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Physiological responses of grapevine (*Vitis vinifera* L.) developing buds to drought stress: A transcriptomic analysis

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ABSTRACT

Drought poses a significant challenge to grapevine (*Vitis vinifera* L.) development, impacting both physiological processes and yield. This study explores the transcriptomic responses of developing buds of cv. Merlot grapevine to drought, focusing on key genetic and physiological adaptations. Grapevines were subjected to a controlled water deficit during critical phenological stages, especially for floral induction, and the transcriptomic analysis revealed complex adaptive mechanisms at the molecular level. Water stress notably affected the grapevine's growth and caused significant leaf necrosis. Bud fertility measurements in the following year showed a differential impact of drought according to node position along the shoot, with basal (nodes 1–3) and distal buds (nodes 8–10) more affected than the central ones. This caused a decrease in the number and weight of grape inflorescences, particularly at basal nodes. Transcriptomic data from nodes 2, 5, and 10 showed significant changes in gene expression, dealing with stress responses, hormonal signalling, and metabolic processes. Notably, upregulation of genes related to calcium signalling, ROS (Reactive Oxygen Species) detoxification, and cell wall modification was observed, which are crucial for maintaining cellular integrity under stress conditions. Differential expression of genes regulating flowering further highlighted the impact of drought on reproductive development. Post-stress recovery analyses showed a partial reversal of stress-induced transcriptomic changes, with significant upregulation of genes involved in photosynthesis and lipid metabolism, suggesting a complex recovery mechanism. These findings may contribute to a deeper understanding of the physiological and molecular basis of drought tolerance in grapevine, providing insights into strategies for improving grapevine resilience in the face of increasing water scarcity due to climate change.

KEYWORDS: floral transition, bud development, gene expression, climate changes, water stress, hormone signal transduction

INTRODUCTION

The ancient multicellular organisms that first faced the formidable challenge of terrestrial life encountered a variety of problems, with the threat of dehydration being paramount among them. Over time, evolution has enabled the development of diverse strategies to cope with this challenge, but among all, plants, as sessile organisms, have perhaps developed some of the most fascinating mechanisms (Akıncı & Lösel, 2012). Grapevine (*Vitis vinifera* L.), like all other plants, is not an exception. When subjected to various levels of water stress, *Vitis vinifera* cv. Merlot demonstrates significant slowdowns in growth, reductions in bunch weight and berry size, and decreases in titratable acidity, all of which contribute to issues concerning the quality of the final product (Shellie, 2006). In recent years, within the general context of climate change, there has been an alarming rise in temperatures (Christensen *et al.*, 2007) and an increase in extreme weather events (Pachauri & Meyer, 2014), such as prolonged droughts, hailstorms, and late frosts. Therefore, there is a pressing need for thorough research regarding plant stress physiology and the strategies employed by plants to manage these challenges effectively.

Flowering in grapevine is not merely a seasonal event but a culmination of a two-year cycle where the primordial reproductive structures are initiated and developed inside the dormant bud, going through five main developmental steps. During the first year, the meristems inside the bud are induced to transit to the reproductive phase thanks to a multitude of signals coming from other organs (floral induction), such as the leaves. Consequently, meristems transit to the reproductive phase (floral transition) and, later on, the anlagen (also known as uncommitted primordia) and the primordial inflorescence are formed (floral initiation) inside the bud (Carmona *et al.*, 2007; Palumbo *et al.*, 2019; Vasconcelos *et al.*, 2009). These reproductive structures remain dormant during winter and resume growth at the beginning of the following growing season, when they undergo further branching and complete floral differentiation, thus resulting in mature flowers through the specification of floral structures and the organisation of the whorl model (Carmona *et al.*, 2007; Palumbo *et al.*, 2019). This complex and critical process significantly influences yield and quality. It is regulated by a complex interplay of environmental, genetic, and hormonal signals. Concerning the environmental factors, flowering is mainly stimulated by high temperatures and high light radiation, with floral meristems developing faster under long-day (LD) photoperiod (Sreekantan *et al.*, 2010).

At the molecular level, the flowering process is controlled at different developmental stages by a small set of so called "floral integrators", whose role consists in merging signals from various environmental cues (light, temperature, photoperiod) and internal factors (hormones) into a single message, telling the meristem when to switch from vegetative to reproductive phase, thereby coordinating flowering time. These integrators activate genes that determine floral meristem identity, initiating the reproductive phase of the plant. In grapevine, the gene *VvMADS8/SOC1* plays a pivotal role in floral evocation and development, especially in the early stages of inflorescence development (Sreekantan & Thomas, 2006). Moreover, among the most important players in grape flowering, *VvFT* and *VvTFL1* act according to a sort of "synergistic antagonism". In the most fertile buds, these genes are expressed according to specific and mutually exclusive time/dosage patterns, with *VvTFL1* early expression aimed at stimulating branching in the uncommitted primordia and *VvFT* late expression aimed at closing the vegetative development to definitely shift to the reproductive phase. When *VvFT* expression increased too early, *VvTFL1* did not have enough time to stimulate branching, and fertility was lower (Crane *et al.*, 2012). Other important genes involved include *FLC*, *VFL*, *CO,* and the *CONSTANS-Like (COL)* family, which regulate flowering through photoperiod sensitivity (Carmona *et al.*, 2002; Wang *et al.*, 2016), and *VvELF4*, a flowering inhibitor (Sreekantan *et al.*, 2010). Finally, among the hormones, gibberellins (GAs) and cytokinins (CKs) are the key regulators, concurring together with floral integrators to determine the formation of inflorescences or tendrils (Carmona *et al.*, 2007).

Among abiotic factors, water stress represents one of the most significant environmental challenges affecting grapevine physiology and productivity. *Vitis vinifera*, although capable of withstanding moderate water stress, can suffer severe consequences when the stress exceeds a certain threshold (Canton *et al.*, 2025). The cavitation threshold for grapevine is approximately –1.1 MPa, beyond which irreversible damage occurs, leading to substantial declines in production and fertility (Salleo *et al.*, 1996). Intense water stress can reduce yield by over 50 %, as observed in Merlot vines subjected to extreme conditions (Korkutal *et al.*, 2011). Water stress affects grapevine at both physiological and biochemical levels, reduces bunch weight and berry size, and decreases titratable acidity, thereby impacting the quality of the final product (Shellie, 2006). Severe water stress also leads to significant changes in leaf composition, including increased levels of secondary metabolites like resveratrol, quercetin, kaempferol, and cyanidin (Griesser *et al.*, 2015), which are part of the plant's defence mechanisms and contribute to stress tolerance.

The timing of water deficit during the vine's two-year reproductive cycle can have profound and lasting effects on vine performance and yield. During the first growing season, water stress can disrupt the branching of the floral meristem, resulting in notable yield variations in the following year (Vasconcelos *et al.*, 2009). This developmental phase is particularly sensitive, with anthesis and post-anthesis identified as the most critical periods (Hardie & Considine, 1976). For the subsequent season's crop, the key developmental stage is the branching phase of the anlagen in cultivars such as Merlot, which typically takes place a few months after bud break during the first season. Water deficits during this period can substantially

reduce bud fertility, ultimately leading to lower yields in the following year (Guilpart *et al.*, 2014). Several studies were carried out using transcriptomics on different grapevine organs to characterise their response to drought (Botton *et al.*, 2022; Cochetel *et al.*, 2020; Ju *et al.*, 2021), yet none have focused on buds—the key determinants of productivity in fruit trees, including grapevines. Although previous transcriptomic studies investigated grapevine bud development (Díaz-Riquelme *et al.*, 2012), they did not account for the positional variation of buds along the shoot (*i.e.*, node position), an important factor that can significantly influence bud fertility. In the present study, a transcriptomic survey was performed through the RNA-Seq technique on buds sampled from different nodes under severe water stress, imposed during floral induction/transition, and after a short period of recovery. The differential gene expression with respect to an unstressed control was analysed to characterise the response of the buds according to their position. Specific analyses were carried out on hormone signal transduction, and specific bioinformatic tools were adopted to find gene modules that characterise the specific response of the buds. Target analyses were also performed on known flowering-related genes used as markers of the floral induction/transition process. The physiological behaviour of the buds at different positions was finally summarised into a working model showing the most important processes that characterise the node-dependent buds' response to drought.

MATERIALS AND METHODS

1. Experimental design and sample collection

The trial was conducted at the experimental farm of the University of Padova (45° 20' 59.0" N 11° 56' 59.3" E), located in Legnaro (Padova, Italy), on 200 *Vitis vinifera* cv. Merlot four-year-old grapevines grafted onto Kober 5BB and grown in pots under semi-controlled conditions in a plastic-covered tunnel. cv. Merlot was chosen due to its economic relevance and its broad international diffusion. The experiment began in 2022 by measuring fertility along the first 10 nodes of one cane previously pruned to 10 buds on purpose. Then, only two homogeneous shoots were left after pruning. Lateral shoots were pruned every 10 days to keep the main shoot dominant throughout the whole season. In 2023, fertility was assessed again, then pruning was performed as described above, and water stress was imposed to evaluate the transcriptional response of the developing buds to drought. To this aim, buds were sampled from different nodes of one of the two shoots in both stressed and unstressed vines. One shoot was kept for fertility measurements to be carried out in 2024.

In detail, vines were grown into 10 L pots filled with a mixture of sand, pumice, and peat (2:2:6 by volume) and followed according to the Integrated Pest Management practices allowed in the EU. Before starting the trial, the vines showing anomalous growing behaviours were discarded. Water stress was imposed at BBCH 75 ("berries pea-sized, bunches hang"; Lorenz *et al.*, 1995) on half of the plants, leaving the other half as controls according to a completely randomised experimental design, similar to previous experiments (Botton *et al.*, 2022). Water supply was completely interrupted for five consecutive days, then the stressed plants were rehydrated with 1 L of water each to avoid their loss, and the stress continued for an additional three days until BBCH 77 ("berries beginning to touch"), when irrigation was restored at the level of the control plants. Phenological phases were assessed in the unstressed control. Two samplings were carried out, one at the end of the stress period (BBCH 77) before rewatering, when stress was supposed to be at maximum (severe) at 8 Days After Stress Imposition (DASI), and a second one at ten days after the end of the stress, which corresponded to veraison (BBCH 84), when the plants appeared to have recovered at 18 DASI. At each sampling, buds of the three biological replicates were excised from the 1st (crown or *bourillon*), 2nd, 3rd, 4th, 5th, and 10th node of five vines per replicate from both stressed and unstressed plants, immediately put in liquid nitrogen, and stored at –80 °C for the following analyses.

2. Fertility measurements

Fertility was measured on at least 50 vines per treatment. All plants were kept under the tunnel during winter and pruned to 10 buds to study the fertility of the first 10 nodes. At BBCH 57 phenological stage ("inflorescences fully developed; flowers separating"; Lorenz *et al.*, 1995), all inflorescences were removed separately for each shoot at each node, counted, and weighed individually. A total of 24 shoots were randomly selected for each node, and the level of inflorescence branching was evaluated.

3. RNA extraction and RNA-Seq analysis

Total RNA was extracted with the RNeasy Plant Mini Kit (Qiagen) starting from 70 milligrams of tissue, with the following modifications made to the protocol provided by the manufacturer: 5 µL of $Ca(OH)_2$ and 0.05 g of PVP (polyvinylpyrrolidone) were added to 750 µL of the extraction buffer RLT. The former was added for its ability to precipitate carbohydrates according to Dal Cin *et al.* (2005), while PVP was added to precipitate polyphenolic compounds. The amount of RNA and the presence of contaminants in the extracted samples were evaluated using NanoDrop 2000 (EuroClone®), and RNA integrity was checked by running the samples in a 1 % agarose gel. RNA extracted from buds of the 2nd, 5th, and 10th nodes was selected to undergo RNA-Seq analyses using an Illumina NextSeq 500 platform (Illumina, San Diego, CA, United States).

4. Bioinformatic analyses

Adaptor-trimmed RNA-Seq reads were mapped onto the PN40024.v4 version of the *Vitis vinifera* genome (Velt *et al.*, 2023). The software HISAT2 v2.2.1 (Zhang *et al.*, 2021) and SAMtools v1.21 (Danecek *et al.*, 2021) were used to carry out genome indexing, read alignment, and file format conversions, while HTSeq v1.0

(Anders *et al.*, 2015) was used to obtain the counts according to gene models available in public GFF3 (Generic Feature Format Version 3) files. A count matrix with merged read counts of each sample was used for the following analyses. Statistical analyses of differential gene expression were performed with the Bioconductor package DESeq2 v1.38.3 (Love *et al.*, 2014) with an FDR (False Discovery Rate) cutoff equal to either 0.01 or 0.05, according to the contrasts, and a minimum fold change of 2. To remove genes with a very low number of counts and high dispersions, the option "Independent filtering of lower counts" was selected. DESeq2 analysis and further functional analyses, such as Pathview (Luo *et al.*, 2017) were carried out through the iDEP bioinformatic platform v2.01 (Ge *et al.*, 2018) for a more integrated approach.

Weighted correlation network analysis (WGCNA; Langfelder & Horvath, 2008) was carried out with the R package BioNERO v1.6.1 (Almeida-Silva & Venancio, 2022). Genes with low levels of expression (min_exp = 10) and low variation were removed from this analysis, keeping only the 4,000 most variable. Outlying samples were removed with the "Pearson" method, and confounding artefacts were corrected with the "PC_correction" function (Parsana *et al.*, 2019).

5. Statistical analysis

Basic statistical analysis packages of R v4.4.1 were used within RStudio v2024.09.0+375 for Principal Component Analysis (PCA), Student's *t*-tests, ANOVA, and various *post hoc* tests as previously described by Vegro *et al.* (2016).

RESULTS AND DISCUSSION

1. Drought application and analysis of fertility

Water stress was applied in 2023 according to the experience gained in previous experiments carried out under the same tunnel infrastructures (Meggio *et al.*, 2020; Botton *et al.*, 2022; Canton *et al.*, 2025). For this reason, the actual stress intensity was monitored only through daily observations of the general status of the vines and a comparison with the unstressed plants. The plants subjected to stress showed marked suffering, with visible wilting tendrils and leaves already at 1 DASI, when the stress was moderate (Figure 1A). A relevant abscission of the basal leaves, at least up to the 5th node, occurred at 8 DASI, when the stress was severe (Figures 1B and 1C). Estimated reference values of leaf water potential (Ψ_{leaf}) at midday, based upon previous trials carried out under similar experimental conditions, can range between –0.5 and –0.6 MPa for well-watered vines, while for the water-stressed plants Ψ_{leaf} may span between –0.8 and –1.0 MPa at 1 DASI (moderate stress), and –1.4 and –1.6 MPa at 8 DASI (severe stress). Analogous values with similar symptoms are also in the literature for vines of different varieties, grown either in pots or open field, undergoing similar water stress conditions (Thorne *et al.*, 2006; Williams *et al.*, 2012; Herrera *et al.*, 2022).

