33	0.84 ± 0.01

highest TAC and TPC levels, while FF wines exhibited the lowest values (Table 1). All differences were statistically significant ($F(2,6) = 288.5$, $p < 0.001$ for TAC, and $F(2,6) = 1418.5$, $p < 0.001$ for TPC). In contrast, AC displayed the opposite pattern, with FF wines showing the highest value (Table 1). Also for AC values, all the differences were statistically significant ($F(2,6) = 5684.9$, $p < 0.001$).

3.3. Transcriptome changes induced by different dehydration techniques

As previously reported, the transcriptomic profiles of berries were compared between FF, PD, and FD conditions at the same °Babo to dissect the effects of the time after harvest and water loss stress (Fig. 1A). Around 10,000 genes were differentially expressed (DEGs) in at least one pairwise comparison (Fig. 2). The most of DEGs exhibited an expression trend of gene clusters significantly different among the three conditions investigated (FF, PD and FD) and compared to the control condition (CT) (Fig. 2A).

Hierarchical clustering of normalized DEG counts highlighted specific profiles for each condition. As expected, the most contrasting profiles was displayed by CT samples, and 43 % and 40 % of down- and up-regulated genes, respectively, were shared among FF, PD, and FD (Fig. 2A; Supplementary Table S2; Supplementary Fig. S1). The high number of common DEGs were displayed by the two different drying methods applied (Fig. 2A; Supplementary Table S2). Among the main five clusters extracted, Cluster 4 and Cluster 5 showed nearly opposite expression trends for the conditions investiageted (FF, PD, and FD) (Supplementary Fig. S1). Interestingly, the expression levels of upregulated genes in Cluster 2 were higher in the FD condition than PD. In addition, a small group of DEGs belonging to Cluster 2 and 5 was downregulated only in *fruttatio* dehydration method (Fig. 2A; Supplementary Table S2; Supplementary Fig. S1). Nine-hundred eighty-five genes were upregulated under both PD and FD; while the expression level of 749 genes were exclusively increased in the FD condition, nearly twice the number observed in the PD and FF (233 and 390, respectively). A similar rank number of DEGs among treatments (FF, PD, and FD) was observed for the downregulated genes (Supplementary Table S2). Regarding the drying methods, FD modulated the highest number of DEGs compared to CT (749 *vs.* 233 up- and 803 *vs.* 195 total down-regulated genes, respectively), while the comparison between PD and FD exhibited 52 unique up- and 77 down-regulated DEGs. Lastly, 1882 up-regulated genes, as well as 1931 down-regulated genes, were affected by all conditions (Fig. 2B).

To validate the transcriptomic results, three DEGs (Supplementary Table S1) were randomly selected and analyzed by RT-qPCR. As expected, their expression profiles were consistent with those obtained from the RNA-Seq experiment ($r = 0.99$, $p = 0.076$) (Supplementary Table S3).

The PCA distinguished all the experimental conditions along with the primary components (Fig. 2C). The PC1, accounting for 69 % of the total variance, effectively distinguished samples based on the sampling time and related treatment. Indeed, the CT samples were separeted on the left part of the axis, while all the treated samples (FF, PD, and FD) formed different private clusters related to the sampling time, highlighting the progressive impact of the treatments on the gene expression changes compared to the control (CT). The PC2 (15 % of the total variance) underlined additional variations that further distinguished the three conditions (FF, PD, and FD) at the same sampling time (Fig. 2C).

DEGs between conditions were classified by a Gene Ontology (GO) enrichment analysis that defined two maps of GO terms, displaying notable differences in enriched functional categories for up- and down-regulated genes (Fig. 3). Nine clusters were identified in the upregulated DEGs dataset with the largest cluster was involved in organelle organization, intracellular sub-compartments (cytoplasm, Golgi apparatus and plastids) and photosynthesis (Fig. 3; Supplementary Table S4). The other significantly enriched categories were related to the cell wall modification and polysaccharide metabolism, followed by cell junctions, protein modification, organic acid metabolism and steroid biosynthesis. Genes upregulated in the FD treatment exhibited the highest number of GO terms related to organelles, intracellular compartments, and biosynthetic processes (Fig. 3; Supplementary Table S4). Nearly all the

