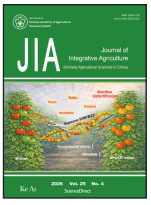
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RESEARCH ARTICLE

Transcriptomic changes and *VvMYBPA1* function analysis reveal the molecular mechanism of drought tolerance in grapevine

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Highlights

- Grafting attenuates drought damage *via* enhanced phytohormones and antioxidant enzymes with reduced oxidative stress.
- Transcriptomic profiling revealed drought-responsive DEGs.
- *VvMYBPA1* overexpression confers drought tolerance validated in *Arabidopsis* and grape calli, revealing novel regulatory mechanisms.

Abstract

Drought stress negatively affects grapevine growth and development. Grafting with rootstock is widely used to improve the quality of grape fruits and confer drought stress tolerance, but the underlying genetics and regulatory mechanisms are unclear. Hence, we investigated the physiologic and transcriptomic profiles in the leaves of grafted SM/1103P (SM shoot/1103P root) and self-rooted SM ('Shine Muscat') as well as roots of grafted SM/1103P and self-rooted 1103P under drought stress conditions. The results indicated that grafted grapevine effectively attenuated drought damage in grape leaves by increasing phytohormone levels and antioxidant enzyme activities, reducing H_2O_2 and MDA contents. Transcriptomic profiling revealed a total of 11,855 and 11,197 differentially expressed genes (DEGs) were identified in grape leaves and roots respectively. Weighted correlation network analysis (WGCNA) was performed based on the RNA-seq data, and five modules (greenyellow, black, turquoise, salmon and blue) were significantly correlated to drought stress. Pathway analysis showed that DEGs were enriched in the plant hormone signal transduction and MAPK signaling pathway. 916 transcription factor genes (TFs) belonging to different gene families were detected that may participate in regulating the drought stress. Quantitative real-time PCR (qRT-PCR) expression analysis of twelve drought stress responsive DEGs was used to verify the transcriptome data. Furthermore, overexpression of *VvMYBPA1* in *Arabidopsis thaliana* and grape callus tissues improved drought tolerance. Our findings provided new insights into to the regulatory mechanism for improving grapevine adaptation to drought.

Keywords: grapevine, physiological change, transcriptome, drought stress, *VvMYBPA1*

1. Introduction

Plants are constantly confronted with various biotic and abiotic stresses in their life that seriously reduce the productivity (Coolen *et al.* 2016). Among them, drought is one of the most significant environmental factors for limiting the plant productivity and survival (Rogiers *et al.* 2011; Li *et al.* 2020). Generally, drought stress can induce adverse reactions in plants, such as photosynthesis reduction, osmotic imbalance, oxidative stress, membrane system damage, and the accumulation of reactive oxygen species (ROS), which reduce the quality and yield of crops and lead to protein denaturation and cell death (Walz *et al.* 2002; Liu *et al.* 2020). Plants responses to drought stress are complex and numerous physiological, biochemical, and molecular levels strategies have evolved in the roots and leaves (Debora *et al.* 2016; Li *et al.* 2020). Notably, functional genes, transcription factors, and protein kinases are differentially expressed in plants in response to drought stress, which form a complex

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signal regulation network.

Grapevine (*Vitis vinifera*) is a major fruit crop and widely grown worldwide (Verdeil *et al.* 2010). Most of the world's emerging grape production areas are arid and semi-arid climates, drought is a widespread and detrimental stress factor during grapevine growth and development, causing significant agricultural losses. Grafting is an important cultivation technique widely used in horticulture to provide resistance to soil pathogens and abiotic stress as well as to control the vigour of the shoot (Lee *et al.* 2010). In viticulture, European grapevines have been cultivated as scions and routinely grafted onto the interspecific hybrid rootstocks to prevent infestation by phylloxera (Mudge *et al.* 2009). In parallel, the rootstocks affect scion by influencing the growth vigor, biomass accumulation, fruit yield and resistance to abiotic stresses (Berdeja *et al.* 2015; Lovisolo *et al.* 2016; Massimiliano *et al.* 2016; Warschefsky *et al.* 2016). Rootstocks are known to confer resistance to abiotic stress, such as drought and salinity, therefore, a rootstock is often to be considered a useful tool for aiding grape to resistance water scarcity (Soar *et al.* 2006; Tramontini *et al.* 2013; Corso and Bonghi 2014; Berdeja *et al.* 2015). In grapevine, 1103 Paulsen (1103P) is one of the most popular rootstocks for conferring to scion higher drought tolerance (Jaume *et al.* 2009).

The MYB family is the most widely distributed transcription factor (TFs) family in plants, and DNA-binding domain is the common feature of MYB protein. Genome-wide analyses have identified and classified the *MYB* gene family in many plants, including *Arabidopsis*, rice, maize, poplar, apple (Dubos *et al.* 2010), peach (Zhang C *et al.* 2018), banana (Pucker *et al.* 2020), tea (Chen *et al.* 2021), grapevine (Wang *et al.* 2023) and sweet cherry(Sabir *et al.* 2022). The MYB transcription family can be divided into four classes based on the number and location of repeats, namely 1R-MYB, R2R3-MYB, R1R2R3-MYB, and 4R-MYB (Dubos *et al.* 2010). In recent years, researchers have identified several *MYB* genes in plants, which play important roles in response to drought stress (Zhang *et al.* 2025). For example, In *Arabidopsis thaliana*, many R2R3-MYB proteins such as MYB96, MYB15 and MYB2 were induced by drought stress and activated the transcription of dehydration responsive genes (Li *et al.* 2015). Overexpression the flavonol synthesis regulators of *PAP1* or *MYB12* in *Arabidopsis* reduces water loss and enhances drought tolerance (Nakabayashi *et al.* 2014). Moreover, several MYB proteins have been found to be involved in drought responses in other species. Overexpression of *MdoMYB121* in tomato improved drought tolerance (Cao *et al.* 2013). In wheat, *TaMYB30* was induced in response to PEG stress, and overexpression of *TaMYB30-B* in *Arabidopsis* increased drought resistance (Zhang *et al.* 2012). Overexpression of *GmMYB84* in soybean exhibited higher survival rate, antioxidant enzymes activities, soluble sugar and proline, which showed stronger drought resistance (Wang N *et al.* 2017). In addition, MYB responded to drought stress by regulating stomatal movement (Zhang *et al.* 2025). In *Arabidopsis*, overexpression *AtMYB20* has no effect on drought resistance, but loss of function mutant *myb20* plants promoted stomatal closure and enhanced drought tolerance (Gao *et al.* 2014). *MYB37*-overexpression improves plant tolerance to drought by inducing stomatal closure in *Arabidopsis* (Yu *et al.* 2016). Overexpression of *TaMYB3R1* in *Arabidopsis thaliana* decreased the stomatal pore size and increased the drought tolerance (Cai *et al.* 2015). Although the functions of many *MYB* genes in drought stress response have been investigated, the role of *MYBPA1* in grapevine drought tolerance remains largely unexplored.

RNA sequencing (RNA-Seq) is an increasingly important tool for comprehensive analyses of the transcriptome profiling (Wang *et al.* 2009). The technology has been widely applied to study the molecular mechanisms of drought stress in many plants, such as *Arabidopsis*, maize, apple, orange and other economic crops (Yu *et al.* 2012; Fu *et al.* 2013; Loraine *et al.* 2013; Muriira *et al.* 2015). However, the expression profiles of dynamically expressed genes have rarely been studied in grafted grapes under drought stress. To clarify the mechanisms underlying the response of grapevine to water deficits, the transcriptome changes both in the leaves of self-rooted SM and grafted SM/1103P and the roots of the self-rooted 1103P and grafted SM/1103P under drought stress were analyzed by RNA-seq in this study. Furthermore, overexpression of *VvMYBPA1* increased drought tolerance in *Arabidopsis thaliana* and grape calli. In contrast to other studies, our work monitored the drought-related genes in grapevines that involves both roots and leaves, which provided new insights into the molecular mechanisms of drought stress. These results can pave the way for future functional study and cultivating high drought tolerant varieties of grape.

2. Materials and methods

2.1. Plant materials and drought stress treatment

Two-year-old grapevines were used as experimental materials, including self-rooted 1103 Paulsen (*V. berlandieri* ×*V. rupestris*), self-rooted 'Shine Muscat' (*V. labrusca*×*V. vinifera*), and grafted vines with 'Shine Muscat' as the scion and 1103 Paulsen as the rootstock (SM/1103P).