Fertility, herein referred to as both the total weight (TWI) and number (NI) of inflorescences per node, was monitored for three years in a row (Figure 2) under normal conditions, pointing out some variability from year to year, as previously reported by several authors (Chloupek *et al.*, 2004; Guilpart *et al.*, 2014; Keller *et al.*, 2004).

Concerning the node-wise trend, a gradual increase in weight was observed from the first to the tenth node across all three years (Figure 2A), with some nodes showing significant differences between years. The number per node (Figure 2B), roughly reported in literature as "equal to 1, more frequently 2" (Meneghetti *et al.*, 2006), was shown to increase on average from node 1 until reaching values ranging from 1.04 to 1.13 inflorescences per node from node 3 to 5, respectively, and then increasing again up to node 10, reaching values close to 2 per node. These data may have relevant implications, especially for taking pruning decisions in a young vineyard, while in a fully productive context, Merlot vines are usually pruned to three or four nodes, according to the clone and, of course, to productive targets, habits, etc. According to statistics, a generally decreasing fertility was observed throughout the three years from node 3 to 5, in terms of both NI and TWI. Such a behaviour may have been due, at least in part, to the tunnel/pot growing conditions, which generally advanced the vine's phenology by about two weeks. In particular, root growth restriction may have affected the physiology of the epigeal part of the plants, as previously demonstrated by Li *et al.* (2022) in terms of hormonal balance in the different parts of the vine. While the aspects related to hormonal balance will be discussed in the next paragraphs, a slight change in the fertility behaviour was shown to occur after the first year of measurements (2022) as shown also by a principal component analysis of all fertility data (Figure 2C); in the first year, the number of inflorescences per node was generally higher than in the following two years, but this difference was statistically significant only for node 4. In the first season, indeed, root growth was not likely to be restricted by the pot, while in the following two, the volume of soil in the pot had probably already been completely explored by the roots, thus representing a sort of restriction, although with only slight effects on fertility, which is the focus of our experiment. Indeed, fertility measurements carried out within a concurrent open field trial on cv. Merlot (data not shown) fully overlap with those of the present experiment, thus supporting the idea that bud fruitfulness is normally not significantly affected by root growth restriction, at least within the first three years in pots.

When the vines were drought-stressed in 2023, their fertility in the following season changed, as shown in Figures 2D–2F. In terms of TWI, the only significant (P = 0.00014) difference was observed at node 2, with a more than three-fold reduction of weight. In general, the three basal nodes were those more affected by drought in terms of weight, while this parameter was slightly higher at nodes 4 and 5 (although with no statistical significance) or closely similar in the other nodes. Water stress notably

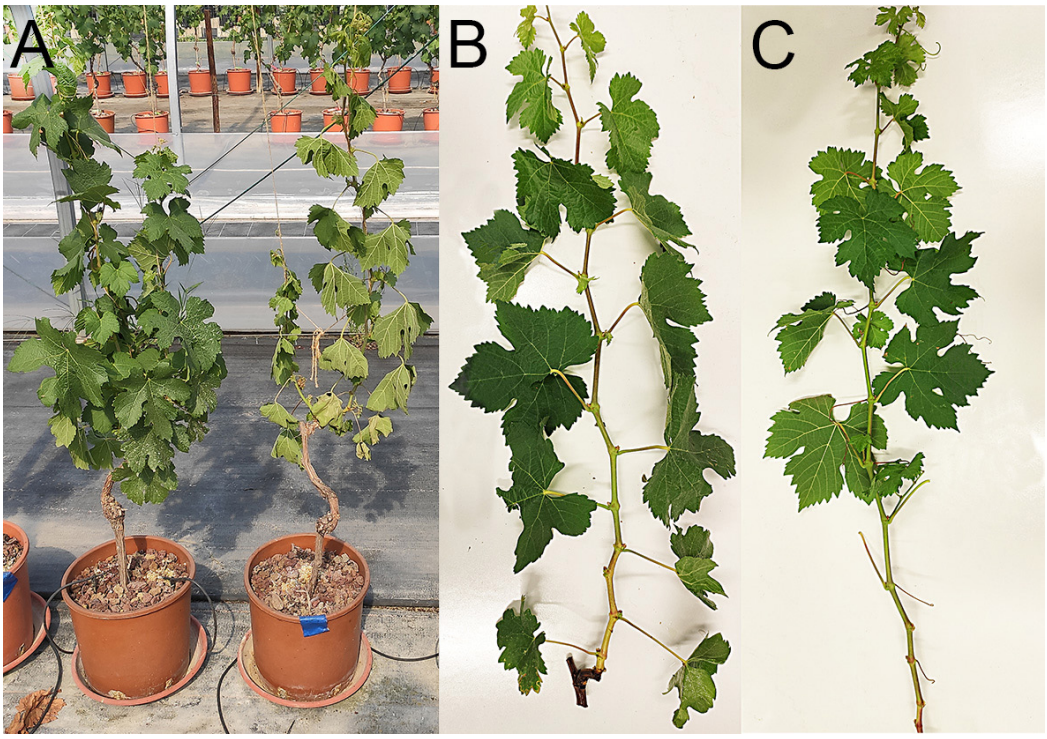


FIGURE 1. Effect of drought on the vines at the beginning and at the end of the experiment.

A) Unstressed (left) and water-stressed (right) vines at 1 Day After Stress Imposition. Wilting leaves are visible in the water-stressed vine. B) Unstressed and C) water-stressed shoots sampled at 8 DASI. Leaves are clearly missing in the first five nodes due to abscission, while bunches were manually removed before taking the pictures.

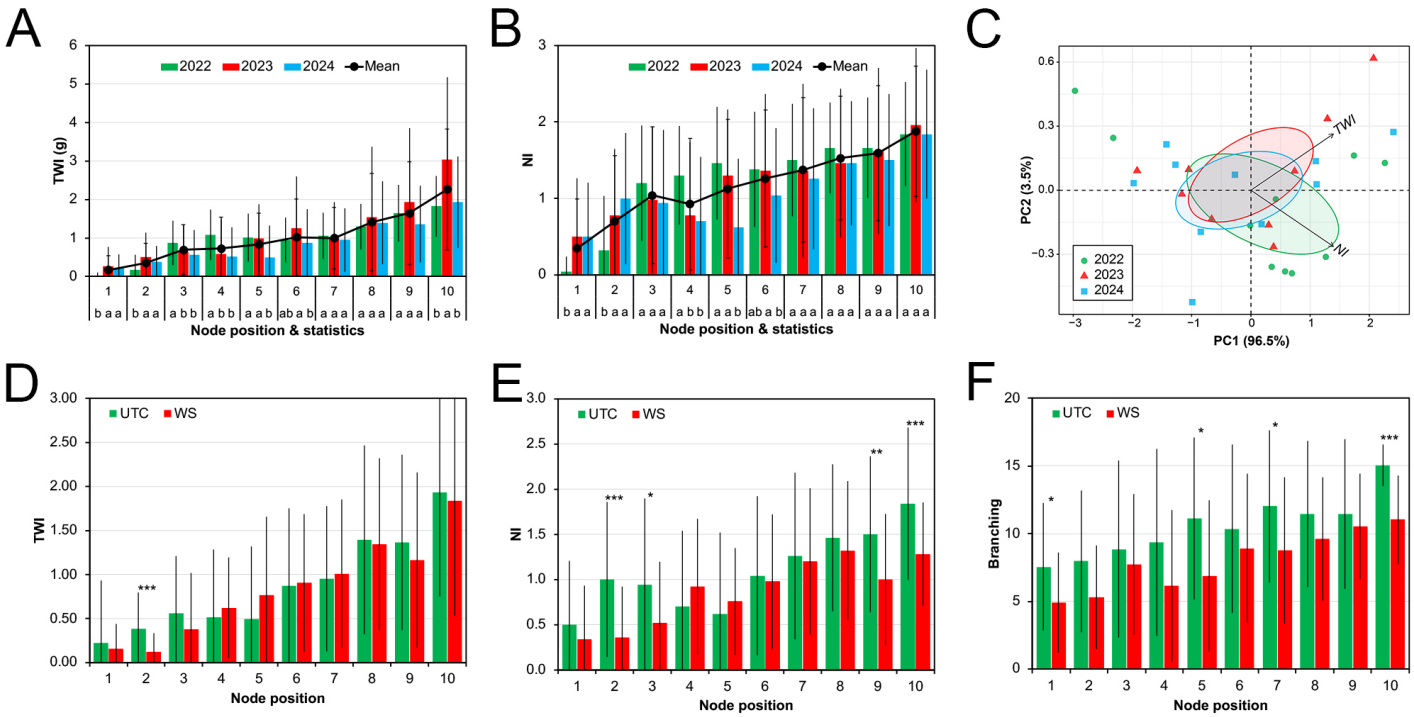


FIGURE 2. Bud fruitfulness of the cv. Merlot in three consecutive years and during the stress trial.

A) Total weight of inflorescences (TWI) and B) number (NI) per node measured in 2022, 2023, and 2024, and as their mean value (black line). Different letters below the nodes show statistically significant differences between the years ($P < 0.05$). Clean and capped bars show the standard deviation ($n = 50$) in single years and mean values, respectively. C) Principal component analysis biplot of the data in A and B without the outlier (node 10 in 2023). Ellipses group samples of the same year according to a 95 % confidence. TWI and NI are shown as the loadings. D) TWI, E) NI, and F) branching degree in unstressed control (UTC, green) and water-stressed (WS, red) vines, as measured in 2024. Bars show standard deviation ($n = 10$). Asterisks on top of the bars are related to the statistical significance at each node (* for $P < 0.05$, ** for $P < 0.01$, and *** for $P < 0.001$).

reduced the number of inflorescences at most node positions, with statistically significant differences observed at nodes 2, 3, 9, and 10. Again, an opposite trend was observed at nodes 4 and 5, although not supported by statistics. Finally, as far as the branching degree is concerned, lower levels were observed in inflorescences coming from all nodes, with statistically significant differences broadly scattered at nodes 1, 5, 7, and 10.

From these data, we can identify three main patterns of response to water stress in terms of fertility, as represented by: i) node 2, showing lower weight and lower number of inflorescences, ii) node 5, showing opposite trends in terms of weight and number, but a decreased branching degree, and iii) node 10, with lower number of inflorescences and lower branching degree, although unaffected in terms of weight. These results suggest that water stress negatively impacts the vine's ability to produce multiple inflorescences of a certain weight, with a more severe effect at basal nodes, most likely due to the damage caused to the corresponding leaves, and, at less extent, the apical ones. The intermediate nodes' fertility was slightly compensating for the loss that occurred in the basal nodes, but not in terms of branching degree, which was shown to be lower.

2. Transcriptomic analysis

Based on the fertility measurements, the samples of the buds coming from positions 2, 5, and 10 were chosen for the following RNA-Seq transcriptomic analyses, as they were representative of three possible physiological backgrounds. On average, the number of RNA-Seq reads obtained was equal to 56.8 million per sample, with a mean overall alignment rate equal to 87.6 % (Table S1**,** supplementary data), thus indicating a good coverage of the transcriptome for all samples. The differences in the number of reads of some samples were because when the yield of the first sequencing turn was low (*i.e.*, < 25 million), the library was re-sequenced, and the RAW files were merged into one. In any case, no bias in the number of RNA-Seq reads was detected with respect to the factors considered in the experiment, *i.e.*, node position, stress, and phenological phase.

The principal component analysis (PCA) of the transcriptomic data is shown in Figure 3, with the couples of the three most informative PCs explaining a total variance of 43.78 % for PC1 and PC2 (Figure 3A), 37.16 % for PC1 and PC3 (Figure 3B; shown inverted in the chart for graphic purposes), and 27.8 % for PC2 and PC3 (Figure 3C), for a total of 54.37 % considering the three PCs together. The relatively low variance represented by the PCA did not prevent us from highlighting the clear correlations existing between PC1 and node position (P = 1.42E–09), with node 2 clearly separated from 5 and 10, the latter clustering more closely.

In addition, PC3 was correlated with the treatment (P = 8.54E–09), showing a clear separation between the unstressed and water-stressed samples, while PC2 correlates with the phenological phase (P = 1.62E–14), with a net separation of the samples of BBCH 77 (severe stress) from those of BBCH 84 (recovery from stress).

The heatmap of the 3,000 most variable genes together with a hierarchical clustering approach showed that both the quality and variability of replicates of the same samples were, in most cases, excellent, although with some exceptions among the water-stressed samples (Figure 3D). Moreover, some groups of genes with expression patterns linked to the experimental factors were already visible in the heatmap. In any case, according to both clustering approaches, the transcriptomes of the different samples consistently represented the experimental context and could thus be used to identify differentially expressed genes (DEGs) and their functional domains.

The number of differentially expressed genes (DEGs) was initially assessed in a selected number of contrasts to point out the differences in gene expression due to node position, stress, and phenological phase/timing after stress imposition, *i.e.*, the three main factors considered in the experiment (Figure 4A). Relevant differences were observed across all comparisons, with a few exceptions, despite the strict FDR cutoff (0.01), and were consistent with PCA results. Concerning the node contrasts, the highest variability was observed between nodes 10 and 2, with 1,225 and 1,562, followed by the contrasts between nodes 5 and 2, with 1,180 and 951 down- and upregulated genes, respectively. The last node contrast, 10 *versus* 5, resulted in the lowest number of DEGs among all the comparisons, with 128 and 79 down- and upregulated genes, respectively. As shown by PCA (Figure 3A), buds at nodes 5 and 10 clustered very closely, while those at node 2 displayed a very distinctive transcriptional background. As far as the water stress effects, 316 and 578 genes were down- or upregulated by drought, respectively. Finally, regarding the phenological phase, the contrast between BBCH 84 and BBCH 77 also showed significant changes, with 128 upregulated and 873 downregulated genes, indicating that the transcriptomes of the buds underwent significant changes during this developmental timeframe.