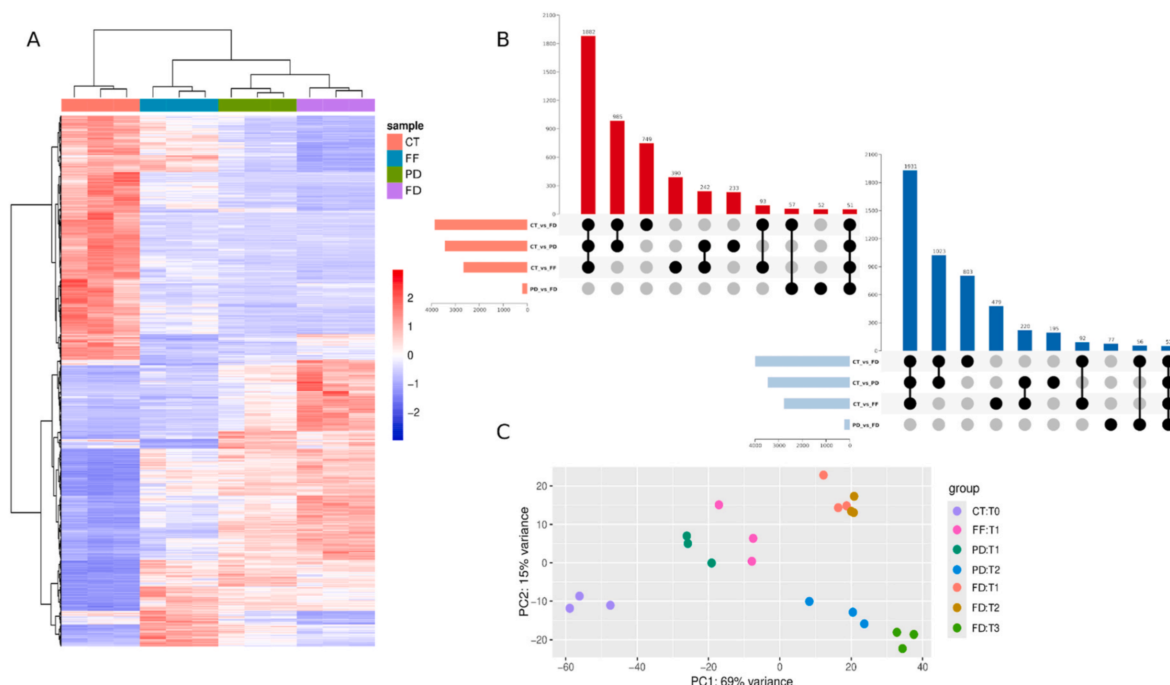

Fig. 2. RNA-Seq analysis of Corvina grapes under dehydration techniques. **A** Heatmap of the Variance Stabilized Data (VSD) for Differentially Expressed Genes (DEGs) at the final time-point for each treatment (T0: control, T1: FF, T2: PD, T3: FD). **B** UpSet plots of upregulated (red) or downregulated (blue) DEGs in the main comparisons. Dots or lines highlight common sets of genes. **C.** Principal Component Analysis. CT: traditional vinification without withering; FF: traditional vinification without withering/fresh fruit. FD: dehydration in *fruttaio*; PD: on-vine dehydration after peduncle twist. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

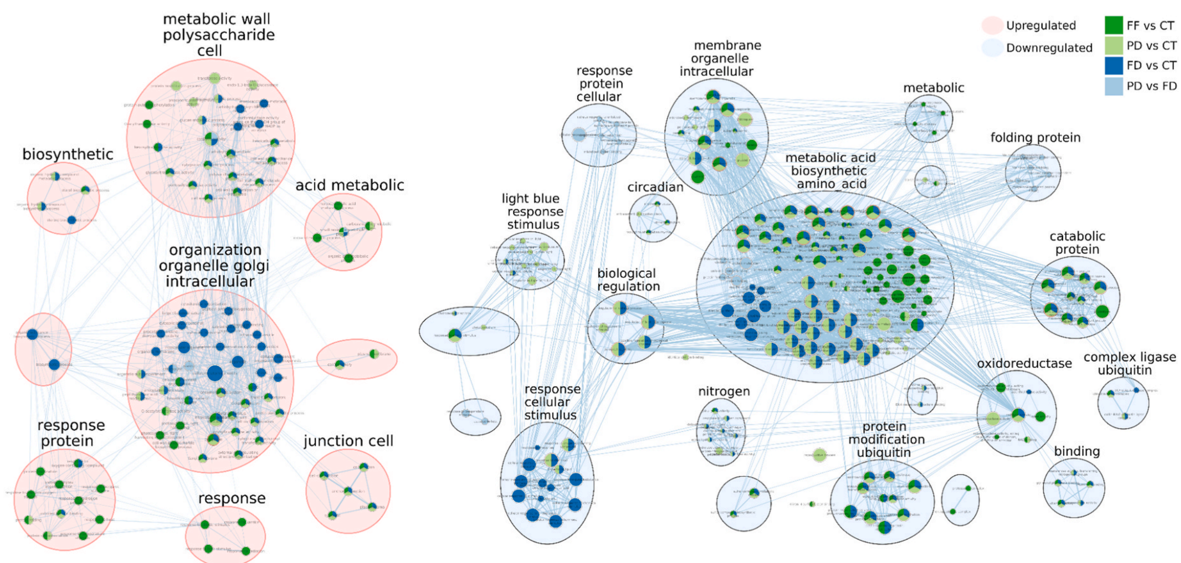

Fig. 3. Enrichment Map-based network of significantly enriched GO terms in both up-regulated (pink) and down-regulated (blue) genes, obtained from RNA-Seq analysis of Corvina grapes under different dehydration techniques. Nodes represent pathways and are connected by edges (lines) indicating shared genes between pathways. Nodes are colored based on the set of DEGs in which that term was significantly enriched, as highlighted in the legend: green - FF *vs.* CT; lightgreen - PD *vs.* CT; blue - FD *vs.* CT; and lightblue - PD *vs.* FD. The size of a node depends on the enrichment score of the pathway, while the thickness of the edges reflects the number of genes shared between the nodes. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

GO terms related to response to stimuli and to oxygen-containing compounds were found in the FF treatment (Supplementary Table S4). In the downregulated DEGs, fifteen (15) clusters were identified (Fig. 3). Among these, the largest cluster encompassed 148 GO terms, mainly related to organic acid metabolism and amino acid biosynthesis. The others were related to protein catabolism, ubiquitination and modification, oxidoreductase activity, response to nitrogen, regulation of cellular processes, intracellular compartments, and response to stimuli, the latter mainly correlated to FD (Fig. 3; Supplementary Table S4).

3.4. Co-expression network analysis

Weighted Gene Co-expression Network Analysis (WGCNA) analysis was conducted on the entire RNA-Seq output from all the pairwise comparisons. DEGs were included in 23 co-expression modules ranging from 43 (palevioletred3) to 4352 (yellow) genes (Supplementary Table S5). The correlation heatmap between co-expression modules and grape phenotypic data (TAC, TPC, and AC), recorded in both berries and wine for each conditions, allowed to select specific modules correlated to investigated phenotypical traits (Fig. 4A; Supplementary Table S6). In detail, the pink, orange, and darkorange2 modules were significantly and positively correlated to several of evaluated traits. By contrast, the darkolivegreen, skyblue3, yellow, darkturquoise, and brown4 were negatively correlated, with statistically significant values. Lightyellow showed a negative and significant correlations to the traits recorded in grape but not in wine. The co-expression modules related to distinct drying techniques were also identified through the module-condition correlation assessment. Seven modules were significantly correlated to different conditions (Fig. 4B; Supplementary Table S5; Supplementary Table S6). Both the pink (3048 genes) and plum1 (1657 genes) modules were downregulated in all three conditions, whereas the yellow (4352 genes), brown4 (785 genes), and sienna3 (606 genes) modules were consistently upregulated in FF, PD, and FD. By contrast, the genes co-expression of blue (3134 genes) and lightyellow (1870 genes) modules appeared strictly correlated to the FD condition. Indeed, the blue and the lightyellow module were significantly down- and up-regulated, respectively, under *fruttatio* dehydration. The seven correlated modules were then characterized through a GO enrichment analysis to identify specific pathways or functions involved in the different dehydration mechanisms (Fig. 4B; Supplementary Fig. S2; Supplementary Table S7). In the Biological Process (BP) category, the most upregulated module (yellow) in all conditions (FF, PD, and FD) compared to control (CT) condition was chiefly related to regulatory processes, gene expression, catabolic processes, and biosynthesis of organic substances. Consistely, the brown4 module showed the same trend of the yellow one, with response to abiotic and light stimuli and GOs involved in nucleic acid metabolism (e.g. nicotinamide nucleotide biosynthetic process) upregulated in all drying condidions. Meanwhile, the pink module, downregulated in FF, PD, and FD, was primarily related to the biosynthesis of organic substances and organonitrogen compounds, as well as protein metabolism and modification. Interestingly, the blue module, downregulated only under FD treatment, was primarily involved in terms related to photosynthesis, small molecule metabolism, and organonitrogen compound biosynthesis. By contrast, the light-yellow module, which was upregulated under FD treatment, was related to cellular component biosynthesis and organization, DNA repair processes, gene expression, and nucleic acid metabolism (Supplementary Table S8).