All plants were grown in a greenhouse at the College of Horticulture, Gansu Agricultural University, Lanzhou, Gansu, China. Each plant was cultivated in a 12 L ceramic pot, according to the ratio of peat moss, perlite and vermiculite=3:1:1, watering every four days before the start of the experiment.

The seedlings were divided into two groups: plants grown under well-watered (CK) condition and plants grown under drought stress (DS) condition. The CK plants were maintained to 60%, whereas for DS plants, the water content of soil in pots was gradually reduced from 60 to 20% and maintained for 40 d. The soil moisture content was measured using a hand-held soil moisture meter (Handi-trase, Zealquest Scientific Technology Co., Ltd.). The required amount of water was maintained at a specific amount for each day, and the DS group was kept to 20% for 40 d. On day 40, leaf samples were collected from the third to fifth leaves (counting from the tip of each branch), and roots were separated from the nutrient-rich zone (15–20 cm depth), immediately frozen in liquid nitrogen and stored at –80°C. The experiments employed three independent biological replicates, with each

replicate consisting of three individual plants.

Arabidopsis thaliana seedlings were grown in growth chambers under controlled conditions ((25±1)°C day/night with a photoperiod of 16/8 h day/night). Additionally, 3-week-old wild-type and *VvMYBPA1*-overexpressing seedlings were kept without irrigation for 14 d, and normal conditions (well-watered) were as controls.

'Pinot Noir' grape calli were subcultured one time at 3-week intervals on B5 medium plus 0.5 mg L^{-1} NAA and 1.0 mg L^{-1} KT at 25°C in the dark. For drought stress, the grape calli were cultivated on a B5 medium supplementing 4% (w/v) PEG6000 for 20 d, and normal conditions (0% PEG6000) were as controls.

2.2. Determination of endogenous hormone concentrations

A total of 1.0 g of leaf and root samples from the suitably treated were used to measure abscsic acid (ABA), indole-3-acetic acid (IAA) and gibberellin (GA). Each sample was ground in liquid nitrogen and extracted with 5 mL 80% chromatographic methanol. After three times washed by solvent, the extract samples were stored at 4°C for 12 h. Then, the supernatant was collected by centrifugation at 12,000 r min^{-1} for 10 min at 4°C. The 2 mL of sample was concentrated by a rotary evaporator (38°C, 1,300 r min^{-1}), and the residue was resuspended with 2 mL 50% chromatographic methanol (v/v, methanol/water). The redissolved fluid then filtered through a 0.22 μm organic membrane and subjected to high-performance liquid chromatography (HPLC, Waters Acquity Arc, USA) for analysis. HPLC determination was performed on an Agilent Zorbax SB-C18 column (5 μm, 250 mm×4.6 mm) at 30°C column temperature. The HPLC conditions were as follows: the mobile phase was methanol and 0.1% phosphoric acid, the flow rate was 1.0 mL min^{-1}, the wavelength was monitored at 254 nm.

2.3. Physiological and biochemical analyses

The contents of hydrogen peroxide (H_2O_2), superoxide anion ($O_2^{-}\cdot$), malondialdehyde (MDA), proline, GSH and the activities of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) were determined using the corresponding commercial assay kits (Keming, Suzhou, China). All of the experiments were conducted following the manufacturer's instructions and analyzed in at least three independent biological repetitions.

For the survival rate experiment, 100 seedlings of wild-type and *VvMYBPA1*-overexpressing plants were kept without irrigation for 14 d, then re-watering for 3 d, followed by assessment of survival rate. The electrolyte leakage was determined according to previous studies (Shi *et al.* 2012). The chlorophyll contents were measured on 0.2 g of fully expanded leaves, which were extracted with 80% acetone and measuring the absorbance at 649 and 665 nm (Gao 2006). The content of soluble protein was determined according to the coomassie brilliant blue staining (Gao 2006). For the relative water content , leaves and roots were weighted after harvesting (FW), then soaked in distilled water at 4°C for 24 h and the turgid weight was measured (TW). Then the leaves and roots were dried in oven at 80°C for 24 h to obtain their dry weight (DW). Relative water content was calculated according to previous studies (Meher *et al.* 2018).

For the water loss assay of *A. thaliana*, the rosette leaves of wild type and transgenic seedlings were cut and placed on a piece of weighting paper. The rosette leaves were periodically weighted at 0, 0.5, 1, 2, 3, 4, 5, 6, 7, and 8 h. Water loss was shown as a percentage of the original fresh weight. At least, four different experiments were performed.

2.4. Germination, cotyledon greening and root growth assay

For evaluation the germination rate and greening seedling, seeds of the WT and *VvMYBPA1*-overexpressing lines were surface-sterilized and placed on 1/2 MS plates with different concentrations of mannitol (0, 50, 100, 150 and 200 mmol L^{-1}) and ABA (0, 0.2, 0.3 and 0.5 μmol L^{-1}), at 23°C with a 16 h light/8 h dark photoperiod. The germination rates of at least 60 seeds from each line were counted at 0–8 d, and cotyledon greening was counted at 10 d. For the root growth assay, 5-d-old transgenic and WT germinated seedlings were carefully transferred to 1/2 MS plates supplemented with mannitol (0, 100, 200 and 300 mmol L^{-1}) and ABA (0, 0.5 and 1.0 μmol L^{-1}) plates, which were placed vertically in a growth chamber at 25°C for 7 d and then measured for the primary root length.

2.5. Stomatal aperture bioassay

The rosette leaves of 3-week-old WT and transgenic seedlings were incubated in stomatal opening solution (10 mmol L^{-1} KCl, 50 μmol L^{-1} $CaCl_2$, 10 mmol L^{-1} MES-KOH, PH 6.15) under light (120 μmol m^{-2} s^{-1}) for 4 h , and then transferred to ABA-containing solution (0, 1 and 10 μmol L^{-1}) for 2 h. Stomata on abaxial surfaces were observed with a microscope (Motic China Group Co. Ltd., China) and stomatal aperture was measured by ImageJ software (Lim *et al.* 2014).

2.6. RNA extraction, cDNA library construction and sequencing

The total RNA was extractedfrom leaf and root tissues using the RNAprep Pure Plant Kit (Tiangen, Beijing, China). The quality and quantity of RNA was assessed by 1% agarose gels, and RNA purity and integrity was assessed using a NanoDrop 2000 spectrophotometer (NanoDrop Technologies, CA, USA) and an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). Subsequently, 24 sequencing libraries were constructed using the NEBNext Ultra RNA Library Prep Kit (NEB, USA), and then constructed libraries were sequenced on an Illumina NovaSeq 6000 platform.

2.7. RNA-seq data analysis

RNA-Seq raw reads were first filtered by eliminating reads containing poly N, reads with adaptors and low-quality reads from raw data to achieve clean data. The paired-end clean reads were aligned to the grape reference genome using *V. vinifera*. IGGP_12x.45 (http://plants.ensembl.org/Vitis_vinifera/Info/Index). A total of 167.62 Gb of clean data were obtained, and all the analyses were based on high quality

clean data. FPKM (fragments per kilobase of transcript per million fragments mapped) was used as a threshold to determine significant differentially expressed genes (DEGs). Transcripts with FPKM≥1 were considered expressed (Anders *et al.* 2015). DESeq2 carried out differential expression analysis using models based on negative binomial distributions. Differential expression genes were detected by the screening standard of fold change≥2 and a threshold of false discovery rate (FDR<0.01) (Love *et al.* 2014). GO (Gene Ontology) enrichment analysis of DEGs was implemented using the GOseq R package. GO terms with corrected *P*-value less than 0.05 were considered significantly enriched in different pairwise comparisons. The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment was performed for identifying the pathways associated with the DEGs (P<0.05).

2.8. qRT-PCR

Total RNA was extracted from leaf and root tissues using a Plant RNA Kit (Omega, USA). First-strand cDNA was synthesized by reverse transcription of 1 μg of total RNA in a 20 μL reaction volume using Evo M-MLV RT Kit II (Accurate Biology, Hunan China). Primer sequences were designed using Primer 6.0 software and synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). qRT-PCR gene expression was carried out using a LightCycle 96 Real-Time PCR System (Roche, USA) with SYBR Green Premix Pro Taq (Accurate Biology, Hunan, China) following the manufacturer's instructions. The grape *actin* gene (XM_002282480.4) and the *A. thaliana actin* gene (AT3G18780) were used as the internal references. Three independent biological replicates were performed for each sample, and the relative expression levels of genes were evaluated using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001) .