Assessing the DEGs within individual comparisons of water-stressed *versus* unstressed buds at the same node, when stress was classed as severe stress (BBCH 77) and during recovery (BBCH 84), may provide a more detailed picture of the bud's stress response according to its position along the shoot (Figure 4B). For these analyses, a less strict FDR cutoff was used, equal to 0.05, to achieve a higher number of DEGs and thus improve the statistical robustness of the following functional analyses. Under severe stress, all the nodes showed a relevant transcriptional response, with nodes 2 and 10 showing the highest number of DEGs. For the former, 997 downregulated and 1,291 upregulated genes were found, while at the tenth node, the DEGs were 581 and 887, respectively. The transcriptional response of buds at node 5 to the stress consisted of 411 and 494 down- and upregulated genes. This pattern of DEGs interestingly overlapped with the fertility trends previously described, with nodes 2 and 10 being the most affected, and node 5 showing a sort of "compensatory behaviour".

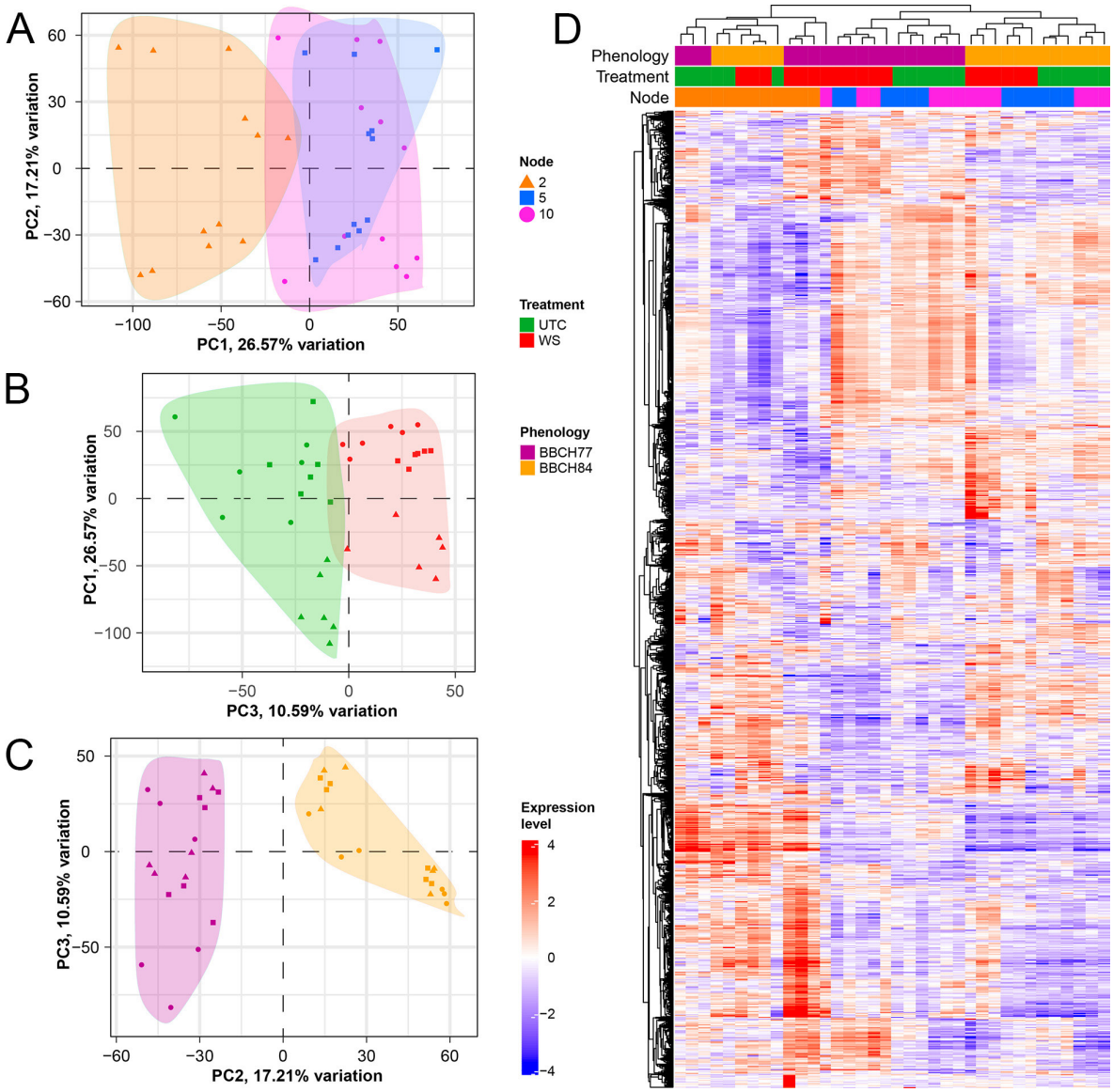


FIGURE 3. Principal component analysis (PCA) and hierarchical clustering of samples.

PCA was applied with A) principal components 1 and 2, B) 3 and 1, C) 2 and 3, respectively. D) Heatmap of the 3,000 most variable genes hierarchically clustered according to average Pearson distance and gene centering. The legends are reported in the middle of the panel, referring to colours and symbols used in both types of clustering methods.

After 10 days of recovery with a normal irrigation regime, the buds at node 10 were still showing a very active transcriptional response, much higher than before, with 768 downregulated and 1,400 upregulated genes, among which 79 (10.3 %) and 204 (14.6 %), respectively, had the same regulatory pattern as BBCH 77, when the stress was severe (Figure S1, supplementary data). This may indicate that those buds were recovering from stress differently with respect to buds at nodes 2 and 5, whose number of DEGs was lower than at BBCH 77. Especially for node 2, the number of DEGs dropped to 338 downregulated and 143 upregulated genes, with 53 (15.7 %) and 25 (17.5 %) DEGs showing the same pattern as in BBCH 77. Moreover, the DEGs at node 5 decreased to lower numbers, consisting of 200 and 236 down- and upregulated transcripts, respectively, with 39 (19.5 %) and 51 (21.6 %) DEGs in common with BBCH 77. According to these statistics, the proportion of DEGs with the same pattern of regulation at both BBCH phases was the highest in buds of node 5, followed by node 2, further supporting the hypothesis that buds at different nodes, and thus at different developmental stages, respond in a different way to water stress. In particular, the buds at node 5 showed a prolonged transcriptional response to water stress, while those at node 10 recovered to a new transcriptional regulation.

3. Enrichment analysis of DEGs

An enrichment analysis was carried out on the DEGs of each contrast (considering only the second list analysed above) to identify the gene functions that were more involved in the stress response. The Gene Ontology Biological Processes (GO-BP) sub-vocabulary was used with the purpose of providing this information, and the results are shown in Figure 5.

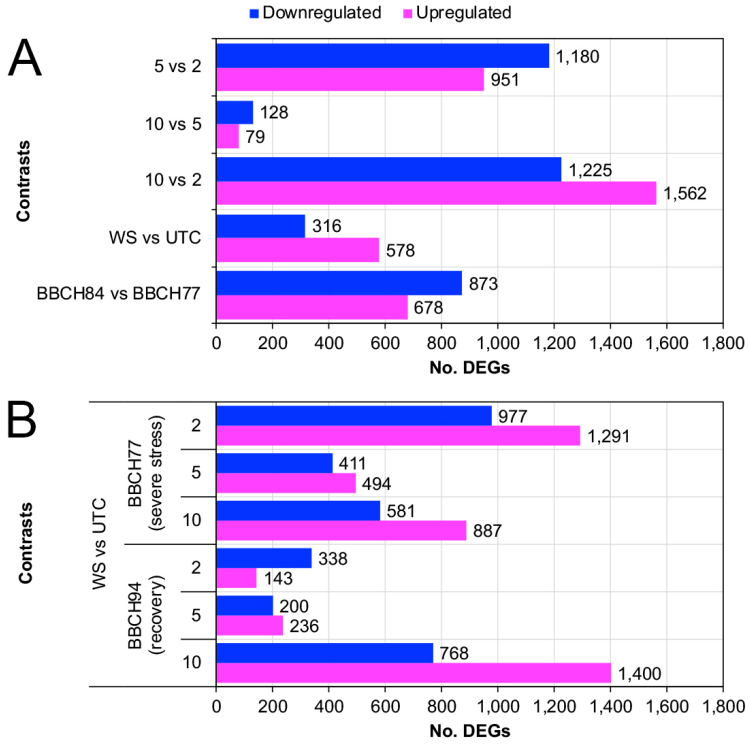


FIGURE 4. Number of differentially expressed genes (DEGs) in the different relevant contrasts.

A) Node 2 *versus* node 5, node 5 *versus* node 10, node 2 *versus* node 10, unstressed (UTC) *versus* water-stressed (WS), BBCH 77 *versus* BBCH 84. In this case, the analysis was carried out with an FDR cutoff equal to 0.01. B) Contrasts were for each node at the same timepoint, between the water-stressed (WS) and the unstressed (UTC) buds, with an FDR cutoff equal to 0.05. The legend at the top of the chart shows the colours used for down- (blue) and up- (magenta) regulated genes.

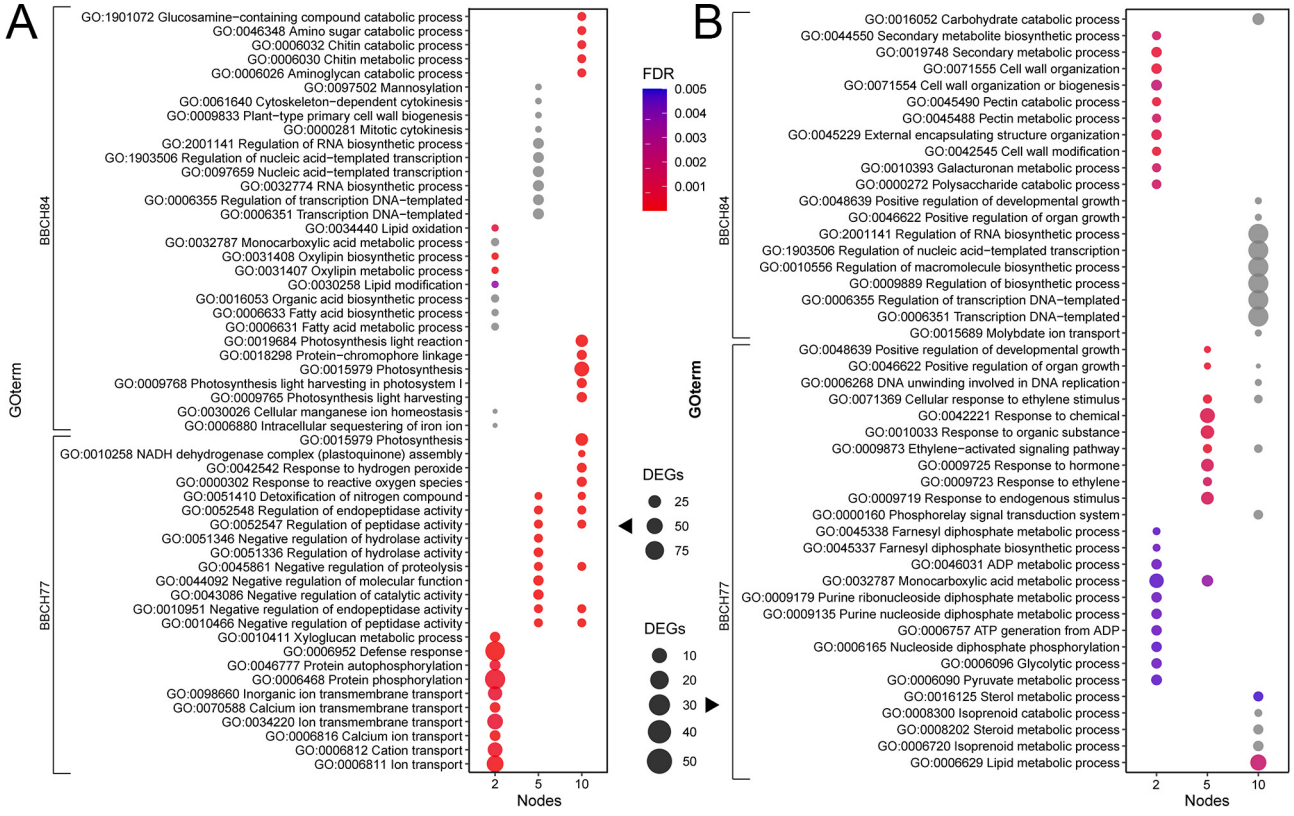


FIGURE 5. Dot plot of the functional enrichment of the DEGs in the contrasts WS *versus* UTC.

The legends are reported in the middle for the FDR colour scale, and the number of DEGs, with an arrowhead indicating which plot the latter legend refers to. Enrichments are shown separately for A) upregulated and B) downregulated genes, timepoint (BBCH 77 or BBCH 84), and node (2, 5, or 10).

The upregulated DEGs identified in buds of node 2 at severe stress (BBCH 77) were enriched with functions related to "ion transport", in particular, calcium and other cations, "protein phosphorylation", "defence response", and "xyloglucan metabolic processes". In buds at node 5, enriched functions dealt with "negative regulation of proteolysis" and endopeptidases/hydrolases activities, but also "detoxification of nitrogen compounds". Buds at node 10 showed an upregulation of genes regarding "photosynthesis", "response to hydrogen peroxide", "response to reactive oxygen species", and, similar to node 5, "negative regulation of proteolysis" and "detoxification of nitrogen compounds" (Figure 5A).

These results confirm the data reported in the literature regarding the role of calcium in mediating defence responses, in general, and water stress, in particular, as reported by Kwak *et al.* (2003), who demonstrated how reactive oxygen species promote the opening of calcium channels (De Nicolo *et al.*, 2023). Xyloglucan metabolism is deeply involved in cell wall formation, a process that is crucial during water stress, as the integrity of the cell wall is essential for plant survival under such conditions (Tenhaken, 2015). In wheat, water stress has been shown to increase the production of this polysaccharide as part of the plant's adaptive response to stress (Leucci *et al.*, 2008). Of particular interest are the processes of "detoxification of nitrogen compounds" and "negative regulation of proteolysis", upregulated at nodes 5 and 10. As a response to protein degradation, which is known to be triggered by water stress, the buds may try to prevent the accumulation of potentially toxic non-functional peptides and to enhance amino acid recycling (van Wijk, 2015; Rowland *et al.*, 2022), concurrently inhibiting proteolytic activity (Moloi & Ngara, 2023). Additionally, it is not surprising to find enrichments in functions related to reactive oxygen species, which are produced in response to stress (Impa *et al.*, 2012). The literature on this topic is extensive, and for further details, the review by Kar (2011) provides an in-depth analysis.