In the Cellular Component (CC) category, both the yellow and pink modules were enriched for terms related to the endomembrane system, protein-containing complexes, and the nucleus (Fig. 4B). Additionally, yellow module was enriched for terms related to nucleus, as well as the lightyellow module. In agreement to BP, the blue module showed enrichment for terms related to mainly chloroplast and plastid in CC. Yellow and pink modules shared Molecular Function (MF) terms related to RNA binding and hydrolase activity (Supplementary Fig. S2). A limited number of KEGG pathways showed significant enrichment, with all the modules, except the lightyellow, that resulted enriched in biosynthesis of secondary metabolites term (Supplementary Fig. S2; Supplementary Table S9).

3.5. Modulation of genes involved in the dehydration process

Many genes commonly associated with responses to dehydration, drought, and heat stress were identified among the differentially expressed genes (DEGs) between applied condictions. Among these, stilbene synthases (41), terpene cyclases (eight), dehydration-induced genes (five), pectinesterases (29), genes related to xyloglucan metabolism (28), sucrose-related genes (14), BURP domain-containing genes

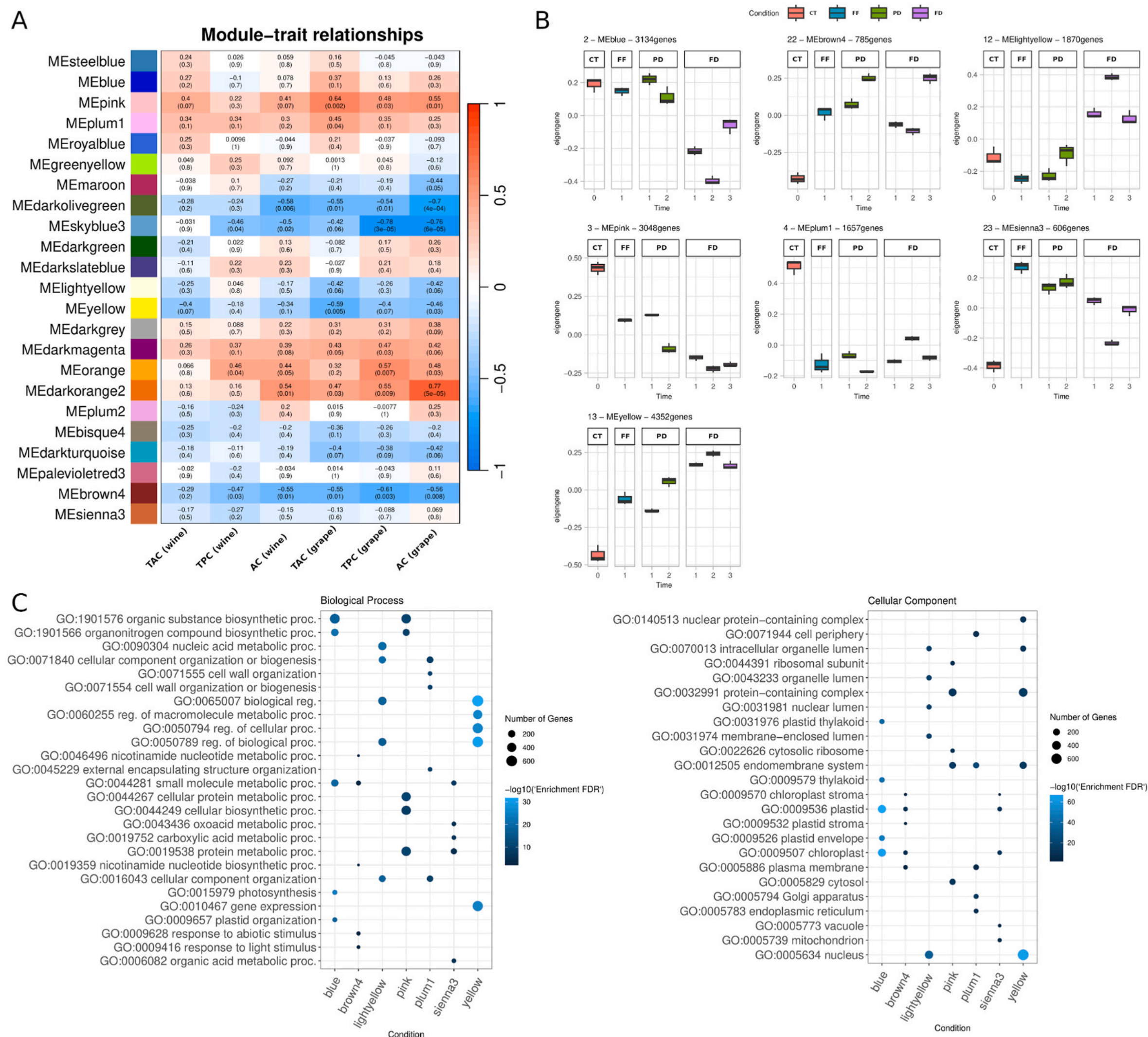

Fig. 4. Weighted Gene Co-expression Analysis (WGCNA) performed on the entire RNA-Seq dataset of Corvina grapes under three dehydration techniques. **A** Heatmap of module-trait relationships. Positive and negative correlations are highlighted using the color scale shown in the legend, and the p-value of the Pearson correlation is indicated in each cell. CT: traditional vinification without withering; FF: traditional vinification without withering/fresh fruit. FD: dehydration in *fruttaio*; PD: on-vine dehydration after peduncle twist; TAC: total antocyanin content [ACN(mg)/skin(g)]; TPC: total polyphenol content [catechin(mg)/skin(g)]; AC: antioxidant capacity [trolox(mM)/grapes(Kg)] **B** Boxplots showing the seven modules related to distinc conditions at each timepoint. **C** Dotplot of the GO enrichment analysis performed on each co-expression module's gene sets, selecting only the module related to specific drying condition. The 5 most enriched GO terms in the Biological Process (left) and Cellular Component (right) categories for each module are shown. The -log10(FDR) of the test is highlighted using a color scale, while the number of genes for each GO category is represented by the dot size. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

(four), and transcription factors involved in abiotic stress responses (36 WRKY and 27 MYB) were included (Fig. 5; Supplementary Table S9). All the stilbene synthase genes were upregulated during berry dehydration, regardless of the dehydration mechanisms (yellow module). By contrast, most genes related to xyloglucan metabolism (21 out of 28), as well as terpene-related genes (seven out of eight) were downregulated during dehydration.

Six DEGs resulted correlated to FD treatment (using a cut-off of ±2 log2FC). Among them, a stilbene synthase, a pectin esterase, a sucrose metabolism related gene and five transcription factors (TFs), one and four belonging to the *WRKY* and *MYB* gene families, respectively, were significantly upregulated by the treatment. By contrast, two terpene cyclase genes were downregulated. The *RESPONSIVE TO DEHYDRATION* 22 (*RD22*) genes, strictly related to dehydration and drought stress response in many plant species, were found significantly upregulated under FD treatment and strongly downregulated under FF. Finally, a gene related to xyloglucan metabolism and another *WRKY* family member were upregulated under the PD treatment (using a cut-off of ±2 log2FC).

3.6. Metabolic pathways involved in the main response to dehydration

To identify the main metabolic pathways involved in dehydration, the two strongly correlated modules to this process, yellow and pink, were analyzed (Supplementary Fig. S3, Supplementary Fig. S4). The yellow module was mainly associated with flavonoid biosynthesis, circadian rhythm, phenylalanine and glutathione metabolism, as well as the secondary metabolites biosynthesis (Supplementary Fig. S3). By contrast, the pink module was primarily related to ribosome function, phagosome formation, biotin metabolism, and steroid biosynthesis (Supplementary Fig. S4).

Genes correlate to specific secondary metabolites biosynthesis were included in both selected modules. Two hundred fourteen (214) genes were annotated within this category, among which 134 up- and 80