2.9. Vector construction and plant transformation

To construct the *35S::VvMYBPA1::EGFP* vector, full-length *VvMYBPA1* cDNA was amplified from the root of 1103P and cloned into the pCAMBIA2300 vector fused with the GFP reporter gene. The recombinant plasmid was transformed into *Agrobacterium tumefaciens* GV3101, which were then used to transform *A. thaliana* (ecotype Columbia-0) according to the floral dip method (Clough and Bent 1998).

The grape 'Pinot Noir' calli were used for genetic transformation. The fresh grape calli were placed on sterilized filter paper and then infected 10 min, screened on B5 selection medium containing 200 mg L^{-1} cephalosporin, 250 mg L^{-1} carbenicillin and 50 mg L^{-1} kanamycin for 30 days in the dark incubator. Newly grown calli were transferred to the same screened media for every 2 weeks, and then the DNA and RNA were extracted to detect positive calli.

2.10. Statistical analysis

The SPSS 25.0 (IBM, USA) was used for statistical analysis, and all figures were performed with Origin 2021 software (OriginLab, USA). The significant differences among samples were indicated using lower-case letters above bars by Duncan's multiple range tests (P<0.05).

3. Results

3.1. Effects of drought stress on the morphology, relative water content, endogenous hormones, MDA, H_2O_2 and antioxidant enzymes in grapevine leaves and roots

The morphology of grapevine leaves is presented in Appendix A. Under normal conditions, the leaves of self-rooted S and grafted G grew well and had no significant differences. Under drought stress conditions, the leaves of S exhibited curling, wrinkling, and developed yellow spots on the leaf edges. While grafted G displayed less degree of leaf damage. Furthermore, roots of self-rooted R and grafted G appeared healthy state under normal conditions, whereas the roots exposed to drought stress became dehydrated. This morphological observation of leaf and root dehydration was substantiated by physiological data, which showed that drought stress significantly reduced the relative water content compared to the well-watered plants (Appendix A).

The endogenous hormone contents were performed both in grapevine leaves and roots of well-watered and drought stress treatment. In leaves, the ABA contents showed no obvious difference in self-rooted S, yet increased sharply after drought stress conditions in grafted G (approximately increased by 6.28 ng g^{-1}). In the roots, the contents of ABA increased significantly both in self-rooted R (approximately increased by 31.87 ng g^{-1}) and grafted G (approximately increased by 21.81 ng g^{-1}) under drought stress condition (Fig. 1-A). Likewise, The IAA contents increased in the leaves by 13.93 ng g^{-1} in S and 68.59 ng g^{-1} in G under drought stress condition relative to that of the control condition, and increased to 93.19 ng g^{-1} in R and 163.28 ng g^{-1} in G of the roots under drought condition, respectively (Fig. 1-B). Differently, the GA contents in leaves decreased in S, but increased in G, and increased dramatically in R (approximately increased by 88.33 ng g^{-1}) and G (approximately increased by 133.67 ng g^{-1}) of the roots under drought stress conditions compared to the well-watered conditions (Fig. 1-C). Taken together, the results indicated that drought stress triggered ABA, IAA and GA changes in the grape leaves and roots, and higher levels were oberved in G than S in leaves.

For further verification, the contents of H_2O_2 and MDA in grape leaves and roots were measured in self-rooted and grafted grapevines (Fig. 1-D and E). The contents of H_2O_2 and MDA were increased both in S and G of grape leaves and roots after drought stress, while its level was much less in G than in S. Next, the activities of antioxidant enzymes (SOD, POD and CAT) were assessed, revealing that the three enzymes activity increased both in leaves and roots, and a higher activity levels were observed in G than S in leaves under drought stress conditions (Fig.1-F–H). This indicated that plants have developed an antioxidant defense system to overcome oxidative stress and maintain redox homeostasis.

3.2. RNA-sequencing analysis

To reveal the molecular mechanisms underlying drought response, 24 cDNA libraries from eight samples of the leaves (L) and roots (R) in self-rooted and grafted

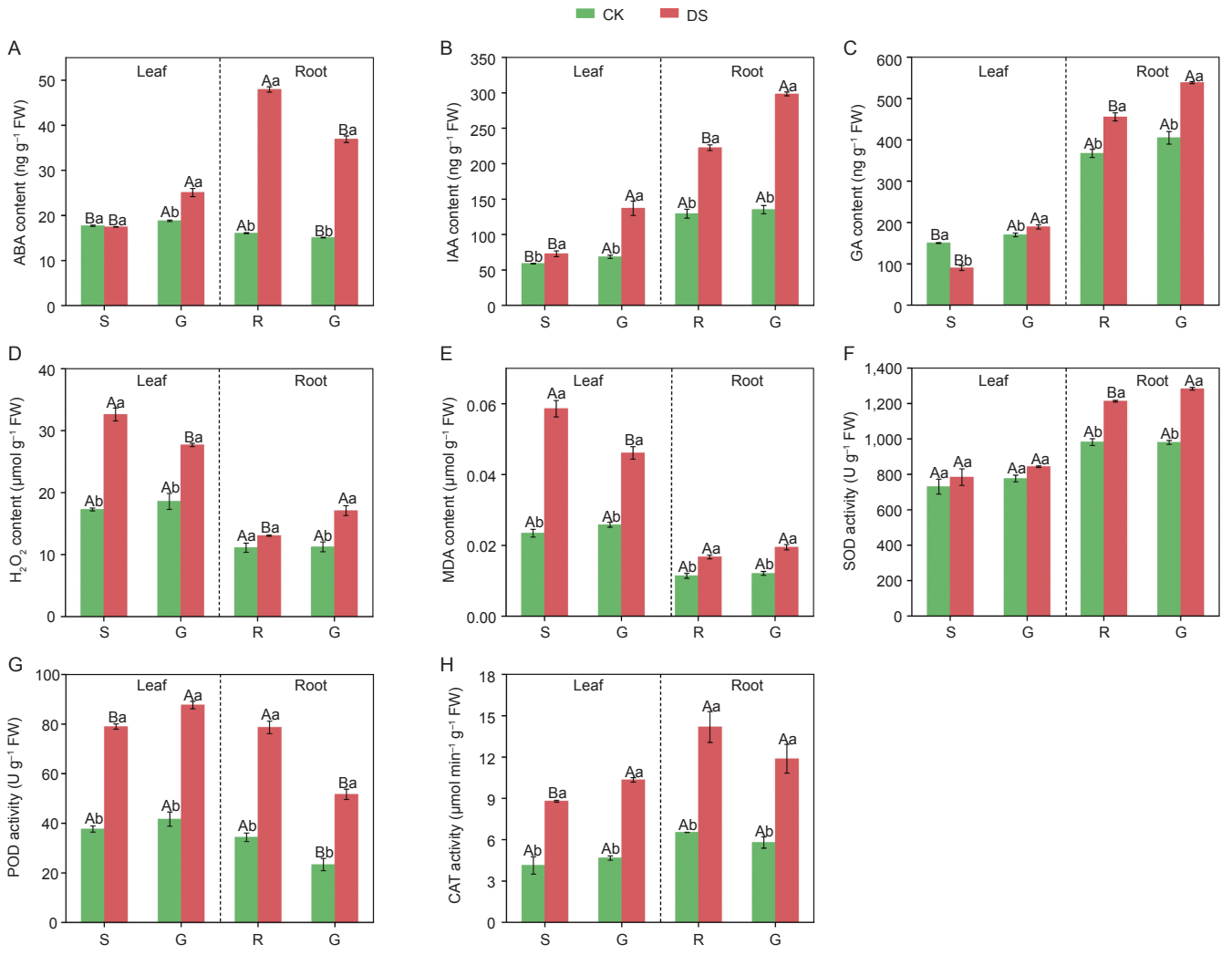


Fig. 1 Effect of drought stress on physiological index in self-rooted and grafted grapevines. A, abscisic acid (ABA) content. B, indole-3-acetic acid (IAA) content. C, gibberellin (GA) content. D, H_2O_2 content. E, malondialdehyde (MDA) content. F, superoxide dismutase (SOD) activity. G, peroxidase (POD) activity. H, catalase (CAT) activity. S, self-rooted table grape cultivar 'Shine Muscat'; G, grafted grape (SM shoot/1103P root); R, self-rooted rootstock '1103 Paulsen'. Different lowercase letters denote the differences between well-watered(CK) and drought stress (DS) treatment of the same grape material, and uppercase letters represent differences between different grape materials under the same treatment (P<0.05). The values are the means±SD (n=3).