Among the gene functions that were downregulated at severe water stress, a long series of terms dealing with energy metabolism, such as the "pyruvate metabolic process", "glycolytic metabolic process", "monocarboxylic acid metabolic process", "ATP generation from ADP", and "ADP metabolic process", were found enriched at node 2. Worthy to note that node 5 showed the presence of downregulated gene functions dealing with "response to ethylene", "ethylene-activated signalling pathways", and "positive regulation of organ/developmental growth". Finally, buds at node 10 pointed out the downregulation of genes involved in the metabolism of lipids, isoprenoids, and steroids, along with those linked to "DNA unwinding involved in DNA replication", "phosphorelay signal transduction", and, similarly to node 5, "ethylene activated signalling pathway" (Figure 5B).

The downregulation of terms related to energy metabolism is well-documented in the literature (Chen *et al.*, 2019; De Vega *et al.*, 2021; Ni *et al.*, 2009). It is known that during water stress, there is a significant reduction in many metabolic activities to conserve the limited energy available for the most essential processes (Ni *et al.*, 2009). The involvement of terms related to ethylene is not surprising, as the role of ethylene in stress responses, particularly water stress, alongside its known function in senescence, has been recognised since the 1970s (Jordan *et al.*, 1972; Kawase, 1974; McMichael *et al.*, 1973). However, since this hormone usually works in concert with abscisic acid (ABA), another key player in stress responses (Salazar *et al.*, 2015), the extensive downregulation of ethylene-related genes at node 5 was unexpected and may resemble a bud-specific protective mechanism against excessive levels of this hormone, which are known to cause problems in the development of floral structures (Iqbal *et al.*, 2017). Regarding the enriched terms "DNA unwinding involved in DNA replication" and "phosphorelay signal transduction", it is well-established that these processes are upregulated primarily during thermal stress (Sharma *et al.*, 2020). However, their role in water stress remains less understood, and further studies are needed to elucidate their involvement in this context.

As far as the recovery timepoint (BBCH 84) is concerned, the genes upregulated by drought in buds at node 2 were enriched by functions dealing with "intercellular sequestering of iron ion", "cellular manganese ion homeostasis", and a long series of terms related to "lipid modification", including "oxylipin biosynthetic process". At node 5, the upregulated transcripts were enriched, although not very significantly, by terms related to "transcription DNA-templated", "mitotic cytokinesis", and "plant-type primary cell wall biogenesis", while at node 10, the functional enrichment included functions like "photosynthesis", and other related terms, and "chitin catabolic process". Only the most representative terms are mentioned, while an exhaustive list can be found in Figure 5.

Iron (Fe) and manganese (Mn) are essential micronutrients critical for plant tolerance to drought stress. Iron is vital for photosynthesis, respiration, and chlorophyll synthesis, and maintaining its homeostasis is crucial for drought tolerance (Rout & Sahoo, 2015). In sorghum, drought stress alters iron accumulation in seeds, and the overexpression of vacuolar iron transporters and ferritin enhances iron accumulation under such conditions (Araki *et al.*, 2022). Interestingly, iron nanoparticles have been shown to increase drought tolerance in canola plants (Rezayian *et al.*, 2023). Manganese, similarly, plays a key role in drought tolerance by ensuring efficient photosynthesis, which is vital for plant growth under water stress (Yue *et al.*, 2023). Mn is also a cofactor for a superoxide dismutase (MnSOD) enzyme, which detoxifies ROS generated during drought stress, helping to maintain cellular redox homeostasis and protect plants from oxidative damage (Ye *et al.*, 2019). Given that cell membranes are involved in water stress perception, the upregulation of lipid metabolisms, including those involving phosphatidic acid and oxylipins, is fully consistent (Sharma & Diwan, 2023). Additionally, exogenous ABA applications can induce lipid biosynthesis (Gai *et al.*, 2020).

Upregulation of photosynthetic genes during recovery from stress likely reflects the plant's attempt to restore damaged photosynthetic functionality, as water stress is known to significantly reduce photosynthetic activity and cause damage to the photosynthetic apparatus (Chaves *et al.*, 2002; Flexas *et al.*, 2006).

Finally, concerning the downregulated DEGs, those at node 2 were functionally enriched with terms dealing with "secondary metabolic process" and, in general, with "cell wall organization and biogenesis", the latter regarding specifically pectin ("pectin metabolic processes"). No functions were found to be enriched in DEGs of buds at node 5, while for those at node 10, the terms "positive regulation of developmental growth" along with "transcription DNA-templated" were found among the enriched ones.

The downregulation of these terms could indicate, at least in buds at node 2, an actual recovery from the stress situation, as enriched terms related to genes that were upregulated during BBCH 77 (severe stress) pertain specifically to cell wall metabolism and xyloglucans, in particular. Regarding the term "secondary metabolic process", it is well known that the biosynthesis of these compounds plays a crucial role in protection against stress (Jogawat *et al.*, 2021). Indeed, some upregulated entries can be observed at BBCH 77 ("isoprenoid metabolic process"). It is therefore reasonable to expect a downregulation during the recovery phase, where metabolism is undoubtedly shifted towards processes of repair and growth (Perrone *et al.*, 2012). Concerning node 10, there are many enriched downregulated terms related to nucleic acid metabolic processes. It is well-documented in the literature that the imposition of water stress causes a massive transcriptional reprogramming of cells (Neill & Burnett, 1999), a finding corroborated by this study. Thus, it is reasonable to hypothesise that once the threat of stress has subsided, all processes activated during BBCH 77 are, in some way, turned off (Zhang *et al.*, 2018), although node 5 buds represent an exception, as pointed out above. As a general remark, the transcriptional shifts observed during the recovery phase at BBCH 84 underscore a transition from stress response mechanisms to growth and repair processes. The upregulation of genes associated with micronutrient homeostasis and lipid metabolism, alongside the downregulation of stress-related pathways such as secondary metabolism and nucleic acid processes, suggests a reallocation of resources toward restoring normal cellular functions and promoting post-stress development. This reprogramming highlights the dynamic and adaptive nature of plant responses to environmental challenges, aiming to optimise resilience and recovery.

4. Gene co-expression network

To understand the regulatory network triggered by drought stress from a different methodological perspective and assess how the stress response varies according to the position of the buds, WGCNA (weighted gene co-expression network analysis; Langfelder & Horvath, 2008) was carried out on the 3,000 most variable genes in all samples, as a complementary approach to DEGs analysis/enrichment. Based on the prerequisites of the approximate scale-free topology, 17 was chosen as the soft threshold power (Figure 6A). Genes were clustered into 15 modules, the most numerous of which was the "darkgreen" with 902 genes, followed by the other modules up to the least numerous, *i.e.*, the "blue", with 32 genes (Figure 6B).

Among the 15 modules obtained, shown in Figure 6C as a hierarchical tree with colour codes according to the canonical WGCNA representation, most had expression patterns correlated with the drought stress, either positively or negatively, with very significant statistics (Figure 6D), while none of them was significantly correlated with the phenological stage/timepoint of the trial. The correlation with node position was analysed, separating the bud samples not only by their reference node but also according to their condition (UTC *vs* WS). In this way, correlated modules were identified for five out of six samples, with the only exception of water-stressed buds at node 2. Worthy to note that the unstressed buds of node 2 pointed out only two correlated modules, the "sienna3" and the "skyblue", whose expression patterns were negatively correlated with $P < 0.01$ and $P < 0.05$, respectively. The former was enriched with the terms "protein phosphorylation" and "regulation of transcription, DNA-templated", while the latter with the term "exocytosis" (Table 1). This would indicate that genes involved in these functions are expressed on average at the lowest levels in these samples, as shown in Figures 7A and 7B.

Unstressed buds of node 5 correlate with six modules. Negative correlations were found with modules "midnightblue" ($P < 0.05$), "darkorange" ($P < 0.01$), "orangered4" ($P < 0.05$), and "grey" ($P < 0.05$), while positive relationships were observed for the "blue" ($P < 0.01$), and "lightcyan" ($P < 0.05$), modules. Among these, the only module with functionally enriched genes was the "midnightblue", with terms like "chlorophyll biosynthetic process", "photosynthesis", "photosynthesis, light harvesting in photosystem I", "protein-chromophore linkage", and "response to light stimulus" (Table 1), also in this case characterising the samples for their low expression levels (Figure 7C).

The stressed buds at the same position negatively correlated with "lightcyan" and positively correlated with "lightcyan1", "darkorange", "brown", "white", "black", and "grey" (Figure 6). Among these seven, only three ("lightcyan1", "brown", and "black") showed significant enrichment with terms dealing with "translation", "mitotic cell cycle", "proteasome-mediated ubiquitin-dependent/independent protein catabolic process", "intracellular protein transport", "protein complex oligomerization", and "response to heat", to cite the most statistically relevant (Table 1). All these processes were upregulated preferentially in water-stressed buds at node 5 (Figure 7).

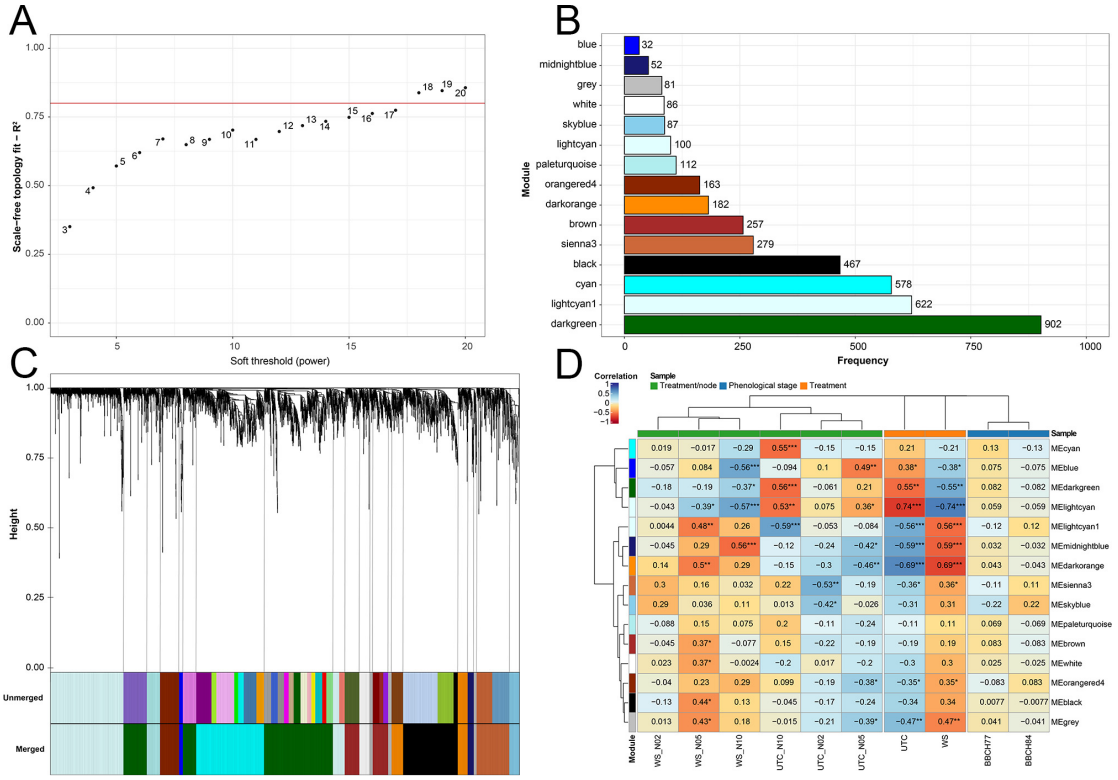


FIGURE 6. Weighted correlation network analysis (WGCNA) of the response of buds to drought stress.

A) Analysis of the scale-free fit index, with the red horizontal line marking the chosen cut-off. Unmerged and merged modules are shown below the tree. B) Colours and number of genes for the WGCNA modules identified. C) Hierarchical cluster tree of co-expression modules based on WGCNA. The different colours correspond to different modules. D) Module-sample correlation matrix. The number in each cell indicates the correlation index (either positive or negative) between the module gene expression and the sample/treatment/condition, according to the colour scale shown in the top left of the matrix. Asterisks indicate the level of statistical significance (* = significant at *alpha* = 0.05; ** = significant at *alpha* = 0.01; *** = significant at *alpha* = 0.001; no asterisk: not significant).

Buds at the 10th node showed the highest and most significant correlations. The unstressed buds correlated with modules "cyan", "darkgreen", "lightcyan", and "lightcyan1", the latter being the only negative relationship. The first two modules were enriched with the functions "gene silencing", "ubiquitin-dependent protein catabolic process", "auxin-activated signalling pathway", "cell wall biogenesis", "cytoplasmic translation", and "mitotic cell cycle", while the "lightcyan1" module was enriched with functions already discussed above. The term regarding auxin is noteworthy, as it is consistent with the proximity of the SAM (shoot apical meristem) that produces high amounts of this hormone, which exerts a greater effect of apical dominance on closer buds, such as those at node 10.

Finally, the stressed buds in the same position are correlated with the "blue" and "lightcyan" (negatively), and "midnightblue" (positively) modules, the latter being enriched with the GO terms "chlorophyll biosynthetic process", "photosynthesis", "protein-chromophore linkage", and "response to light stimulus", implying that these stressed buds showed an upregulation of these processes, most likely to counteract the damages to photosynthetic apparatus caused by the scarcity of water.

Taken together, these results add further information about the specific physiological background of each node and support the enrichment analysis of the DEGs shown above. In summary, buds at node 2 were characterised by a more advanced stage of differentiation, as indicated by the downregulation of genes controlling both the transcriptional processes and protein phosphorylation, the latter being known to be strongly related to cell differentiation (Aguilar-Hernández *et al.*, 2020). Buds at node 5 were progressively losing their ability to carry out photosynthesis (buds are initially constituted by green tissues), as indicated by the downregulation of photosynthesis-related genes. The same buds under water stress were characterised by a more active response, including basic processes such as translation, mitosis, and heat responses, whose genes were upregulated preferentially at this node. Finally, node 10 buds were notably affected by the proximity of the SAM, as pointed out by the upregulation of auxin signalling-related genes. Nevertheless, in contrast to buds at node 5, node 10 is characterised by a low basal expression of genes dealing with ROS and heat stress responses. When subjected to drought, these buds showed an upregulation of photosynthesis-related genes that was consistent with the previous DEGs analysis, most likely to recover their photosynthetic capacity, still active at that stage of development, unlike node 5 buds.

TABLE 1. Gene ontology biological process (GO-BP) terms enriched in the WGCNA modules.