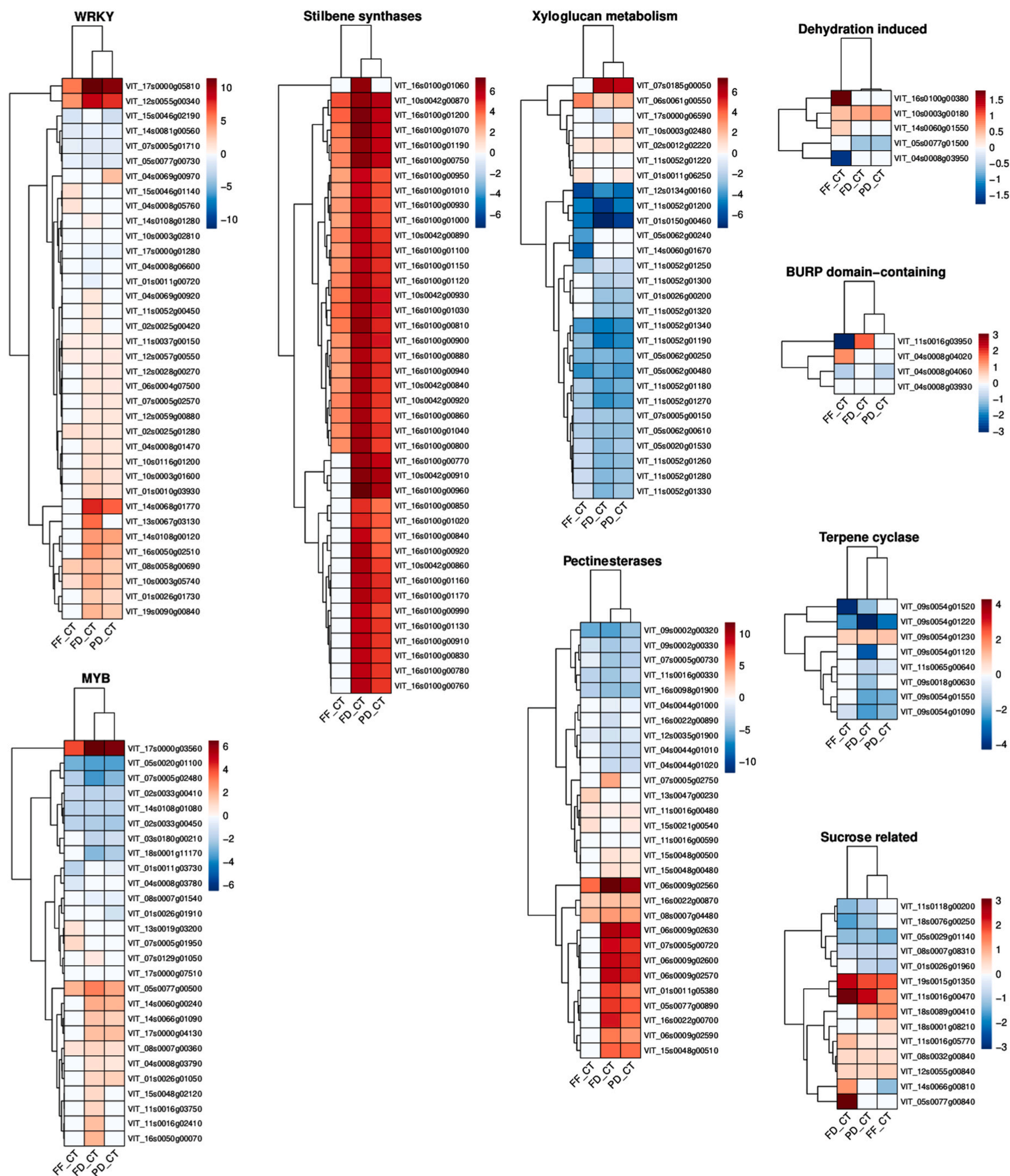

Fig. 5. Heatmaps of Differentially Expressed genes involved in the main pathways modulated during the dehydration process in Corvina grapes. The log2FC for each comparison (FF vs CT, PD vs CT and FD vs CT) are showed. Hierarchical clustering was performed on both rows and columns. CT: traditional vinification without withering; FF: traditional vinification without withering/fresh fruit. FD: dehydration in *fruttaio*; PD: on-vine dehydration after peduncle twist.

down-regulated in the yellow and pink module, respectively. Most of the upregulated DEGs are included in the terpenoid biosynthesis, starch and glucose metabolism, pentose phosphate pathway, phenylpropanoid biosynthesis and glyoxylate as well as dicarboxylate metabolism. Among downregulated DEGs, the most are included in steroid and phenylpropanoid biosynthesis and glyoxylate, as well as the dicarboxylate metabolism (Supplementary Fig. S5).

4. Discussion

In climacteric fruits, the ripening and senescence processes can continue after harvest, with a significant increase in metabolic changes and respiration process, as well as ethylene synthesis that impact on several traits. Nevertheless, postharvest processes in non-climacteric fruits, like grape, have also been shown to involve active metabolisms, including phytohormone signaling, transcriptional regulation, and a range of physiological and biochemical modifications, in which several modifications are governed mainly by abscisic acid (ABA) (Zenoni et al., 2020, 2023). Withering is a post-harvest process commonly used to produce sweet or fortified wines, as well as some high-structured dry wines. To produce these wines, like Amarone wines, different grape dehydration techniques can be adopted, differing for water loss level, as well as the environmental conditions (Sanmartin et al., 2021). Although the technology of post-harvest fruit dehydration has been extensively

studied at both physiological and transcriptomics level (Zenoni et al., 2020), on-vine dehydration remains poorly investigated. Indeed, a direct comparison between on-vine withering and controlled post-harvest dehydration has been unexplored until now. Therefore, this study investigates the relationship between transcriptomic changes and dehydration kinetics in grapes subjected to two commonly used withering techniques, emphasizing the novelty of our approach. Here, the dehydration technologies on-vine after peduncle twist (PD) and in *fruttaio* (FD) were compared, alongside to their differences relative to the natural maturation of grapes ripening on plants, identifying physiological and transcriptional responses and their ultimate impact on wines.

4.1. Impact of dehydration techniques on grape composition and properties

The withering process leads to berries water loss. Concurrently to a water content decrease, an increase of sugar levels was previously observed (Mencarelli et al., 2010; Panceri et al., 2013). Furthermore, temperature, relative humidity, and air flow, as well as berry size, shape, and skin surface, were reported to affect the intensity and rate of post-harvest water loss (Mencarelli and Bellincontro, 2013). In our experiments, the environmental conditions strongly impacted on the effect of both withering processes on berry weight and sugar content. FD and PD treatments determined a berry weight reduction and an increase in sugar level, as expected. In addition, FD led to a faster total soluble solid concentration, achieving a peak (24 °Babo) within 50 days compared to PD that required a longer