grapevine plants were analyzed by high throughout RNA-seq, including SCK-L, SDS-L, GCK-L, GDS-L, RCK-R, RDS-R, GCK-R and GDS-R (Fig. 2-A; Appendix B). In total, 1,119,924,308 raw reads were obtained from 24 cDNA libraries, of which an average of 88.31% raw reads were mapped to the reference genome. The unique and multiple mapping rates were 80.2–90.74% and 2.22–4.25%, respectively. The mapped data to exons ranged from 87.03 to 90.78% (Appendix B). After filtering out the low quality reads and trimming the adapter sequences, approximately 559,962,154 clean reads were extracted for further analysis. The GC content of 24 libraries ranged from 45.01 to 47.32%, and the Q20 and Q30 values of all the samples exceeded 96.88 and 91.98%, respectively (Appendix C). The expression levels of biological replicates from the same tissues under the same treatment conditions were highly correlated (R^2=approximately 0.99, P<0.01) (Appendix D). Additionally, Principal component analysis (PCA) was also carried out to visualize the variation among samples (Appendix D). The PCA results showed that the distance between three biological replications for each sample clustered closely, suggesting that the data were highly quality. A clear separation was detected between the leaves and roots, indicating that the transcriptomic of plant tissue showed various difference. Moreover, the arrays used for CK samples and DS samples were also completely separated, which revealing that the drought treatment comprised the main source of variation.

3.3. Analysis of DEGs

To further elucidate the transcriptional response to drought stress, DEGs in leaves and roots among three grapevine materials were calculated. A total of 23,052 DEGs were identified of the 24 cDNA libraries. Of these DEGs, 10,886 up-regulated and 12,166 down-regulated DEGs were quantified among the eight comparison groups. Specifically,

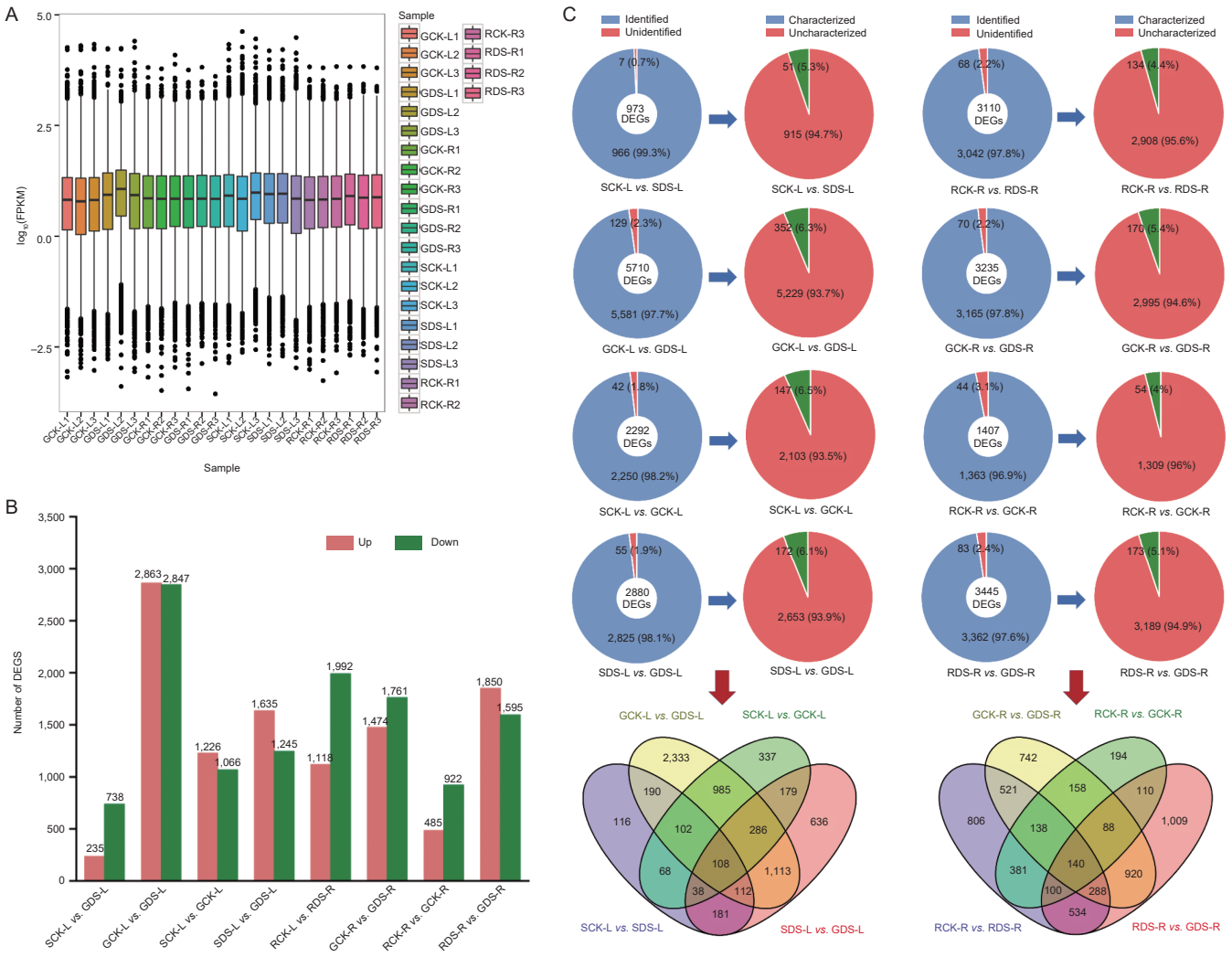


Fig. 2 Analysis of identified differentially expressed genes (DEGs) in self-rooted and grafted grapevines under drought stress. A, total gene expression in each sample based on $\log_{10}$FPKM values. B, the number of DEGs between leaves and roots in the indicated groups. C, distribution and classification of DEGs in leaves and roots of the indicated groups.

11,855 DEGs were identified in the leaves of four comparison groups, 11,197 DEGs were identified in the root of the other four groups (Fig. 2-B). Additionally, a comparison of the two groups between control and drought treatment in leaves and roots was performed (Fig. 2-B). In leaves, 973 DEGs in SM and 5,710 DEGs in grafted SM/1103P, suggesting that the drought responsive transcriptomic program is predominant in grafted grapevine. Furthermore, in root samples, 3,110 DEGs in 1103P and 3,235 DEGs in SM/1103P. Comparison of self-rooted and grafted grapevine (SCK-L *vs.* GCK-L and RCK-R *vs.* GCK-R) showed that the expression level of 2,292 and 1,407 genes changed in response to grafting with 1103P rootstock, and the number of up-regulated DEGs was 1,226 in leaves and 485 in roots, down-regulated DEGs were1,066 in leaves and 922 in roots, indicating that a large number of grafting-responsive genes were identified and a significant influence of 1103P rootstock on gene expression level in SM scion. In addition, DS-responsive DEGs in self-rooted and grafted grapevine were identified, revealing that 2,880 and 3,445 DEGs were found in leaves and roots, respectively (Fig. 2-B). The analysis revealed that the number of DEGs in roots were higher than the number of leaves, confirming that roots are the first organs to perceive water deprivation, undergoing more complex gene regulation during water deprivation than leaves. In addition, volcano plots of the DEGs in the two groups illustrate their distinct transcriptional profiles (Appendix E).

After data compilation, 973, 5,710, 2,292, and 2,880 genes were differentially expressed at SCK-L *vs.* SDS-L, GCK-L *vs.* GDS-L, SCK-L *vs.* GCK-L, and SDS-L *vs.* GDS-L, respectively. Of these genes, 966, 5,581, 2,250, and 2,825 genes in leaves have been identified in GO, KEGG, KOG, Pfam, SwissProt and NR database. Among these identified genes, 915, 5,229, 2,103 and 2,653 genes showed characterized functions (Fig. 2-C). In addition, DEGs in root comparison groups (RCK-R *vs.* RDS-R, GCK-R *vs.* GDS-R, RCK-R *vs.* GCK-R and RDS-R *vs.* GDS-R) identified 3,042, 3,165, 1,363 and 3,362 genes in above database, respectively. Of the identified DEGs, respectively 2,098, 2,995, 1,309 and 3,189 genes presented characterized

functions (Fig. 2-C).