GO ID	GO term	N° genes	N° Bkg genes	*P*-value	FDR	Module
GO:0002181	cytoplasmic translation	3	18	0.001641	0.046089	black
GO:0001732	formation of the cytoplasmic translation initiation complex	3	9	0.000185	0.010372	black
GO:0006886	intracellular protein transport	6	92	0.001454	0.044533	black
GO:0000463	maturation of LSU-rRNA from tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA, LSU-rRNA)	4	16	4.94E–05	0.004163	black
GO:0010499	proteasomal ubiquitin-independent protein catabolic process	6	9	4.34E–10	4.87E–08	black
GO:0043161	proteasome-mediated ubiquitin-dependent protein catabolic process	10	42	1.57E–10	2.65E–08	black
GO:0051726	regulation of the cell cycle	3	17	0.001381	0.044533	black
GO:0000027	ribosomal large subunit assembly	3	11	0.000356	0.017121	black
GO:0000028	ribosomal small subunit assembly	3	9	0.000185	0.010372	black
GO:0042254	ribosome biogenesis	3	13	0.000604	0.025457	black
GO:0006412	translation	29	150	2.94E–25	9.89E–23	black
GO:0006413	translational initiation	4	36	0.001296	0.044533	black
GO:0006886	intracellular protein transport	6	92	6.09E–05	0.020524	brown
GO:0031047	gene silencing by RNA	5	16	4.45E–06	0.001441	cyan
GO:0000122	negative regulation of transcription by RNA polymerase II	4	9	8.55E–06	0.001441	cyan
GO:0006511	ubiquitin-dependent protein catabolic process	7	76	0.000258	0.028969	cyan
GO:0009734	auxin-activated signalling pathway	7	56	0.000557	0.023483	darkgreen
GO:0042546	cell wall biogenesis	4	13	0.000256	0.012344	darkgreen
GO:0002181	cytoplasmic translation	9	18	1.84E–10	6.20E–08	darkgreen
GO:0000226	microtubule cytoskeleton organisation	8	28	3.61E–07	4.05E–05	darkgreen
GO:0000278	mitotic cell cycle	8	28	3.61E–07	4.05E–05	darkgreen
GO:0006334	nucleosome assembly	5	14	1.82E–05	0.001227	darkgreen
GO:0006412	translation	16	150	1.74E–06	0.000146	darkgreen
GO:0006414	translational elongation	6	26	4.18E–05	0.002346	darkgreen
GO:0030433	ubiquitin-dependent ERAD pathway	4	18	0.000991	0.037093	darkgreen
GO:0034599	cellular response to oxidative stress	4	15	0.000114	0.007663	lightcyan1
GO:0001732	formation of the cytoplasmic translation initiation complex	3	9	0.000428	0.020623	lightcyan1
GO:0051259	protein complex oligomerisation	5	19	1.62E–05	0.00273	lightcyan1
GO:0006457	protein folding	7	72	0.000287	0.016115	lightcyan1
GO:0042026	protein refolding	4	12	4.30E–05	0.004637	lightcyan1
GO:0009408	response to heat	6	31	1.52E–05	0.00273	lightcyan1
GO:0042542	response to hydrogen peroxide	5	24	5.50E–05	0.004637	lightcyan1
GO:0009651	response to salt stress	5	38	0.00053	0.022322	lightcyan1
GO:0015995	chlorophyll biosynthetic process	2	14	0.000193	0.013028	midnightblue
GO:0015979	photosynthesis	6	38	2.08E–11	1.75E–09	midnightblue
GO:0009768	photosynthesis, light harvesting in photosystem I	6	11	3.58E–15	1.21E–12	midnightblue
GO:0018298	protein-chromophore linkage	7	34	5.33E–14	8.98E–12	midnightblue
GO:0009416	response to light stimulus	6	32	6.86E–12	7.71E–10	midnightblue
GO:0006357	regulation of transcription by RNA polymerase II	5	127	5.61E–05	0.018918	paleturquoise
GO:0006468	protein phosphorylation	17	725	7.98E–05	0.013438	sienna3
GO:0006355	regulation of transcription, DNA-templated	16	568	1.48E–05	0.004993	sienna3
GO:0006887	exocytosis	3	20	1.62E–05	0.005465	skyblue

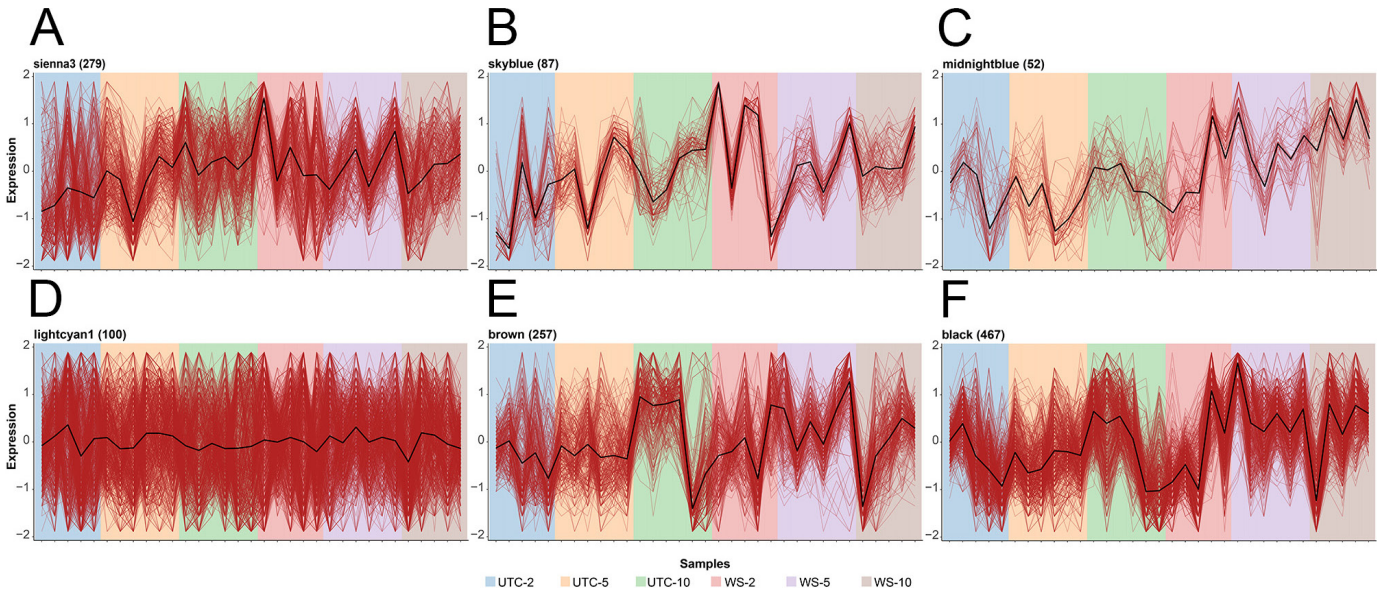


FIGURE 7. Expression patterns of the genes belonging to relevant WGCNA modules.

The numbers in brackets indicate the number of genes for each module. The sample colour legend is reported below the charts, with the treatment code (UTC, untreated control; WS, water-stressed) followed by the node number.

5. Hormone signal transduction analysis

A Pathview analysis was used to visualise on KEGG maps the changes in the expression patterns of the genes dealing with hormone signal transduction. A broad phytohormonal signalling is known to play a relevant role during response to water stress in crops, involving all the known plant hormones, and several studies were carried out in grapevine regarding the hormonal signalling pathways activated upon water stress (Botton *et al.*, 2022; Jiao *et al.*, 2024). As a general consideration, the signalling pathways of some of these hormones, namely brassinosteroids (BRs), gibberellins (GAs), cytokinins (CKs), and auxin, are generally repressed, while those of abscisic acid (ABA), salicylic acid (SA), ethylene, jasmonates (JAs), and strigolactones (SLs) are thought to be stimulated (Jogawat *et al.*, 2021). However, according to the crop, the specific organ and its developmental phase, the entity of the stress and its duration, most of these up/down trends can change and eventually revert. Moreover, while analysing the hormone signal transduction, the potential impact of root growth restriction due to the pot growth conditions should also be considered, as Li *et al.* (2022) showed that these conditions affect the hormonal balance in different parts of the vine, pointing out variations of every single hormone, not always consistent in the organs considered.

Concerning auxin, a broad downregulation of both its influx and signalling pathway was pointed out under severe water stress (BBCH 77), especially at nodes 2 and 5, in particular regarding the *AUX1* influx carrier genes, the *GH3* genes, and the *Aux/IAA* genes (Figure 8), the latter known to be robust indicators of auxin signalling activation (Abel *et al.*, 1994). Oppositely, buds at node 10 showed an upregulation of *Aux/IAA* genes, most likely due to the proximity of the shoot apical meristem, as pointed out also by WGCNA. At recovery (BBCH 84), the situation slightly changed at node 5, where some of the genes cited above were upregulated in the stressed vines, indicating an actual and prompt recovery, despite the lower levels of this hormone measured in all the parts of the vine under root growth restriction (Li *et al.*, 2022).

CK signalling was stimulated under severe water stress, as pointed out by the genes encoding the type-A regulators (RRA; negative regulators; Hwang *et al.*, 2012), which were upregulated at nodes 2 and 5 and unaffected at node 10, confirming the different behaviours of the nodes.

Transcription of *RRA* genes is known to be rapidly induced by CKs (D'Agostino *et al.*, 2000), most likely to exert a negative feedback regulation on CK signalling and decrease the sensitivity to the hormone upon a severe water stress, when roots may mobilise CKs to the shoot (Jogawat *et al.*, 2021), especially in potted vines that were shown to have enhanced levels of CKs in roots (Li *et al.*, 2022). At BBCH 84, the regulatory pattern changed, with *RRA* genes upregulated at node 10, repressed at node 5, and unaffected at node 2. At node 10, the wave of stress-related CKs likely arrived from roots later, when water flow in the xylem recovered, so that the same upregulation of *RRA* genes previously observed at nodes 2 and 5 was also detected in the apical buds.

A substantially neutral situation was observed for gibberellins at nodes 2 and 10 concerning the *DELLA* genes, which are usually transcriptionally active when the GA response is triggered (Middleton *et al.*, 2012). Worthy to note that, under severe stress, *DELLA* genes were downregulated in buds at node 5, leading to hypothesised lower levels of these hormones in these buds. Later, during recovery, the same downregulation was observed at node 10.

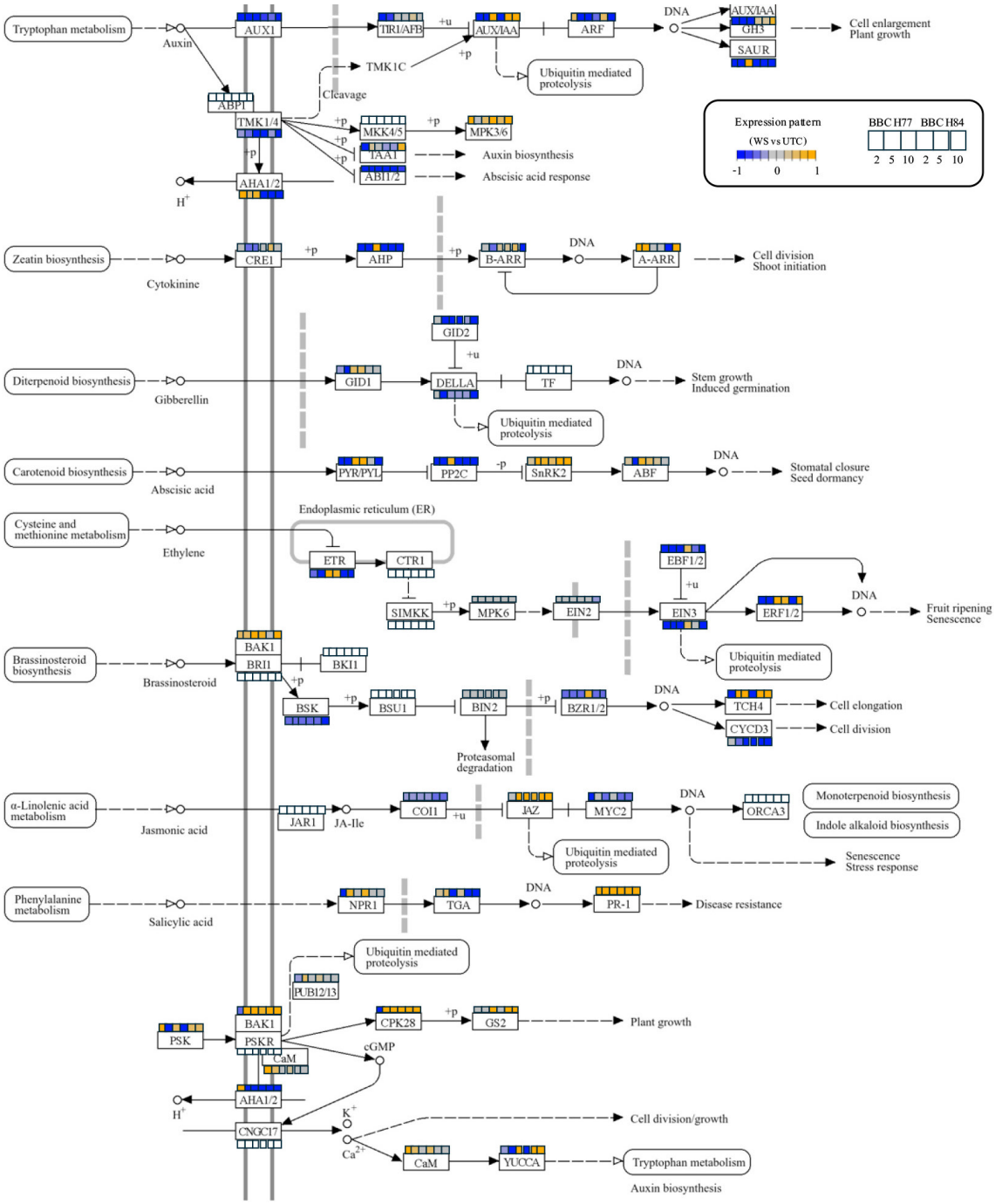


FIGURE 8. Pathview analysis of hormone signal transduction through KEGG mapping (map04075).

The colour scale and a basic legend are reported within the figure. Briefly, the expression pattern of each gene/multigene family is shown as a water-stressed *vs* UTC ratio through the six squares above or below the gene name according to the colours and position shown in the legend (two timepoints and three nodes for each timepoint).