time (about 55 days) to reach a 22 °Babo sugar content. In detail, FD-treated berries experienced a significant weight reduction, losing 20 % of their initial weight within 48 days. A similar postharvest dehydration kinetic, reaching approximately 20 % weight loss after 50 days (27 °Brix = 23 °Babo) and around 30 % weight loss after 100 days (33 °Brix = 28 °Babo) was previously observed in Corvina berries (Zenoni et al., 2016). By contrast, PD-treated berries showed a slower rate of dehydration, retaining about 80 % of their original weight after 55 days. These results suggested that FD compared to PD method sped up both sugar concentration and weight loss, that could be due to a constant temperature (15.6 °C) and relative humidity (65.5 %) conditions under which the grapes are maintained in the FD treatment.

The TAC increased during both withering processes. However due to water loss, this did not significantly impact the final anthocyanin content. A similar trend was observed for TPC. Thus, berry weight decreased and concurrently TAC and TPC remained relatively unchanged. This leads to a concentration effect at the end of the dehydrating process already observed in many cultivars, although varying by genotype (Figueiredo-González et al., 2013; Mencarelli et al., 2010; Panceri et al., 2013; Zenoni et al., 2016).

The antioxidant capacity, namely the ability to neutralize reactive oxygen species (ROS) or free radicals, prevented the oxidative damage to cells and tissues (Capanoglu et al., 2022). Antioxidant power is directly related to the presence of antioxidant compounds, such as polyphenols and mainly flavonoids, thus this ability increases with the concentration of these class of compounds rises in grapes during ripening (Figueiredo-González et al., 2013). In our experimental conditions, the antioxidant capacity showed an increasing trend in non-withered grapes (FF), by contrast, it significantly declined in both withering treatments, faster in PD compared to FD. However, the final values of FD and PD grapes did not differ significantly, suggesting that despite differences in dehydration kinetics, both methods ultimately converge toward a similar depletion of antioxidant activity. This finding indicates that the structural transformations of polyphenols occurring during withering may override the effect of the dehydration rate, leading to comparable antioxidant outcomes at the end of the process.

4.2. Impact of dehydration techniques on wine composition and properties

As for the grapes, TAC and TPC values were higher in wines obtained from FD and PD grapes, although the differences became much more pronounced in the corresponding wines. This result can be explained by two concurrent processes. First, grape dehydration reduces the must volume, thereby increasing the concentration of solutes, including phenolic compounds (Figueiredo-González et al., 2013; Mencarelli et al., 2010; Panceri et al., 2013; Zenoni et al., 2016). Second, wines from dehydrated grapes generally exhibit higher ethanol levels due to the elevated sugar content at vinification (24 °Babo for FD grapes and 22 °Babo for PD grapes in our experimental conditions). Ethanol acts as an efficient solvent, enhancing the extraction of skin-derived phenolics during maceration and fermentation (Muñoz-Bernal et al., 2020). The combined effects of solute concentration and enhanced extraction efficiency thus account for the significantly higher anthocyanin and polyphenol content observed in wines produced from dehydrated grapes compared to wines from fresh controls.