Furthermore, a Venn diagram analysis of characterized DEGs among the leaves and roots comparison groups uncovered drought stress effect on self-rooted and grafted grapevine transcript abundance changes (Fig. 2-C). As shown in the Fig. 2-C, 108 genes were present in four comparison groups of leaves, and 140 genes were expressed in roots comparison groups. The 108 genes co-existing in leaves were enriched in the BP terms "regulation of jasmonic acid mediated signaling pathway, galactose metabolic process, regulation of defense response", CC terms "nucleus", and MF terms "DNA binding, transmembrane transporter activity, transcription factor activity, protein binding" (Appendix F). The 140 DEGs overlap in root were enriched BP terms "lignin catabolic process, iron ion homeostasis and transport, cell wall organization, response to oxidative stress", CC terms "apoplast, extracellular region, plasma membrane", MF terms "hydroquinone:oxygen oxidoreductase activity, copper ion binding, ferroxidase activity, transcription factor activity" (Appendix G).

3.4. GO and KEGG enrichment analysis of DEGs

For the functional annotation based on DEGs, the genes in grape leaves and roots were classified into biological process, cellular component, and molecular function categories. In the cellular component category of leaves and roots comparison groups, cell (GO: 0005623), cell part (GO: 0044464), organelle (GO: 0043226), and membrane (GO: 0016020) were significantly enriched items. In the molecular function, the DEGs were annotated mainly binding (GO: 0005488) and catalytic activity (GO: 0003824). In the biological processes category, the most common groups were metabolic process (GO: 0008152), cellular process (GO: 0009987), single-organism process (GO: 0044699) and biological regulation (GO: 0065007) (Appendix H).

To further explore the functions of DEGs, the KEGG pathways enrichment analysis was performed (Appendix I). The DEGs in the leaves of comparison groups were mainly concentrated in the plant hormone signal transduction (ko04075), MAPK signaling pathway-plant (ko04016), flavonoid biosynthesis (ko00941), starch and sucrose metabolism (ko00500). The drought-responsive genes in the roots of grape affected plant hormone signal transduction (ko04075), MAPK signaling pathway-plant (ko04016) and phenylpropanoid biosynthesis (ko00940). Taken together, based on the results of KEGG, the DEGs significantly affected by both leaves and roots had important functions in the plant hormone signal transduction and MAPK signaling pathway-plant. This indicated that these biological processes played crucial roles in response to drought stress in the grapes lines.

3.5. Heat-map and K-means clustering in grape leaves and roots after drought stress

To determine the grape gene expression profiles during drought stress conditions and to identify DEGs that may have key regulatory roles influencing drought tolerance in grafted vines, we identified 10,503 DEGs based on the RNA-seq data (Appendix J). All of DEGs revealed two conserved groups, up-regulated in roots and down-regulated in leaves, or the opposite expression trend under drought stress (Appendix K). K-means clustering was used to group the 10,503 unique genes into 15 clusters (Appendix K). The clusters of K2 (138 DEGs), K4 (462 DEGs), K6 (862 DEGs), K7 (1,055 DEGs), K10 (539 DEGs) and K12 (1,056 DEGs) were predominantly expressed in roots, and the gene from the K1 (313 DEGs), K5 (891 DEGs), K9 (561 DEGs), K11 (98 DEGs) and K14 (359 DEGs) were expressed firstly in leaves. Furthermore, the expression levels of DEGs in K1 (313 DEGs), K13 (1,301 DEGs) and K15 (934 DEGs) were up-regulated under drought stress compared to the normal condition. In contrast, The 138 DEGs in K2 exhibited down-regulated expression levels under drought stress.

3.6. Gene co-expression network analyses

To classify the gene regulatory network that are associated with drought stress in leaves and roots of self-rooted and grafted grapevine, the weighted correlation network analysis (WGCNA) was performed to identify correlated hub genes or modules using 10,503 transcripts (Fig. 3). A gene cluster scheme was determined with a power value of 6, and hierarchal clustering tree was constructed using Dynamic Hybrid Tree Cut algorithm. All genes were clustered into 11 modules (black, turquoise, orange, greenyellow, brown, darkgreen, darkgrey, blue, salmon, green and lightgreen) (Fig. 3-A and B). The gene numbers in module ranging from 39 to 1,391, the largest number of genes (1,391) fell into the blue module. Modules of black, turquoise, brown, green and lightgreen contained more than 500 genes, and the other 5 modules contained between 39 and 494 genes (Appendix L). The correlations between the modules and 24 samples were recognized. Five intriguing modules were screened for the genes in these modules that were significantly correlated to drought stress. The analysis of the module and samples correlations revealed that the greenyellow had greater correlations with SDS-L, black and turquoise modules were significantly correlated to GDS-L, salmon modules with RDS-R, and blue modules with GDS-R, indicating that the genes in these five modules played important roles in response to drought stress (Fig. 3-B and C). The KEGG pathway functional classifications of each module showed that plant hormone signal transduction (ko04075), MAPK signaling pathway (ko04016), plant Starch and sucrose metabolism (ko00500) were enriched in most drought stress samples, and protein processing in endoplasmic reticulum (ko04141) and plant hormone signal transduction (ko04075) were the most significantly enriched pathway in GDS-L and GDS-R (Fig. 3-D).

3.7. Expression of genes related to ABA and auxin hormone biosynthesis and signaling pathways

Several genes involved in ABA and IAA biosynthesis and signaling pathways were significantly differentially expressed under drought stress (Appendices M, N and O). The drought treatment resulted in the differential expression of 31 DEGs involved in ABA biosynthesis and signaling pathways. Two *ZEPs* (*VIT_18s0001g01110*, *VIT_04s0044g00230*) were