Given the importance of gibberellins and cytokinins for the differentiation of uncommitted primordia into floral structures (Crane *et al.*, 2012), the hormonal balance pointed out by this analysis indicates that cytokinins were more active in basal nodes during severe water stress and in apical nodes during recovery.

Concerning ABA, the most important hormonal player in plants' drought response (Osakabe *et al.*, 2014), a clear upregulation was pointed out by *SnRK2* genes at both timepoints, more markedly in buds at nodes 5 and 10. These genes encode for key positive regulators of ABA response and were recently shown to be pivotal in grapevine drought tolerance (Lan *et al.*, 2024). Therefore, their extended upregulation in all the buds at both the timepoints was not surprising and further confirmed the ongoing water stress response.

Regarding ethylene, the *ERF* genes can be considered the best indicator of its involvement in drought stress (Müller & Munné-Bosch, 2015). According to Pathview analysis, ethylene signalling was shown to be repressed when the

stress was severe, but only in buds at nodes 2 and 5. At node 10, ethylene signalling was active at both timepoints, and so was node 2 at recovery, while buds at node 5 still displayed a marked downregulation. These results do not fully match the DEGs analysis regarding the timing, although in both cases the prevalent response was a downregulation of ethylene signalling, with a marked repression displayed by buds at node 5, either at BBCH 77 in the case of DEGs or at both timepoints for Pathview analysis. This may be due to the different methods adopted, especially the high stringency of the statistical parameters used in DEGs analysis and their enrichment.

As far as the BRs' signalling is concerned, a different behaviour was pointed out by *TCH4* and *CYCD3* genes, the two BR transcriptional targets represented in the Pathview map. Since the transcription of *TCH4* in *Arabidopsis* was shown to be activated regardless of the presence of BRs by several environmental stimuli (Iliev *et al.*, 2002), among which drought, its upregulation in nodes 5 and 10 may not surprise. In node 2, on the other hand, its transcription is repressed. This pattern is conserved at both timepoints. In addition, *CYCD3* pointed out an extended downregulation in all samples at both timepoints. According to these results, BRs may not be involved in the bud's drought response, while the transcriptional changes regarding *TCH4* may have bypassed BR signalling and, since this gene codes for a xyloglucan endotransglucosylase/hydrolase (Purugganan *et al.*, 1997), may reflect a recruitment of cell wall-modifying activity in response to water stress, as shown to occur by enrichment analysis of DEGs.

Both the signalling pathways of JA and SA were shown to be activated in all samples and both timepoints, as pointed out by Pathview analysis, in particular regarding the expression of *JAZ* and *PR-1* genes, both known indicators of JA- and SA-related transcription (Santner & Estelle, 2007) respectively, also in the grapevine (Rahman *et al.*, 2022). Moreover, grapevine *JAZ* genes were previously shown to transcriptionally respond to several environmental stimuli, including water stress (Zhang *et al.*, 2012), and drought-responsive elements were found in the promoter of the *VvPR1* gene of *Vitis vinifera* (Rahman *et al.*, 2022).

6. Expression of flowering-related genes

Based on the studies carried out by Almada *et al.* (2009), Carmona *et al.* (2007), and Palumbo *et al.* (2019), and a search which was carried out on PN40024.v4 genome annotations (Velt *et al.*, 2023), eight genes dealing with the flowering process were selected and their expression represented in a heatmap (Figure 9).

When the stress was severe (BBCH 77), few functionally relevant differences emerged between unstressed (UTC) and water-stressed (WS) buds. Statistically significant variations (Student's *t*-test with $P < 0.01$) in gene expression were observed in buds at node 2, with the downregulation of three genes in water-stressed buds: *VITVI06G01473*, encoding TFL1A, *VITVI07G04487*, encoding FT, and *VITVI17G00021*, encoding VFL (the *LEAFY* ortholog). All these genes were previously shown to play a pivotal role in grapevine flower bud differentiation and were demonstrated to fully complement their *Arabidopsis* orthologs. Moreover, in grapevine, they show peaks of expression during the floral induction/transition period. In summary, *VvTFL1A* is expressed at early bud development in uncommitted primordia to enable branching and prevent them from forming terminal flowers (Boss *et al.*, 2006), with a higher expression in apical buds correlated with higher fertility (Crane *et al.*, 2012). Expression of *VvFT* in the uncommitted primordia definitely closes the vegetative development and triggers flower differentiation; therefore, a higher bud fruitfulness is achieved when its transcription rate increases after the peak of *VvTFL1* (*i.e.*, mutually exclusive) as the axis of the inflorescence has undergone branching for longer (Carmona *et al.*, 2007; Crane *et al.*, 2012). Finally, *VvVFL* expression is thought to prolong the undifferentiated phase of the anlagen and is higher in apical buds that are more fertile (Carmona *et al.*, 2002; Crane *et al.*, 2012). Therefore, their downregulation in buds at the 2nd node upon water stress is fully consistent with the significant loss of fertility shown by buds at this node, both in terms of weight and number of inflorescences. Another significant change was displayed by *VITVI15G00774*, encoding *VvMADS8/VvSOC1*, whose expression upon drought stress increased in buds of the 5th node, further enhancing the specific physiological background pointed out for these buds by previous analyses and supporting the hypothesis that node 5 might be somehow protected from the effects of water stress. Such a protection may derive from the multiple and apparently contradictory functions of *VvMADS8/VvSOC1*, which was shown to be involved not only in the promotion of floral transition (Sreekantan & Thomas, 2006), but also in the repression of floral identity genes and induction of genes coding for cuticle biosynthetic enzymes (Jollife *et al.*, 2024). Although without any statistical significance, both the *CONSTANS-Like* genes (*VvCO* and *VvCOL1*) were upregulated by drought at node 10, indicating a sort of "phase shift" of expression that might have interfered with the flowering process, consistent with the lower fertility measured in these buds in terms of both the number of inflorescences and degree of branching.

Passing from BBCH 77 to BBCH 84, expression levels of these genes in the buds of well-watered vines pointed out some significant changes. *VvFLC (VITVI01G01673)* dropped in all samples, consistent with its inhibitory role on flowering, and the transcripts of both *VvVFL* at the 2nd node and *VvTFL1A* at the 10th node decreased through the experiment, while they remained constant in the 5th node. Although not statistically significant, *VvFT* expression was concurrently increasing. Based upon previous studies (Crane *et al.*, 2012), the timing of our trial was chosen correctly, *i.e.*, it was positioned exactly in the most critical moment of flower bud differentiation, between the peaks of expression of *VvTFL1A* and *VvFT*. To further support this fact, the expression of *VvCO* decreases in buds at node 2, differently from the behaviour of *VvCOL1*, as previously shown by Almada *et al.* (2009).

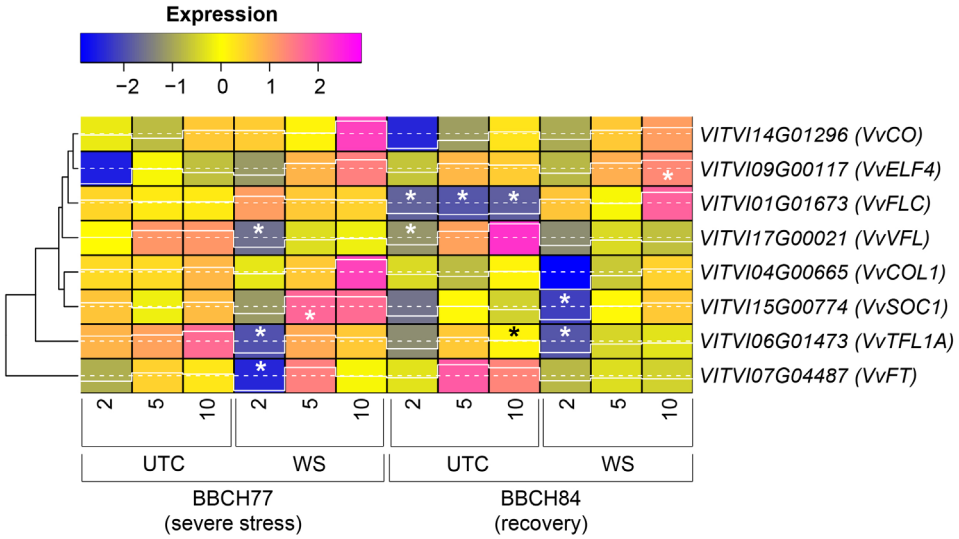


FIGURE 9. Expression heatmap of eight flowering-related genes.

The gene names are shown in brackets after the gene ID, and the line traces within the boxes represent the expression levels. The colour legend is shown at the top. Asterisks show statistically significant (Student's *t*-test at $P < 0.01$) differences according to the following order: BBCH *77*-WS *vs* BBCH *77*-UTC, BBCH 84-UTC *vs* BBCH *77*-UTC, BBCH 84-WS *vs* BBCH 84-UTC. UTC, unstressed control; WS, water-stressed; 2–5–10, buds at nodes 2, 5, and 10, respectively.

In buds recovering from water stress, the differences between nodes 2 and 10 on one side and node 5 on the other side were again evident, with *VvSOC1* and *VvTFL1A* downregulated by drought at node 2, and *VvELF4*, a flowering inhibitor (Sreekantan *et al.*, 2010), upregulated in buds of node 10. These observations were fully consistent with the reduced fertility observed, especially for buds at the 2nd and 10th node.

CONCLUSION

In the present study, the physiological and molecular responses of grapevine buds to water stress were analysed, revealing how node position affects the response to drought imposed at a critical phenological stage. The transcriptomic analysis provided an overview of the adaptive mechanisms across nodes subjected to water stress, allowing the definition of a working model for the buds' response to drought, as related also to their developmental and physiological background (Figure 10). Buds located at basal and apical nodes were more affected by water stress compared to those at central positions, herein represented by the 5th node, which showed a greater capacity to mitigate the effects of drought thanks to specific physiological and molecular mechanisms linked to their stage of development. Indeed, RNA-Seq data and WGCNA revealed distinct molecular profiles associated with node position, with unstressed basal buds showing a clearly different transcriptomic pattern linked to a more advanced developmental stage. The low expression levels of photosynthesis-related genes were a good developmental marker, especially for unstressed buds at node 5, while those at node 10 were showing the influence of the SAM, which kept the auxin signalling active in this node.

The number of DEGs under severe stress was well correlated with fertility outputs in the following year, with nodes 2 and 10 being the most affected and node 5 showing the lowest number of DEGs, along with a more prolonged transcriptional response during the recovery phase.

Node-specific responses involved hormone signal transduction pathways, with significant changes in auxin, cytokinins, ABA, gibberellins, and ethylene regulation. Auxin signalling was reduced at nodes 2 and 5, in contrast to cytokinins. ABA response was upregulated at nodes 5 and 10, whereas gibberellin signalling was lowest at node 5. Considering the negative role of gibberellins on grape floral induction (Boss & Thomas, 2002), these data were fully consistent with fertility measured in the following year. WGCNA identified gene expression modules correlated with drought stress. Water-stressed buds at node 5 showed higher expression of genes related to metabolism, cell cycle, heat response, and protein transport, indicating both a higher metabolic activity and a stronger level of protection, which were consistent with the higher fertility measured one year after. The expression of flowering-related genes supported these results, with node 5 showing stable and increased expression of *VvSOC1* under severe stress, with a putative protective role, while the floral integrators transcript profiles in the other nodes were compatible with a compromised situation for flowering.

Overall, the stress response limited its phenotypic consequences, particularly in central nodes. These results suggest that the position of buds along the shoot and the consequent developmental phase shifts influenced their ability to cope with water deficit and maintain fertility. This study contributes to understanding grapevine adaptation mechanisms and provides a basis for future research aimed at improving resilience to drought through molecular and agronomic approaches.

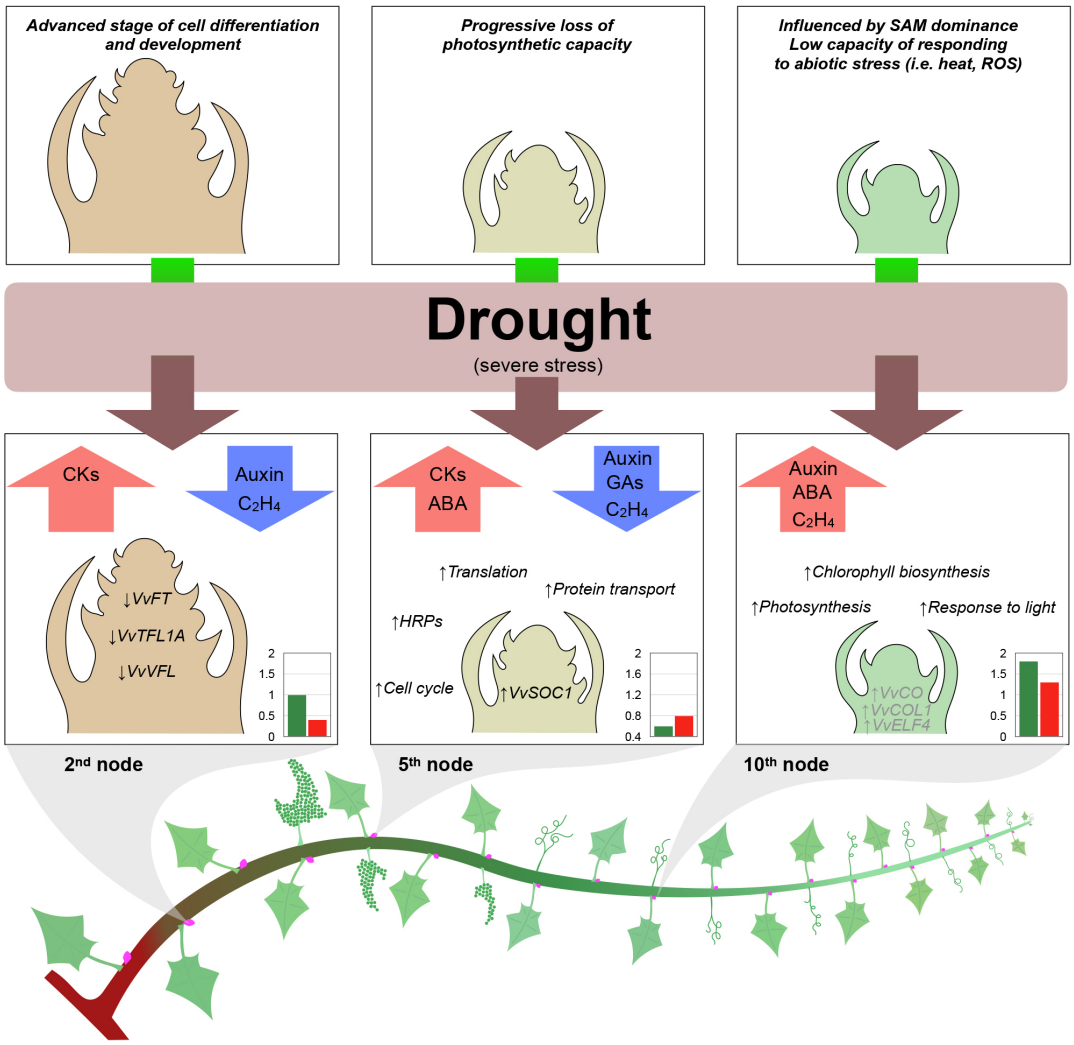


FIGURE 10. Working model for the response of grapevine buds to drought.