Several studies have indicated that the overall concentration of phenolic compounds in wine does not always correlate with its antioxidant activity (Heinonen et al., 1998). Conversely, other authors have reported a positive relationship between phenolic levels and antioxidant capacity (Sato et al., 1996). This apparent discrepancy can be explained by the fact that both antioxidant potential and sensory attributes are influenced not only by the quantity but also by the qualitative profile and structural characteristics of polyphenols (Sun et al., 2011). In our experimental conditions, since no positive correlation was observed between antioxidant capacity and total polyphenol content in wines, it is likely that the determining factor lies in the quality and structural features of the polyphenols, such as the degree of tannin polymerization, with both withering processes acting in this direction. Condensed tannins generally display lower radical-scavenging ability compared to hydrolysable tannins, but their polymerization contributes positively to the sensory profile of wines, such as astringecy (Paissoni et al., 2022). Indeed, wines with higher levels of polymerized tannins exhibited greater sensory appeal, characterized by reduced astringency and a smoother mouthfeel (Pavez et al., 2022). Taken together, our findings suggest that wines produced from FD-treated grapes may better align with consumer preferences due to their enhanced tannin polymerization.

4.3. Transcriptomic variations revealed overlapping responses governing grapevine ripening and dehydration processes

Grape berry development is usually divided into two phases, a lag phase (phase I), in which the berry size increase is evident, while the phase II is the ripening stage, where berries soften and begin to change color (a stage called veraison). Veraison is characterized by rapid cell expansion and sugar accumulation, concurrently with the increase of phenolic and flavors compounds biosynthesis up to the ripening (Mullins et al., 1992). Ripening is characterized by the expression of known target genes, previously characterized (Zenoni et al., 2010), belonging to the glutathione-*S*-transferases family, responsible for the anthocyanin accumulation in the vacuole, stilbene synthases, responsible for the synthesis of resveratrol, and several transcription factors, like *MYB* involved in the regulation of flavonoid biosynthesis (Zenoni et al., 2010).

In this experiment, at the end of each condition (FF:T1, PD:T2, FD: T3), several shared DEGs, regardless the condition, have been observed. DEGs analysis highlighted the activation of several classes of genes, among others stilbene synthases and terpene cyclases, belonged to dehydration-induced genes, pectinesterases, xyloglucan metabolism and sucrose-related genes, BURP domain-containing genes, as well as *WRKY* and *MYB* transcription factors (TF). Interestingly, all these isolated genes and TFs are involved in grapevine ripening as well as responses to biotic stress. Indeed, stilbene synthases belongs to a gene family

responsible for the synthesis of stilbenes, a class of polyphenols that play a key role in defending against both biotic and abiotic stresses, such as drought (Marant et al., 2024). In grapevine berries, stilbenes primarily accumulate in the skins, and their presence strongly depends on genotype, ripening stage, and agronomic practices (Benbouguerra et al., 2021). Furthermore, in agreement to our results, stilbene biosynthesis in grapevine berries is reported to be regulated by *WRKY* and *MYB* TFs (Mu et al., 2023). Then, terpene cyclases are a specific class of terpene synthases that catalyze cyclization reactions (Christianson, 2017). Terpenes play a crucial role in grape flavor and aroma, with their biosynthesis increasing from the preveraison stage to veraison and consequently decreased toward full maturity (Wang et al., 2021). This behavior explained the observed downregulation of terpene synthases at the end of ripening process (CT:T0) in our samples. Pectinesterases and genes related to xyloglucan metabolism are responsible for modifying the fruit cell wall structure, contributing to softening during ripening. In grapevine, these enzymes are active from the preveraison stage through to full ripening and also play an important role in the plant response to both biotic and abiotic stresses (Malacarne et al., 2024; Matus et al., 2014). The genes related to sucrose metabolism play essential roles in regulating sugar metabolism and transport, contributing to berry ripening, and stress responses (Walker et al., 2021).

The core set of genes listed above is involved in grapevine berry ripening, whose expression remains consistent regardless the dehydration process, whether it occurs on the vine, after rachis detachment (PD), or in a drying facility, such as *fruttaio* (FD). This suggests that, despite the stress undergone to the berries, they play a crucial role in the maturation process, ensuring the preservation of key metabolic and structural changes essential for grape quality.

4.4. Water loss as the primary driver of transcriptomic changes in withering berries

The PCA provided an overview of the global transcriptomic variations, explaining 84 % of the total variance, that included the key transcriptional differences across samples. The clustering observed along with the first PC indicated a close transcriptional relationship between PD and FD samples collected at the end of each condition (T2 and T3 for PD and FD, respectively), suggesting that both dehydration treatments, although with different dynamics, timing and magnitude of gene expression, triggered similar molecular responses. Although PD:T2 and FD:T3 samples were collected at different times after harvesting, they showed comparable berry weight, suggesting that the overall progression of gene expression changes is more influenced by the degree of water loss rather than the withering method applied. To our knowledge, this is the first study that compared the impact of on-vine and in *fruttaio* withering on the berry transcriptome. Recently, a relevant study examined two drying methods in *fruttaio* differing in dehydration rate (slow *vs.* fast), and different rates of water loss on Corvina grapes by the comparison between the two dehydration methods were reported (Zenoni et al., 2020). These findings revealed a closer relationship between berry samples collected at the same sampling time after harvesting than samples showing the same degree of weight loss, indicating that some genes respond similarly to duration, but they were not affected by the degree of water loss. The differences compared to our data may lie in the dehydration methods, emphasizing the significance of both kinetics and environmental conditions when the molecular adaptations to withering were monitored by whole gene expression.