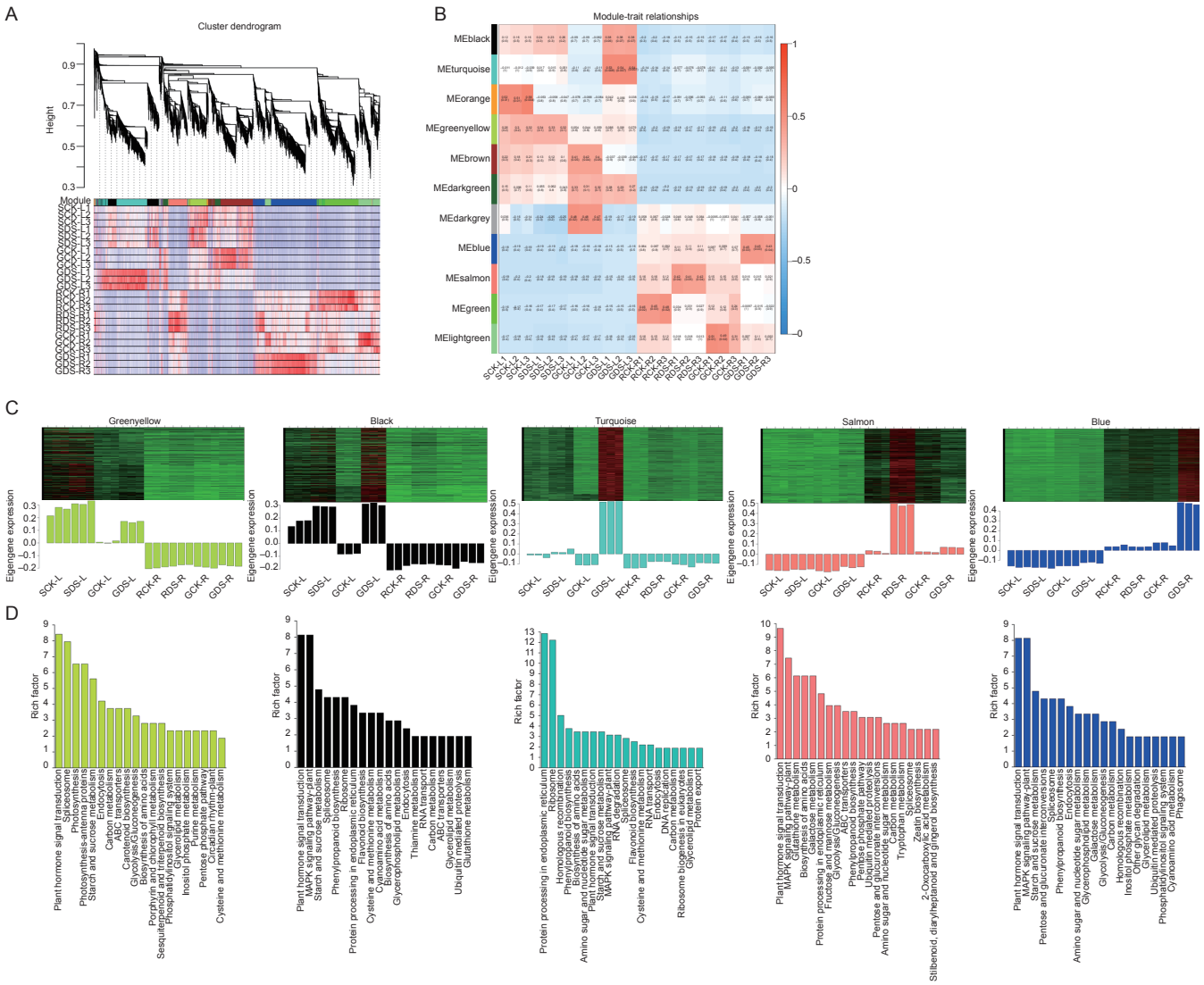


Fig. 3 WGCNA analysis. A, hierarchical clustering tree showing co-expression modules based on WGCNA analysis. Each branch in the phylogenetic tree indicate interconnected gene modules, each leaf corresponds to an individual gene within each module, and each module is color coded. B, module-trait association analysis of expressed genes. Each row corresponds to a consensus module. Each cell contained the corresponding correlation and *P*-value. C, heatmap indicating expression levels of the co-expressed genes in each module. D, the KEGG pathway classification of the DEGs in the five modules corresponding to (C).

up-regulated in GCK-L *vs*. GDS-L, but down-regulated in GCK-R *vs*. GDS-R. Four NCEDs, the committing step in ABA synthesis, were up-regulated induced by GCK-R *vs*. GDS-R. The *PYR/PYL* genes (*VIT_08s0058g00470*, *VIT_13s0067g01940*), which were key factors in the ABA signaling pathway, were up-regulated in SCK-L *vs*. GCK-L and RCK-R *vs*. GCK-R. *PP2Cs* represented the largest number of DEGs, four of which (*VIT_06s0004g05460*, *VIT_11s0016g03170*, *VIT_13s0019g02200*, *VIT_16s0050g02680*) were up-regulated in the SCK-L *vs*. SDS-L and GCK-L *vs*. GDS-L. The expression of a *SnRK2* gene (*VIT_02s0236g00130*) exhibited up-regulation in drought stress in both the leaves and roots. Finally, one *ABF* gene (*VIT_18s0072g00470*) were down-regulated in leaves and roots of self-rooted grape, but up-regulated in grafted grape under drought treatment (Appendix M).

YUCCA flavin monooxygenases (*VIT_03s0038g03180*, *VIT_07s0104g01250*) controls the auxin biosynthesis, were hightly induced in GCK-L *vs*. GDS-L and RCK-R *vs*. RDS-R, respectively. Two *UGT74B1* genes (*VIT_06s0004g07280*, *VIT_17s0000g07790*) were up-regulated in leaves comparison groups. Two *ALDH7A1* genes (*VIT_06s0004g02060*, *VIT_14s0060g02270*) were induced in the GCK-L *vs*. GDS-L and GCK-R *vs*. GDS-R, and three transcripts (*VIT_11s0016g00900*, *VIT_17s0000g08070*, *VIT_01s0026g00220*) were up-regulated in SCK-L *vs*. SDS-L. The auxin receptor *TIR1* gene was induced in RCK-R *vs*. RDS-R. *AUX1* (*VIT_06s0061g01210*, *VIT_08s0007g02030*, *VIT_13s0067g00330*) can promote auxin transport to leaf microtubules, were down-regulated in SCK-L *vs*. SDS-L and GCK-L *vs*. GDS-L, while up-regulated in SCK-L *vs*. GCK-L. The similar expression trend was found in the most of *AUX/IAA* genes, which were down-regulated in GCK-L *vs*. GDS-L and up-regulated in SCK-L *vs*. GCK-L group. ARFs are

important transcription factors which is involved in plant development auxin-mediated response. Most of them were down-regulated in leaves group GCK-L *vs*. GDS-L, but up-regulated in roots group GCK-R *vs*. GDS-R. SAURs are the family of early auxin-responsive genes, which exhibited down-regulation expression in SCK-L *vs*. SDS-L and GCK-L *vs*. GDS-L, but up-regulated in SCK-L *vs*. GCK-L and SDS-L and GDS-L (Appendix M). In conclusion, dynamic changes of DEGs from the ABA and IAA signaling pathway played a crucial role in response to drought stress in grafted grape.

3.8. Expression of genes related to MAPK signaling pathways

Among 10,503 DEGs affected by drought , a total of 60 DEGs were involved in MAPK pathways, of which 18, 11, 17, 8 and 6 genes were involved in the subpathways of ehylene biosynthesis, jasmonicacid acid biosynthesis, abscisic acid biosynthesis, Ca^{2+} signaling pathway and EPF signaling pathway, respectively (Appendix P). For the ehylene biosynthesis pathway, the expression level of *ETR/ERS* gene was up-regulated induced by SDS-L *vs*. GDS-L. Seven *CTR1* genes were differentially expressed: two genes (*VIT_05s0051g00660*, *VIT_19s0085g00550*) were significantly up-regulated in GCK-L *vs*. GDS-L group, and down-regulated in SCK-L *vs*. GCK-L, four genes (*VIT_01s0011g01490*, *VIT_05s0094g01080*, *VIT_11s0052g01480*, *VIT_18s0001g07700*) exhibited the opposite expression trend in the two comparison groups. Similarly, *MKK9* and *EIN3* exhibited down-regulated expression levels in GCK-L *vs*. GDS-L, and up-regulated expression in SCK-L *vs*. GCK-L and GCK-R *vs*. GDS-R. Interestingly, the expression levels of *ERF1* (*VIT_07s0005g03230*) were down-regulated in roots comparison groups, and another *ERF1* (*VIT_05s0049g00510*) were up-regulated in leaves groups. In addition, three of *ChiBs* (*VIT_04s0008g00140*, *VIT_05s0062g01320*, *VIT_05s0077g01250*) had similar expression that were down-regulated in SCK-L *vs*. SDS-L and GCK-L *vs*. GDS-L groups but up-regulated in SDS-L *vs*. GDS-L group. As for the Jasmonicacid acid biosynthesis pathway, the basic helix-loop-helix (bHLH) transcription factor *MYC2* genes (*VIT_02s0012g01320*, *VIT_07s0141g01060*, *VIT_15s0046g00320*, *VIT_15s0048g02820*, *VIT_15s0046g02560*) involved in the modulation of diverse jasmonate signaling of plants, had down-regulated expression levels in GCK-L *vs*. GDS-L. Differently, three other *MYC2* genes (*VIT_00s0927g00010*, *VIT_11s0052g00100*, *VIT_18s0001g08040*) were significantly down-regulated in SCK-L *vs*. SDS-L, and up-regulated in SDS-L *vs*. GDS-L. For the abscisic acid biosynthesis pathway, the expression of two *MAPKKK17/18* (*VIT_07s0031g00530*, *VIT_19s0090g00820*) were down-regulated in GCK-L *vs*. GDS-L group, while up-regulated in GCK-R *vs*. GDS-R, showing the different expression pattern in leaves and roots. As to the Ca^{2+} signaling pathway, three *CaM4* genes had significantly expression levels in leaves groups, which one transcript (*VIT_04s0069g00960*) were up-regulated and two genes (*VIT_05s0077g00810*, *VIT_14s0108g01000*) were down-regulated in GCK-L *vs*. GDS-L. The expression level of *MPK8* (*VIT_01s0011g04920*) were significantly up-regulated in GCK-R *vs*. GDS-R group. The *RbohD* genes (*VIT_19s0014g02830*) were up-regulated by drought stress in grafted grape in both the leaves and roots. In the EPF signaling pathway, four *MKK4/5* genes had similar expression pattern, which exhibited down-regulated expression levels in leaves comparison groups but up-regulated expression level in roots comparison groups.