For each of the three nodes represented in the panels, the situation in unstressed buds is shown at the top, along with their main physiological features as deduced from transcriptomic data. The specific responses to severe drought are shown below in terms of fertility (as number of inflorescences per node, in the bottom right chart), hormone signalling (above in the red arrows—stimulation; in the blue arrows—inhibition), gene functions belonging to both the WGCNA modules and the DEGs (labelled around and outside the bud), and the flowering-related genes (inside the bud; the grey text at node 10 is used for genes with no statistical significance).

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REFERENCES

Abel, S., Oeller, P. W., & Theologis, A. (1994). Early auxin-induced genes encode short-lived nuclear proteins. *Proceedings of the National Academy of Sciences*, *91*(1), 326–330. https://doi.org/10.1073/pnas.91.1.326

Aguilar-Hernández, V., Brito-Argáez, L., Galaz-Ávalos, R. M., & Loyola-Vargas, V. M. (2020). Post-translational modifications drive plant cell differentiation. *Plant Cell, Tissue and Organ Culture*, *143*, 1–12. https://doi.org/10.1007/s11240-020-01908-0

Akıncı, Ş., & Lösel, D. M. (2012). Plant Water-Stress Response Mechanisms. In I. M. M. Rahman (Ed.), *Water Stress* (pp. 15–42). InTechOpen. https://doi.org/10.5772/29578

Almada, R., Cabrera, N., Casaretto, J. A., Ruiz-Lara, S., & Villanueva, E. G. (2009). *VvCO* and *VvCOL1*, two *CONSTANS* homologous genes, are regulated during flower induction and dormancy in grapevine buds. *Plant Cell Reports*, *28*(8), 1193–1203. https://doi.org/10.1007/s00299-009-0720-4

Almeida-Silva, F., & Venancio, T. M. (2022). BioNERO: an all-in-one R/Bioconductor package for comprehensive and easy biological network reconstruction. *Functional and Integrative Genomics*, *22*(1), 131–136. https://doi.org/10.1007/s10142-021-00821-9

Anders, S., Pyl, P. T., & Huber, W. (2015). HTSeq-A Python framework to work with high-throughput sequencing data. *Bioinformatics*, *31*(2), 166–169. https://doi.org/10.1093/bioinformatics/btu638

Araki, R., Takano, Y., Miyazaki, H., Ii, H., & An, P. (2022). Drought stress alters iron accumulation in *Sorghum bicolor* seeds. *Environmental and Experimental Botany*, *204*, 105093. https://doi.org/10.1016/j.envexpbot.2022.105093

Boss, P. K., Sreekantan, L., & Thomas, M. R. (2006). A grapevine TFL1 homologue can delay flowering and alter floral development when overexpressed in heterologous species. *Functional Plant Biology*, *33*(1), 31-41. https://doi.org/10.1071/FP05191

Boss, P. K., & Thomas, M. (2002). Association of dwarfism and floral induction with a grape 'green revolution' mutation. *Nature*, *416*, 847–850. https://doi.org/10.1038/416847a

Botton, A., Girardi, F., Ruperti, B., Brilli, M., Tijero, V., Eccher, G., Populin, F., Schievano, E., Riello, T., Munné-Bosch, S., Canton, M., Rasori, A., Cardillo, V., & Meggio, F. (2022). Grape Berry Responses to Sequential Flooding and Heatwave Events: A Physiological, Transcriptional, and Metabolic Overview. *Plants*, *11*(24). https://doi.org/10.3390/plants11243574

Canton, M., Mirone, F., Meggio, F., Pichierri, A., Casolo, V., Tornielli, G. B., & Pitacco, A. (2025). Consecutive Moderate and Severe Drought Stresses Affect Chlorophyll Fluorescence and Non-structural Carbohydrates Dynamics in Grapevine Leaves. *Physiologia Plantarum*, *177*(5). https://doi.org/10.1111/ppl.70535

Carmona, M. J., Calonje, M., & Martínez-Zapater, J. M. (2007). The *FT/TFL1* gene family in grapevine. *Plant Molecular Biology*, *63*(5), 637–650. https://doi.org/10.1007/s11103-006-9113-z

Carmona, M. J., Cubas, P., & Martínez-Zapater, J. M. (2002). *VFL*, the Grapevine *FLORICAULA/LEAFY* Ortholog, is Expressed in Meristematic Regions Independently of Their Fate. *Plant Physiology*, *130*(1), 68–77. https://doi.org/10.1104/pp.002428

Chaves, M. M., Pereira, J. S., Maroco, J., Rodrigues, M. L., Ricardo, C. P. P., Osório, M. L., Carvalho, I., Faria, T., & Pinheiro, C. (2002). How Plants Cope with Water Stress in the Field? Photosynthesis and Growth. *Annals of Botany*, *89*(7), 907. https://doi.org/10.1093/aob/mcf105

Chen, D., Shao, Q., Yin, L., Younis, A., & Zheng, B. (2019). Polyamine function in plants: Metabolism, regulation on development, and roles in abiotic stress responses. *Frontiers in Plant Science*, *9*, 429326. https://doi.org/10.3389/fpls.2018.01945

Chloupek, O., Hrstkova, P., & Schweigert, P. (2004). Yield and its stability, crop diversity, adaptability and response to climate change, weather and fertilisation over 75 years in the Czech Republic in comparison to some European countries. *Field Crops Research*, *85*(2–3), 167–190. https://doi.org/10.1016/S0378-4290(03)00162-X

Christensen, J. H., Carter, T. R., Rummukainen, M., & Amanatidis, G. (2007). Evaluating the performance and utility of regional climate models: the PRUDENCE project. *Climatic Change*, *81*, 1–6. https://doi.org/10.1007/s10584-006-9211-6

Cochetel, N., Ghan, R., Toups, H. S., Degu, A., Tillett, R. L., Schlauch, K. A., & Cramer, G. R. (2020). Drought tolerance of the grapevine, *Vitis champinii* cv. Ramsey, is associated with higher photosynthesis and greater transcriptomic responsiveness of abscisic acid biosynthesis and signalling. *BMC Plant Biology*, *20*(1), 1–25. https://doi.org/10.1186/s12870-019-2012-7

Crane, O., Halaly, T., Pang, X., Lavee, S., Perl, A., Vankova, R., & Or, E. (2012). Cytokinin-induced *VvTFL1A* expression may be involved in the control of grapevine fruitfulness. *Planta*, *235*(1), 181–192. https://doi.org/10.1007/s00425-011-1497-6

D'Agostino, I. B., Deruère, J., & Kieber, J. J. (2000). Characterization of the response of the Arabidopsis response regulator gene family to cytokinin. *Plant Physiology*, *124*(4), 1706–1717. https://doi.org/10.1104/pp.124.4.1706

Dal Cin, V., Danesin, M., Rizzini, F. M., & Ramina, A. (2005). RNA extraction from plant tissues: the use of calcium to precipitate contaminating pectic sugars. *Molecular Biotechnology*, *31*(2), 113–119. https://doi.org/10.1385/MB:31:2:113

Danecek, P., Bonfield, J. K., Liddle, J., Marshall, J., Ohan, V., Pollard, M. O., Whitwham, A., Keane, T., McCarthy, S. A., Davies, R. M., & Li, H. (2021). Twelve years of SAMtools and BCFtools. *GigaScience*, *10*(2). https://doi.org/10.1093/gigascience/giab008

De Nicolo, B., Cataldi-Stagetti, E., Diquigiovanni, C., & Bonora, E. (2023). Calcium and Reactive Oxygen Species Signalling Interplays in Cardiac Physiology and Pathologies. *Antioxidants*, *12*(2), 353. https://doi.org/10.3390/antiox12020353

De Vega, J. J., Teshome, A., Klaas, M., Grant, J., Finnan, J., & Barth, S. (2021). Physiological and transcriptional response to drought stress among bioenergy grass *Miscanthus* species. *Biotechnology for Biofuels*, *14*(1), 1–13. https://doi.org/10.1186/s13068-021-01915-z

Díaz-Riquelme, J., Grimplet, J., Martínez-Zapater, J. M., & Carmona, M. J. (2012). Transcriptome variation along bud development in grapevine (*Vitis vinifera* L.). *BMC Plant Biology*, *12*(1), 1–13. https://doi.org/10.1186/1471-2229-12-181

Flexas, J., Bota, J., Galmé, J., Lito Medrano, H., & Ribas-Carbó, M. (2006). Keeping a positive carbon balance under adverse conditions: responses of photosynthesis and respiration to water stress. *Physiologia Plantarum*, *127*, 343–352. https://doi.org/10.1111/j.1399-3054.2006.00621.x

Gai, Z., Wang, Y., Ding, Y., Qian, W., Qiu, C., Xie, H., Sun, L., Jiang, Z., Ma, Q., Wang, L., & Ding, Z. (2020). Exogenous abscisic acid induces the lipid and flavonoid metabolism of tea plants under drought stress. *Scientific Reports*, *10*(1), 1–13. https://doi.org/10.1038/s41598-020-69080-1

Ge, S. X., Son, E. W., & Yao, R. (2018). iDEP: An integrated web application for differential expression and pathway analysis of RNA-Seq data. *BMC Bioinformatics*, *19*(1). https://doi.org/10.1186/s12859-018-2486-6

Griesser, M., Weingart, G., Schoedl-Hummel, K., Neumann, N., Becker, M., Varmuza, K., Liebner, F., Schuhmacher, R., & Forneck, A. (2015). Severe drought stress is affecting selected primary metabolites, polyphenols, and volatile metabolites in grapevine leaves (*Vitis vinifera* cv. Pinot noir). *Plant Physiology and Biochemistry*, *88*, 17–26. https://doi.org/10.1016/j.plaphy.2015.01.004

Guilpart, N., Metay, A., & Gary, C. (2014). Grapevine bud fertility and number of berries per bunch are determined by water and nitrogen stress around flowering in the previous year. *European Journal of Agronomy*, *54*, 9–20. https://doi.org/10.1016/j.eja.2013.11.002

Hardie, W. J., & Considine, J. A. (1976). Response of Grapes to Water-Deficit Stress in Particular Stages of Development. *American Journal of Enology and Viticulture*, *27*(2), 55–61. https://doi.org/10.5344/ajev.1976.27.2.55

Herrera, J. C., Calderan, A., Gambetta, G. A., Peterlunger, E., Forneck, A., Sivilotti, P., Cochard, H., & Hochberg, U. (2022). Stomatal responses in grapevine become increasingly more tolerant to low water potentials throughout the growing season. *The Plant Journal*, *109*, 804-815. https://doi.org/10.1111/tpj.15591

Hwang, I., Sheen, J., & Müller, B. (2012). Cytokinin signalling networks. *Annual Review of Plant Biology*, *63*, 353–380. https://doi.org/10.1146/annurev-arplant-042811-105503

Iliev, E. A., Xu, W., Polisensky, D. H., Oh, M. H., Torisky, R. S., Clouse, S. D., & Braam, J. (2002). Transcriptional and Posttranscriptional Regulation of Arabidopsis*TCH4* Expression by Diverse Stimuli. Roles of cis Regions and Brassinosteroids. *Plant Physiology*, *130*(2), 770–783. https://doi.org/10.1104/pp.008680

Impa, S. M., Nadaradjan, S., & Jagadish, S. V. K. (2012). Drought Stress Induced Reactive Oxygen Species and Anti-oxidants in Plants. *Abiotic Stress Responses in Plants*, 131–147. https://doi.org/10.1007/978-1-4614-0634-1_7

Iqbal, N., Khan, N. A, Ferrante, A., Trivellini, A., Francini, A., & Khan, M. I. R. (2017). Ethylene Role in Plant Growth, Development and Senescence: Interaction with Other Phytohormones. *Frontiers in Plant Science*, *8*, 475. https://doi.org/10.3389/fpls.2017.00475

Jiao, Q., Bai, H., Zada, A., & Hu, X. (2024). Intrinsic Signalling Pathways and key Regulatory Factors of Stomatal Development. *Journal of Plant Biology*, *67*(3), 185–194. https://doi.org/10.1007/s12374-024-09427-7

Jogawat, A., Yadav, B., Chhaya, Lakra, N., Singh, A. K., & Narayan, O. P. (2021). Crosstalk between phytohormones and secondary metabolites in the drought stress tolerance of crop plants: A review. *Physiologia Plantarum*, *172*(2), 1106–1132. https://doi.org/10.1111/ppl.13328

Jolliffe, J. B., Moser, C., Pilati, S., & Lashbrooke, J. G. (2024). The grapevine *SOC1* homolog, *VviMADS8/SOC1a*, regulates floral organ specification in tomato. *Fruit Research*, *4*, e029. https://doi.org/10.48130/frures-0024-0023

Jordan, W. R., Morgan, P. W., & Davenport, T. L. (1972). Water Stress Enhances Ethylene-mediated Leaf Abscission in Cotton. *Plant Physiology*, *50*(6), 756–758. https://doi.org/10.1104/pp.50.6.756

Ju, Y.-L., Min, Z., Zhang, Y., Zhang, K.-K., Liu, M., & Fang, Y. L. (2021). Transcriptome profiling provide new insights into the molecular mechanism of grapevine response to heat, drought, and combined stress. *Scientia Horticulturae*, *286*, 110076. https://doi.org/10.1016/j.scienta.2021.110076

Kar, R. K. (2011). Plant responses to water stress: role of reactive oxygen species. *Plant Signalling & Behavior*, *6*(11), 1741–1745. https://doi.org/10.4161/psb.6.11.17729

Kawase, M. (1974). Role of Ethylene in Induction of Flooding Damage in Sunflower. *Physiologia Plantarum*, *31*(1), 29–38. https://doi.org/10.1111/j.1399-3054.1974.tb03673.x