4.5. Enhanced transcriptional response in berries during in fruttaio withering

Overall, although a common transcriptional profile was observed between FD and PD, our findings revealed a differential responses triggered by the dehydration methods investigated. Indeed, the presence of distinct transcriptional responses, involving nearly 1000 genes upregulated in both PD and FD conditions and 242 genes exclusively upregulated in the control (CT), highlighting the existence of treatment-specific regulatory pathways. These evidences suggested that PD and FD conditions triggered partially overlapping responses, influenced by the dehydration treatment as reported by Zenoni et al. (2020). By contrast, the CT condition elicited a distinct gene expression profile, as expected, reflecting the physiological processes related to grape ripening, as previously reported in Fasoli et al. (2012). However, some differences in the transcriptomic profiles were also observed among the withered samples. Interestingly, the expression levels of shared expressed genes were more pronounced in FD compared to PD samples, suggesting that FD induced a more significant transcriptional response to treatment. The differences concerned mainly genes included in the lightyellow module, such as DNA replication licensing factor MCM3, belonging to DNA repair, cellular response to DNA damage stimulus, and cellular response to stress categories, or modulating the gene expression, like MADS-BOX protein, grouped in the gene expression and regulating the gene expression GO terms, or protein-serine/threonine phosphatases, included in regulation of primary metabolic and regulation of cellular metabolic processes, all involved in abiotic stress tolerance. Indeed, DNA replication machinery was previously shown to have a key role in promoting stress tolerance in crop plants (Dang et al., 2011) and, in agreement, MADS-box transcription factor worked as a positive stress-responsive factor in several abiotic (osmotic, salt, and cold) stress signaling pathways in pepper (Chen et al., 2019), as well as different phosphatase gene families (including protein-serine/threonine phosphatase) played crucial roles in cellular stress signal transduction (Sahoo et al., 2020). These differences could be due to the different dehydration procedures, differing for the withering kinetics and environmental conditions. Regarding withering kinetics, the controlled drying room (*fruttaio*) method exhibited a faster dehydration rate compared to on-vine withering. This speeded water loss can significantly influence the higher expression level of the berries transcriptomic response. However, our findings contrasted with other studies, where an accelerated dehydration appeared to suppress the berries transcriptomic changes, but comparing a forced and natural in *fruttaio* dehydration process (Zenoni et al., 2020). Both studies investigated different withering kinetics, but in our experiment, one withering methods occurs in *fruttaio*, while the other takes place on the vine. This may explain the discrepancy, as the vine could act as a physiological buffer, that modulate the stress response permitting a more gradual adaptation to dehydration. In on-vine withering, residual connections to the vascular system, due to incomplete damage of vascular bundles during peduncle twist, might enable a partial exchange of water and metabolites, reducing the suddenness of the dehydration-induced stress and leading to a more sustained transcriptomic response. By contrast, berries in *fruttaio* are subjected to a more extreme shift in their physiological state, underlined by the increase in gene expression of gene linked to stress tolerance.

Finally, the on-vine and *fruttaio* withering methods also differed in terms of environmental conditions, mainly temperature and relative humidity. In the *fruttaio*, these factors were carefully controlled and maintained constant throughout the withering process (15.6 °C and 65.5 % relative humidity). On-vine withering exposed the berries to fluctuating environmental conditions, with temperature and humidity varying both diurnally and throughout the whole withering stage. This may influence the berry transcriptome, as grapes in the *fruttaio* are deprived of the natural temperature shifts they would typically experience. The evidences were in agreement with the expression level of genes involved in photosynthesis process and oxidative phosphorylation (e.g. NADH dehydrogenase, ferredoxin-NADP reductase, photosystem I reaction center), belonged to blue module, down-regulated in FD condition comparing PD one. Such fluctuations could play a regulatory role in metabolic and stress-response pathways, meaning that berries withered under stable conditions might activate different transcriptional programs compared to those exposed to diurnal temperature variability

on the vine. Indeed, the berry transcriptome was reported highly sensitive to temperature and circadian rhythms fluctuations, which should play a crucial role in regulating key metabolic and physiological processes (Lecourieux et al., 2017; Pastore et al., 2017; Rienth et al., 2014).

4.6. Molecular responses to dehydration: insights into stilbene, pectin, and terpene pathways

The dehydration process significantly influenced the expression of stilbene synthase genes, which play a critical role in grapevine metabolism, mainly in the synthesis of stilbenes, a class of polyphenolic compounds involved in stress responses (Parage et al., 2012). In our experimental conditions, out of the 41 identified stilbene synthase genes, 16 resulted solely expressed in the dehydrated samples, highlighting their specific induction during dehydration process. In agreement, Zenoni et al. (2020) observed that one of the major molecular events during grapevine dehydration is the massive upregulation of genes encoding stilbene synthases. In grapevine, stilbenes are synthesized as a response to water stress (Corso et al., 2015). During berry dehydration, a pronounced water deficit is induced, leading to the activation of the stilbene biosynthetic pathways. This observation suggested that dehydration triggered the activation of specific stilbene biosynthetic pathways, which may enhance the defense mechanisms against berries water deficit. The selective higher expression of these 16 genes underscored the role of stilbene accumulation as a protective response during the dehydration.

Another class of DEGs related to the responses to dehydration are the pectinesterases. Nine out of 29 are strongly upregulated in PD and FD samples. A significant upregulation of pectinesterase-encoding genes is one of the key molecular events during grapevine dehydration was already reported (Zenoni et al., 2020). However, this molecular event appeared cultivar-specific, indeed, Corvina showed a notable impact on skin pectin metabolism during dehydration, while this phenomenon was not observed in the cultivars Sangiovese and Oseleta (Zoccatelli et al., 2013). Pectines, responsible of grape berry textural features, are also critical for determining the polyphenol extractability (Malacarne et al., 2024). In our experimental conditions, the polyphenol content increased in the dehydrated samples, but DEGs related to the polyphenol biosynthetic pathway were not detected in dehydrated Corvina grapes. Thus, the higher anthocyanin and total polyphenol content may be attributed to an enhanced polyphenol extractability, resulting from increased pectin degradation driven by pectinesterase activity, together with berry weight reduction due to water loss that determined a metabolites concentration. Indeed, the use of pectolytic-based maceration enzyme favored the phenolic extraction of must (Osete-Alcaraz et al., 2022). The higher polyphenol levels observed in the FD samples compared to the PD samples could be explained by the greater upregulation of pectinesterase genes in the FD-treated berries.