3.9. Transcription factor genes (TFs) under drought stress

To investigate the regulatory networks of drought stress in self-rooted and grafted grape, we identified transcription factor genes that were differentially expressed in the leaves and roots (Fig. 4; Appendices Q and R). A number of 916 TFs belonging to different gene families were differentially expressed in grape leaves and roots. The AP2, bHLH, MYB, NAC, WAKY, C2H2, bZIP, HSF families of TFs have been previously identified to response to drought stress, of which AP2 (114; 12%), bHLH (82; 9%), MYB (81; 9%) and NAC (58; 6%) were the most identified DEGs (Fig. 4-A). As shown in Fig. 4-B, 213 and 281 TF genes were differentially expressed in the leaves and roots of grape, and the common genes were 211. Most of *AP2/ERF-ERF* were down-regulated in GCK-L *vs*. GDS-L, and up-regulated in SCK-L *vs*. GCK-L and SDS-L *vs*. GDS-L. Additionally, a similar expression levels trend were observed in *C2H2* genes. The expression level of *MYB* gene (*VIT_07s0005g02480*) was up-regulated in the roots of 4 comparison groups, while in leaves exhibited the opposite expression pattern. Five of *NAC* genes (*VIT_19s0014g03290*, *VIT_19s0027g00230*, *VIT_10s0003g00350*, *VIT_15s0048g02290*, *VIT_15s0048g02320*) were down-regulated in GCK-L *vs*. GDS-L, while up-regulated in GCK-R *vs*. GDS-R, indicating that different plant tissue showed multiple expression pattern in grafted grape. Four members of the *HSF* family genes (*VIT_01s0011g05970*, *VIT_08s0007g03900*, *VIT_00s0179g00150*, *VIT_04s0008g01110*) exhibited significantly up-regulated expression in GCK *vs*. GDS both leaves and roots after drought treatment. Furthermore, the *HSF* gene (*VIT_02s0025g04170*) were up-regulated in leaves and down-regulated in roots groups. In addition, eight of *bHLH* genes (*VIT_17s0000g08150*, *VIT_15s0046g00320*, *VIT_11s0037g00040*, *VIT_00s0927g00010*, *VIT_06s0004g01740*, *VIT_15s0048g02820*, *VIT_14s0006g02850*, *VIT_12s0028g01110*) were significantly down-regulated in GCK-L *vs*. GDS-L, but up-regulated in GCK-R *vs*. GDS-R. Finally, most of genes belonging to *WAKY* families exhibited the similar expression levels, which were down-regulated in SCK-L *vs*. SDS-L and GCK-L *vs*. GDS-L, up-regulated in other three comparison groups (SDS-L *vs*. GDS-L, GCK-R *vs*. GDS-R, RDS-R *vs*. GDS-R) (Fig. 4-C). Taken together, the results indicated that various differentially expressed TFs help regulating diverse biological processes in response to drought stress of grapevine lines.

3.10. Expression verification of DEGs by qRT-PCR

Twelve drought stress responsive DEGs were randomly selected to verify the reliability of transcriptome data by

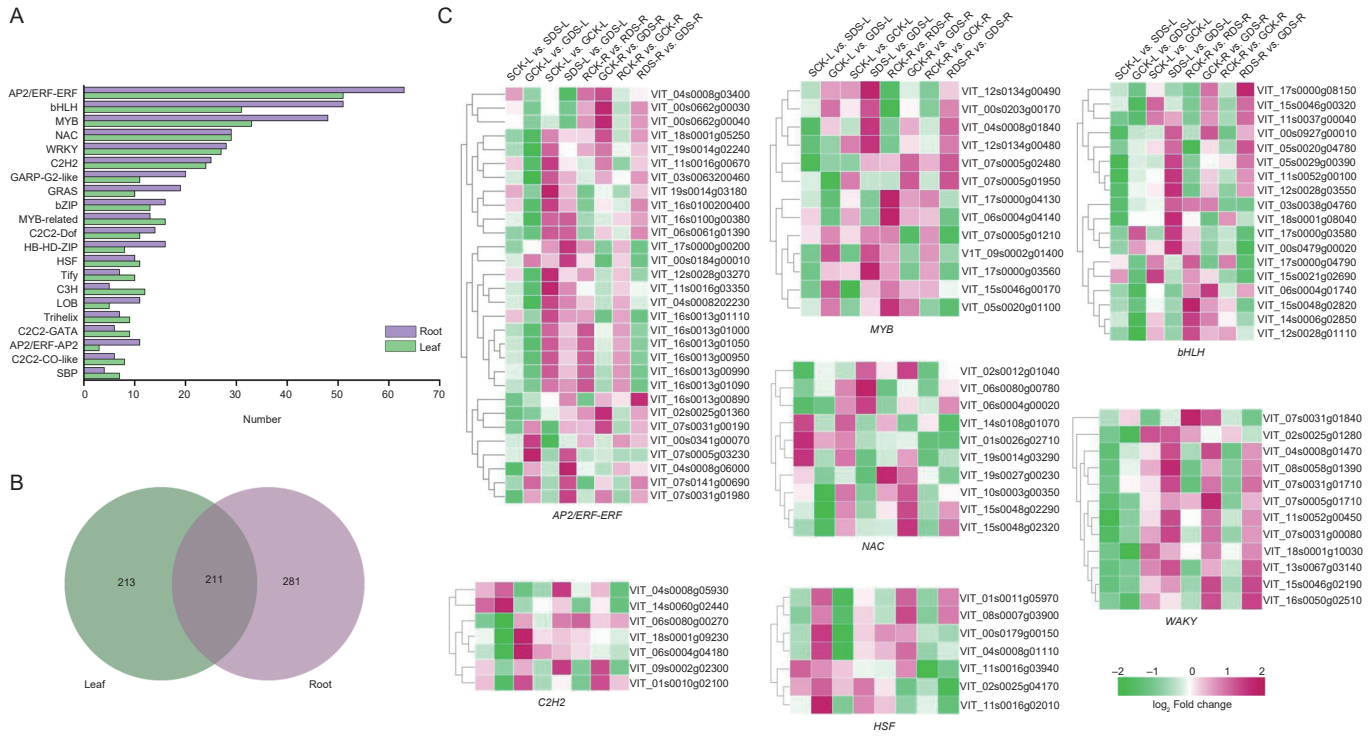


Fig. 4 Transcription factors (TF) were differentially expressed under drought stress in self-rooted and grafted vines. A, the number of predicted transcription factors. B, Venn diagram showing TFs among the differentially expressed genes (DEGs) in grape leaves and roots. C, heatmap of the common differentially expressed TFs.

qRT–PCR (Appendices S and T), including *MYBPA1* (*VIT_15s0046g00170*), *MYB94* (*VIT_17s0000g06190*), *HB7* (*VIT_15s0048g02870*), *ERF61* (*VIT_02s0025g01360*), *DEAR3* (*VIT_07s0031g00190*), *XER* (*VIT_12s0057g01330*), *THI1* (*VIT_19s0177g00140*), *AFP3* (*VIT_14s0068g00300*), *PRP* (*VIT_02s0154g00280*), *HSP18.1* (*VIT_13s0019g03000*), *HSP18.2* (*VIT_13s0019g02840*) and *HSP22* (*VIT_12s0035g01910*). The results showed that the expression patterns were consistent with the RNA-Seq trends, which indicated that the RNA-Seq results were credible and reproducible.