Keller, M., Mills, L. J., Wample, R. L., & Spayd, S. E. (2004). Crop Load Management in Concord Grapes Using Different Pruning Techniques. *American Journal of Enology and Viticulture*, *55*(1), 35–50. https://doi.org/10.5344/ajev.2004.55.1.35

Korkutal, I., Bahar, E., & Carbonneau, A. (2011). Growth and yield responses of cv. Merlot (*Vitis vinifera* L.) to early water stress. *African Journal of Agricultural Research*, *6*(29), 6281–6288. https://doi.org/10.5897/AJAR11.1893

Kwak, J. M., Mori, I. C., Pei, Z. M., Leonhard, N., Angel Torres, M., Dangl, J. L., Bloom, R. E., Bodde, S., Jones, J. D. G., & Schroeder, J. I. (2003). NADPH oxidase *AtrbohD* and *AtrbohF* genes function in ROS-dependent ABA signalling in *Arabidopsis*. *The EMBO Journal*, *22*(11), 2623–2633. https://doi.org/10.1093/emboj/cdg277

Lan, Y., Song, Y., Liu, M., & Luo, D. (2024). Genome-wide identification, phylogenetic, structural and functional evolution of the core components of ABA signalling in plant species: a focus on rice. *Planta*, *260*(3), 1–21. https://doi.org/10.1007/s00425-024-04475-2

Langfelder, P., & Horvath, S. (2008). WGCNA: An R package for weighted correlation network analysis. *BMC Bioinformatics*, *9*(1), 1–13. https://doi.org/10.1186/1471-2105-9-559

Leucci, M. R., Lenucci, M. S., Piro, G., & Dalessandro, G. (2008). Water stress and cell wall polysaccharides in the apical root zone of wheat cultivars varying in drought tolerance. *Journal of Plant Physiology*, *165*(11), 1168–1180. https://doi.org/10.1016/j.jplph.2007.09.006

Li, J., Li, D., Liu, B., Wang, R., Yan, Y., Li, G., Wang, L., Ma, C., Xu, W., Zhao, L., Li, X., & Wang, S. (2022). Effects of root restriction on phytohormone levels in different growth stages and grapevine organs. *Scientific Report, 12*, 1323. https://doi.org/10.1038/s41598-021-04617-6

Lorenz, D. H., Eicchorn, K. W., Bleiholder, H., Klose, R., Meier, U., & Weber, E. (1995). 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. *Australian Journal of Grape and Wine Research*, *1*(2), 100–103. https://doi.org/10.1111/j.1755-0238.1995.tb00085.x

Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology*, *15*(12). https://doi.org/10.1186/s13059-014-0550-8

Luo, W., Pant, G., Bhavnasi, Y. K., Blanchard, S. G., & Brouwer, C. (2017). Pathview Web: user friendly pathway visualization and data integration. *Nucleic Acids Research*, *45*(W1), W501–W508. https://doi.org/10.1093/nar/gkx372

McMichael, B. L., Jordan, W. R., & Powell, R. D. (1973). Abscission Processes in Cotton: Induction by Plant Water Deficit. *Agronomy Journal*, *65*(2), 202–204. https://doi.org/10.2134/agronj1973.00021962006500020005x

Meggio, F., Trevisan, S., Manoli, A., Ruperti, B., & Quaggiotti, S. (2020). Systematic Investigation of the Effects of a Novel Protein Hydrolysate on the Growth, Physiological Parameters, Fruit Development and Yield of Grapevine (*Vitis vinifera* L., cv. Sauvignon Blanc) under Water Stress Conditions. *Agronomy*, *10*, 1785-1802. https://doi.org/10.3390/agronomy10111785

Meneghetti, S., Calò, A., & Gardiman, M. (2006). Flower Biology of Grapevine. A Review. *Advances in Horticultural Science*, *20*(4), p. 317–325. https://doi.org/10.1400/56453

Middleton, A. M., Úbeda-Tomás, S., Griffiths, J., Holman, T., Hedden, P., Thomas, S. G., Phillips, A. L., Holdsworth, M. J., Bennett, M. J., King, J. R., & Owen, M. R. (2012). Mathematical modeling elucidates the role of transcriptional feedback in gibberellin signalling. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(19), 7571–7576. https://doi.org/10.1073/pnas.1113666109

Moloi, S. J., & Ngara, R. (2023). The roles of plant proteases and protease inhibitors in drought response: a review. *Frontiers in Plant Science*, *14*, 1165845. https://doi.org/10.3389/fpls.2023.1165845

Müller, M., & Munné-Bosch, S. (2015). Ethylene Response Factors: A Key Regulatory Hub in Hormone and Stress Signalling. *Plant Physiology*, *169*(1), 32–41. https://doi.org/10.1104/pp.15.00677

Neill, S. J., & Burnett, E. C. (1999). Regulation of gene expression during water deficit stress. *Plant Growth Regulation*, *29*(1–2), 23–33. https://doi.org/10.1023/A:1006251631570

Ni, F.-T., Chu, L.-Y., Shao, H.-B., & Liu, Z.-H. (2009). Gene Expression and Regulation of Higher Plants Under Soil Water Stress. *Current Genomics*, *10*(4), 269. https://doi.org/10.2174/138920209788488535

Osakabe, Y., Yamaguchi-Shinozaki, K., Shinozaki, K., & Tran, L. S. P. (2014). ABA control of plant macroelement membrane transport systems in response to water deficit and high salinity. *New Phytologist*, *202*(1), 35–49. https://doi.org/10.1111/nph.12613

Pachauri, R. K., & Meyer, L. A. (2014). *AR5 Synthesis Report: Climate Change 2014 — IPCC*. Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. https://www.ipcc.ch/report/ar5/syr/

Palumbo, F., Vannozzi, A., Magon, G., Lucchin, M., & Barcaccia, G. (2019). Genomics of flower identity in grapevine (*Vitis vinifera* L.). *Frontiers in Plant Science*, *10*, 443330. https://doi.org/10.3389/fpls.2019.00316

Parsana, P., Ruberman, C., Jaffe, A. E., Schatz, M. C., Battle, A., & Leek, J. T. (2019). Addressing confounding artifacts in reconstruction of gene co-expression networks. *Genome Biology*, *20*(1), 1–6. https://doi.org/10.1186/s13059-019-1700-9

Perrone, I., Pagliarani, C., Lovisolo, C., Chitarra, W., Roman, F., & Schubert, A. (2012). Recovery from water stress affects grape leaf petiole transcriptome. *Planta*, *235*(6), 1383–1396. https://doi.org/10.1007/s00425-011-1581-y

Purugganan, M. M., Braam, J., & Fry, S. C. (1997). The Arabidopsis TCH4 Xyloglucan Endotransglycosylase (Substrate Specificity, pH Optimum, and Cold Tolerance). *Plant Physiology*, *115*(1), 181–190. https://doi.org/10.1104/pp.115.1.181

Rahman, F. U., Khan, I. A., Aslam, A., Liu, R., Sun, L., Wu, Y., Aslam, M. M., Khan, A. U., Li, P., Jiang, J., Fan, X., Liu, C., & Zhang, Y. (2022). Transcriptome analysis reveals pathogenesis-related gene 1 pathway against salicylic acid treatment in grapevine (*Vitis vinifera* L.). *Frontiers in Genetics*, *13*, 1033288. https://doi.org/10.3389/fgene.2022.1033288

Rezayian, M., Niknam, V., & Arabloo, M. (2023). Iron nanoparticle regulate succinate dehydrogenase activity in canola plants under drought stress. *Scientific Reports*, *13*(1), 1–14. https://doi.org/10.1038/s41598-023-36105-4

Rout, G. R., & Sahoo, S. (2015). Role of iron in plant growth and metabolism. *Reviews in Agricultural Science*, *3*(0), 1–24. https://doi.org/10.7831/ras.3.1

Rowland, E., Kim, J., Friso, G., Poliakov, A., Ponnala, L., Sun, Q., & van Wijk, K. J. (2022). The CLP and PREP protease systems coordinate maturation and degradation of the chloroplast proteome in *Arabidopsis thaliana*. *New Phytologist*, *236*, 1339–1357. https://doi.org/10.1111/nph.18426

Salazar, J. A., Rubio, M., Ruiz, D., Tartarini, S., Martínez-Gómez, P., & Dondini, L. (2015). SNP development for genetic diversity analysis in apricot. *Tree Genetics & Genomes*, *11*(15). https://doi.org/10.1007/s11295-015-0845-2

Salleo, S., Lo Gullo, M. A., De Paoli, D., & Zippo, M. (1996). Xylem recovery from cavitation-induced embolism in young plants of *Laurus nobilis*: a possible mechanism. *New Phytologist*, *132*(1), 47–56. https://doi.org/10.1111/j.1469-8137.1996.tb04507.x

Santner, A., & Estelle, M. (2007). The JAZ proteins link jasmonate perception with transcriptional changes. *The Plant Cell*, *19*(12), 3839–3842. https://doi.org/10.1105/tpc.107.056960

Sharma, N., Geuten, K., Giri, B. S., & Varma, A. (2020). The molecular mechanism of vernalization in Arabidopsis and cereals: role of Flowering Locus C and its homologs. *Physiologia Plantarum*, *170*(3), 373–383. https://doi.org/10.1111/ppl.13163

Sharma, R., & Diwan, B. (2023). Lipids and the hallmarks of ageing: From pathology to interventions. *Mechanisms of Ageing and Development*, *215*, 111858. https://doi.org/10.1016/j.mad.2023.111858

Shellie, K. C. (2006). Vine and Berry Response of Merlot (*Vitis vinifera* L.) to Differential Water Stress. *American Journal of Enology and Viticulture*, *57*(4), 514–518. https://doi.org/10.5344/ajev.2006.57.4.514

Sreekantan, L., Mathiason, K., Grimplet, J., Schlauch, K., Dickerson, J. A., & Fennell, A. Y. (2010). Differential floral development and gene expression in grapevines during long and short photoperiods suggests a role for floral genes in dormancy transitioning. *Plant Molecular Biology*, *73*(1–2), 191–205. https://doi.org/10.1007/s11103-010-9611-x

Sreekantan, L., & Thomas, M. R. (2006). VvFT and VvMADS8, the grapevine homologues of the floral integrators FT and SOC1, have unique expression patterns in grapevine and hasten flowering in Arabidopsis. *Functional Plant Biology: FPB*, *33*(12), 1129–1139. https://doi.org/10.1071/FP06144

Tenhaken, R. (2015). Cell wall remodeling under abiotic stress. *Frontiers in Plant Science*, *5*(JAN). https://doi.org/10.3389/fpls.2014.00771

Thorne, E. T., Stevenson, J. F., Rost, T. L., Labavitch, J. M., & Matthews, M. A. (2006). Pierce's Disease Symptoms: Comparison with Symptoms of Water Deficit and the Impact of Water Deficits. *Americal Journal of Enology and Viticulture*, *57*, 1-11. https://doi.org/10.5344/ajev.2006.57.1.1

van Wijk, K. J. (2015). Protein maturation and proteolysis in plant plastids, mitochondria, and peroxisomes. *Annual Review in Plant Biology*, *66*, 75–111. https://doi.org/10.1146/annurev-arplant-043014-115547

Vasconcelos, C. M., Graven, M., Winefield, C. S., Trought, M. C. T., & Raw, V. (2009). The Flowering Process of *Vitis vinifera*: A Review. *American Journal of Enology and Viticulture*, *60*(4), 411–434. https://doi.org/10.5344/ajev.2009.60.4.411

Vegro, M., Eccher, G., Populin, F., Sorgato, C., Savazzini, F., Pagliarani, G., Tartarini, S., Pasini, G., Curioni, A., Antico, A., & Botton, A. (2016). Old Apple (*Malus domestica* L. Borkh) Varieties with Hypoallergenic Properties: An Integrated Approach for Studying Apple Allergenicity. *Journal of Agricultural and Food Chemistry*, *64*(48), 9224–9236. https://doi.org/10.1021/acs.jafc.6b03976

Velt, A., Frommer, B., Blanc, S., Holtgräwe, D., Duchêne, É., Dumas, V., Grimplet, J., Hugueney, P., Kim, C., Lahaye, M., Matus, J. T., Navarro-Payá, D., Orduña, L., Tello-Ruiz, M. K., Vitulo, N., Ware, D., & Rustenholz, C. (2023). An improved reference of the grapevine genome reasserts the origin of the PN40024 highly homozygous genotype. *G3 (Bethesda, Md.)*, *13*(5). https://doi.org/10.1093/g3journal/jkad067

Wang, Y., Wang, Y., Song, Z., & Zhang, H. (2016). Repression of *MYBL2* by Both microRNA858a and HY5 Leads to the Activation of Anthocyanin Biosynthetic Pathway in *Arabidopsis*. *Molecular Plant*, *9*(10), 1395–1405. https://doi.org/10.1016/j.molp.2016.07.003

Williams, L. E., Baeza, P., & Vaughn, P. (2012). Midday measurements of leaf water potential and stomatal conductance are highly correlated with daily water use of Thompson Seedless grapevines. *Irrigation Science*, *30*, 201–212. https://doi.org/10.1007/s00271-011-0276-2

Ye, Y., Medina-Velo, I. A., Cota-Ruiz, K., Moreno-Olivas, F., & Gardea-Torresdey, J. L. (2019). Can abiotic stresses in plants be alleviated by manganese nanoparticles or compounds? *Ecotoxicology and Environmental Safety*, *184*. https://doi.org/10.1016/j.ecoenv.2019.109671

Yue, L., Xie, B., Cao, X., Chen, F., Wang, C., Xiao, Z., Jiao, L., & Wang, Z. (2023). The Mechanism of Manganese Ferrite Nanomaterials Promoting Drought Resistance in Rice. *Nanomaterials*, *13*(9), 1484. https://doi.org/10.3390/nano13091484

Zhang, X., Lei, L., Lai, J., Zhao, H., & Song, W. (2018). Effects of drought stress and water recovery on physiological responses and gene expression in maize seedlings. *BMC Plant Biology*, *18*(1). https://doi.org/10.1186/s12870-018-1281-x

Zhang, Y., Gao, M., Singer, S. D., Fei, Z., Wang, H., & Wang, X. (2012). Genome-wide identification and analysis of the *TIFY* gene family in grape. *PloS One*, *7*(9). https://doi.org/10.1371/journal.pone.0044465

Zhang, Y., Park, C., Bennett, C., Thornton, M., & Kim, D. (2021). Rapid and accurate alignment of nucleotide conversion sequencing reads with HISAT-3N. *Genome Research*, *31*(7), 1290–1295. https://doi.org/10.1101/gr.275193.120