An additional class of genes mirroring the expression of stilbene synthases and pectinesterase was two classes of TFs, *WRKY* and *MYB*. *WRKY* and *MYB* genes are known as genes that monitor the expression of stilbene synthases and pectinesterase (Mu et al., 2023; Zhang et al., 2022).

By contrast to the findings of other studies (Piombino et al., 2025; Shmuleviz et al., 2023; Zenoni et al., 2016, 2020), our experimental conditions did not reveal a positive regulation of genes involved in terpene biosynthesis. Among the DEGs, we identified eight terpene cyclases, which were predominantly downregulated across all the experimental conditions. Although terpene content was not directly measured in this study, Slaghenaufi et al. (2020) analyzed the volatile organic compounds (VOCs) in wines produced from the same experimental setup. Their results indicated that the levels of eight identified terpenes did not differ significantly among Corvina wines produced from non-dried, dried on the vine, and dried grapes in *fruttaio*. These results can be explained by the relatively high temperatures (from 16 °C to 7 °C for FD grapes and room temperature for PD grapes) in which grapes were exposed in our experimental conditions. According to Shmuleviz et al. (2023), high temperatures negatively affected the expression of terpene synthase genes, whereas lower temperatures (approximately 8 °C) triggered the expression of genes related to grape quality.

Among the dehydration related- and BURP domain-containing-genes, two *RESPONSIVE TO DEHYDRATION 22* (*RD22*) genes (VIT_04s0008g03950 and VIT_11s0016g03950), known for playing an important role in the plant response to abiotic stress, seemed highly involved in the response of grapes to the dehydration process. They are also reported as dehydration-responsive genes, involved in the ABA-mediated stress responses (Matus et al., 2014). Interestingly, as non-climacteric fruits, after detachment grapes started the senescence, a process influenced by ABA, well explaining the *RD22* involvement in the dehydration process (Tonutti and Bonghi, 2013).

4.7. A potential role of on-vine withering in modern viticulture

Based on our data, the two withering methods resulted in a comparable composition of both berries and wine. These findings provided novel valuable insights into the potential applications of these techniques in commercial winemaking. In particular, they highlight two key aspects for consideration: the decoupling of phenolic and technological maturation and the sustainability achievable by selecting one method over the other.

In viticulture, the concept of decoupling between technological and phenolic maturity has gained increasing attention, mainly in the context of climate change and evolving winemaking techniques. Technological maturity refers to the accumulation of sugars and the reduction of acidity, while phenolic maturity involves the development of tannins and anthocyanins, which are crucial for the color, structure, and mouthfeel of the wine (Palliotti et al., 2014). Under conventional ripening conditions, these two processes often progress concurrently, but external factors such as high temperatures and water stress can accelerate sugar accumulation while slowing down phenolic development, leading to an imbalance in the grape composition. Indeed, high temperatures caused a desynchronization between sugar accumulation and anthocyanin development in Tempranillo (Arrizabalaga et al., 2018) and Syrah (Sadras and Moran, 2013), resulting in wines with higher alcohol content, that no longer align with consumer preferences, due to negative social and health-related outcomes (Bucher et al., 2020). The on-vine withering method applied in our experiment, by means of peduncle twist can represent an effective strategy to mitigate this decoupling by halting sugar accumulation while allowing phenolic compounds to continue evolving. When grapes remain on the vine after the peduncle twist, water loss leads to a concentration of phenolic compounds without further accumulation of sugars. This process could help a production of wines with greater softness and balance. These wines tend to enhance the complexity and smoother tannins, making them particularly appealing.

The on-vine withering method could offer a more energy-efficient alternative to withering methods in controlled drying rooms (*fruttaio*), increasing the winemaking sustainability. By leveraging environmental climatic conditions, the on-vine methods are considered to reduce the carbon footprint of the winemaking process and aligns with sustainable viticultural practices (Pinto da Silva & Esteves da Silva, 2022). In addition, they minimize the infrastructure required for post-harvest drying, making it an attractive option for wineries aiming to optimize resource use.

5. Conclusions

Our study highlighted the significant impact of different withering techniques on the composition, transcriptomic profile, and resulting wine quality of Corvina grapes, comparing on-vine and in *fruttaio* withering methods. Our findings revealed that, despite differences in withering methods and in some molecular pathways, concerning mainly

genes involved in stress tolerance and signaling, the molecular responses governing grape dehydration and ripening exhibited significant overlapping transcriptomic signatures, suggesting a shared regulatory framework. This shared transcriptional signature is mirrored by the comparable features of both grapes and the resulting wines at the end of the withering techniques applied.

Overall, on-vine withering showed both qualitative and ecological advantages, ensuring that winemakers can achieve the desired phenolic profile limiting energy consume, making it a compelling technique for both premium winemaking and sustainable viticulture. Although on-vine withering offers several advantages, it also presents potential challenges and limitations that must be carefully considered. Despite its qualitative and ecological benefits, environmental variability can significantly affect the consistency and predictability of the dehydration process. Indeed, unexpected rainfall and high humidity may promote uncontrolled fungal infections, leading to lower grape quality or yield losses. Furthermore, yield loss and longer vineyard management could pose economic constraints for producers.

Despite these challenges, our findings provide valuable insights into the phenotypic and molecular adaptations induced by different withering techniques. This deeper understanding of their impact on grape and wine offers guidance for optimizing postharvest practices in viticulture, balancing both quality enhancement and sustainability.

CRediT authorship contribution statement

Davide Bianchi: Writing – review & editing, Writing – original draft, Methodology, Formal analysis. **Guglielmo Puccio:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis. **Valentina Ricciardi:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis. **Carola Pozzoli:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis. **Giovanni Tinervia:** Methodology, Formal analysis. **Maria Teresa Sardina:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis. **Francesco Sunseri:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Conceptualization. **Lucio Brancadoro:** Writing – review & editing, Writing – original draft, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Francesco Mercati:** Writing – review & editing, Writing – original draft, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Gabriella De Lorenzis:** Writing – review & editing, Writing – original draft, Funding acquisition, Formal analysis, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2025.110523.

Data availability

All sequenced data produced were deposited in the Sequence Read Archive (SRA) of NCBI (National Center for Biotechnology Information) with the identifier: PRJNA1237280

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