3.11. *VvMYBPA1* transgenic seedlings increased resistance to drought stress

Transcriptome data showed that the expression of *VvMYPA1* is significantly up-regulated in leaves and roots of grafted grape under drought stress condition. To further investigate the function of *VvMYBPA1* in response to drought stress, we overexpressed the *VvMYBPA1* in *Arabidopsis thaliana* using the pCAMBIA2300, and an empty vector (EV) used as control. Validation of the transgenic *A. thaliana* was performed by PCR and qRT-PCR, and the expression of *VvMYBPA1* was higher in *VvMYBPA*-overexpressing lines than WT and EV lines (Appendix U). To investigate the role of *VvMYBPA1* in response to drought stress, the seed germination and green cotyledons were examined supplementing with different concentrations of mannitol (0, 50, 100, 150 and 200 mmol L^{-1}). The results showed that the germination rate on 0 mmol L^{-1} media have no difference, however, the germination rate of transgenic lines was significantly higher than WT at different concentration for mannitol (Fig. 5-A and B). In addition, the green cotyledons rates of the *VvMYBPA1*-overexpression lines were higher than that of WT seeds at 100,150 and 200 mmol L^{-1} mannitol (Fig. 5-C). Furthermore, the primary root growth of transgenic and WT *Arabidopsis* was measured at different concentrations of mannitol (0, 100, 200 and 300 mmol L^{-1}). The results showed that there was no significant difference between transgenic and WT lines on 0 mmol L^{-1} mannitol medium, whereas the root length of *VvMYBPA1*-overexpressing was longer than that of WT seedlings under 100, 200 and 300 mmol L^{-1} mannitol treatment (Fig. 5-D and E).

3.12. Overexpression of *VvMYBPA1* increased ABA sensitivity

To investigate whether the drought tolerance of *VvMYBPA1* was by regulating ABA signal transduction, WT and *VvMYBPA1*-overexpressing seeds were planted on 1/2 MS medium containing different concentrations (0, 0.2, 0.3 and 0.5 μmol L^{-1}) ABA for evaluating the germination percentages, green cotyledons, root growth, and stomatal apertures. As shown in Fig. 6-A–C, there was no significant difference of the germination rate and green cotyledons between WT and transgenic lines on 0 μmol L^{-1} ABA medium. The germination rates and green cotyledons of transgenic *Arabidopsis* showed hypersensitivity to ABA on the medium supplemented with different concentrations ABA. The primary root length of *VvMYBPA1*-overexpressing was less than that of WT on medium containing 0.5 and 1.0 μmol L^{-1} ABA (Fig. 6-D and E).

Furthermore, 3-week-old rosette leaves of *Arabidopsis thaliana* were collected and submerged in stomatal opening

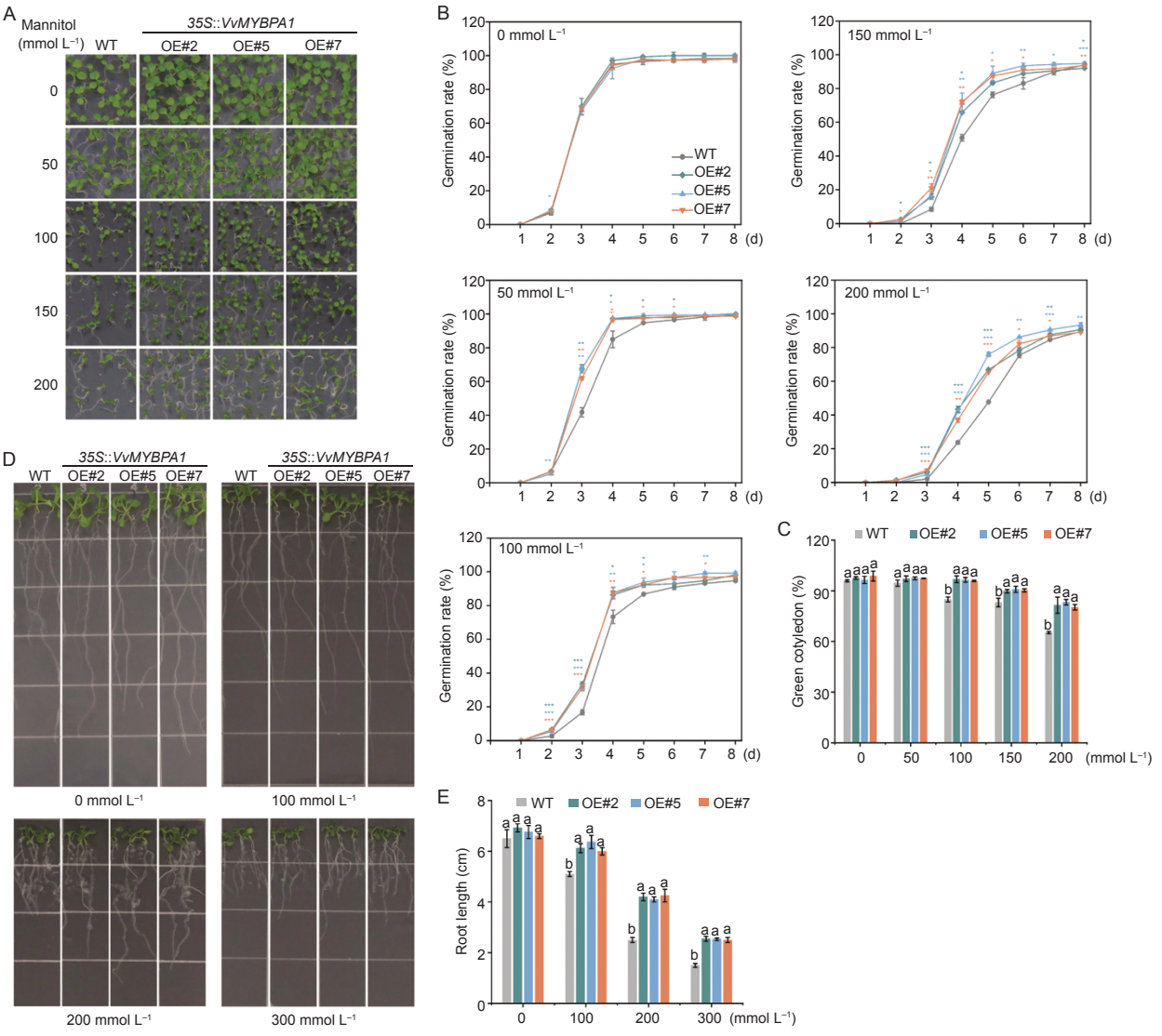


Fig. 5 Germination, cotyledon greening and root growth analysis of *VvMBYPA1*-overexpressing and wild-type (WT) lines under different concentrations of mannitol. A, photographs of seeds from WT and transgenic lines cultivated on MS medium containing different concentrations of mannitol (0, 50, 100, 150, 200 mmol L^{-1}). B, germination rate. C, statistics of green cotyledons from transgenic and WT seedlings in 10 d. D, photographs of seeds from WT and transgenic lines were grown for 5 d on MS medium and transferred to MS medium containing different concentrations of mannitol (0, 100, 200, 300 mmol L^{-1}). E, statistical analysis of root length of seedlings. Asterisks (B) and lowercase letters (C, E) denote significant differences between overexpression lines and WT based on Duncan's test (P<0.05).

solution with different concentrations of ABA (0, 1, and 10 μmol L^{-1}) to observe stomatal closure. As shown in Fig. 6-F and G, the stomatal pore size (width/length) of WT and transgenic lines were approximate 0.47 in the absence of ABA. With the increase of ABA concentration, the stomatal aperture was reduced continuously. In the presence of 1.0 μmol L^{-1} ABA, the stomatal pore size of transgenic and WT *Arabidopsis* was 0.28 and 0.37, respectively. The pore size of transgenic *Arabidopsis* was 0.17, while that of WT was 0.27 in the presence of 10 μmol L^{-1} ABA. Thus, these data suggested that *VvMYBPA1*-overexpressing increased drought tolerance by promoting stomatal closure and reducing water loss.

3.13. Overexpression of *VvMYBPA1* enhanced the drought stress tolerance in *Arabidopsis*

To further investigate the function of *VvMYBPA1* response to drought stress, we subjected 3-week-old WT, (EV) empty vector and *VvMYBPA1*-OE (#2, #5 and #7) plants to drought stress by withholding water for 14 d, followed by re-watering. The results showed that WT and EV lines displayed a more wilted phenotype than *VvMYBPA1*-OE lines after drought stress (Fig. 7-A). After re-watering for 3 d, only approximately 35% of WT and 40% of EV plants were survived, while most of transgenic plants showed a high survival rates (OE#2 and #5 was 93%, #7 was 86%) (Fig. 7-B). We also measured the relative water loss of rosette leaves of 3-week-old plants, and observed that *VvMYBPA1*-OE plants lost water more slowly than that of WT and EV plants (Fig. 7-C). In addition, *VvMYBPA1*-OE plants exhibited higher relative water content, Chlorophyll content, proline content and soluble protein, but lower electrolyte leakage and MDA content under drought stress than did the WT and EV plants (Fig. 7-D–I). To

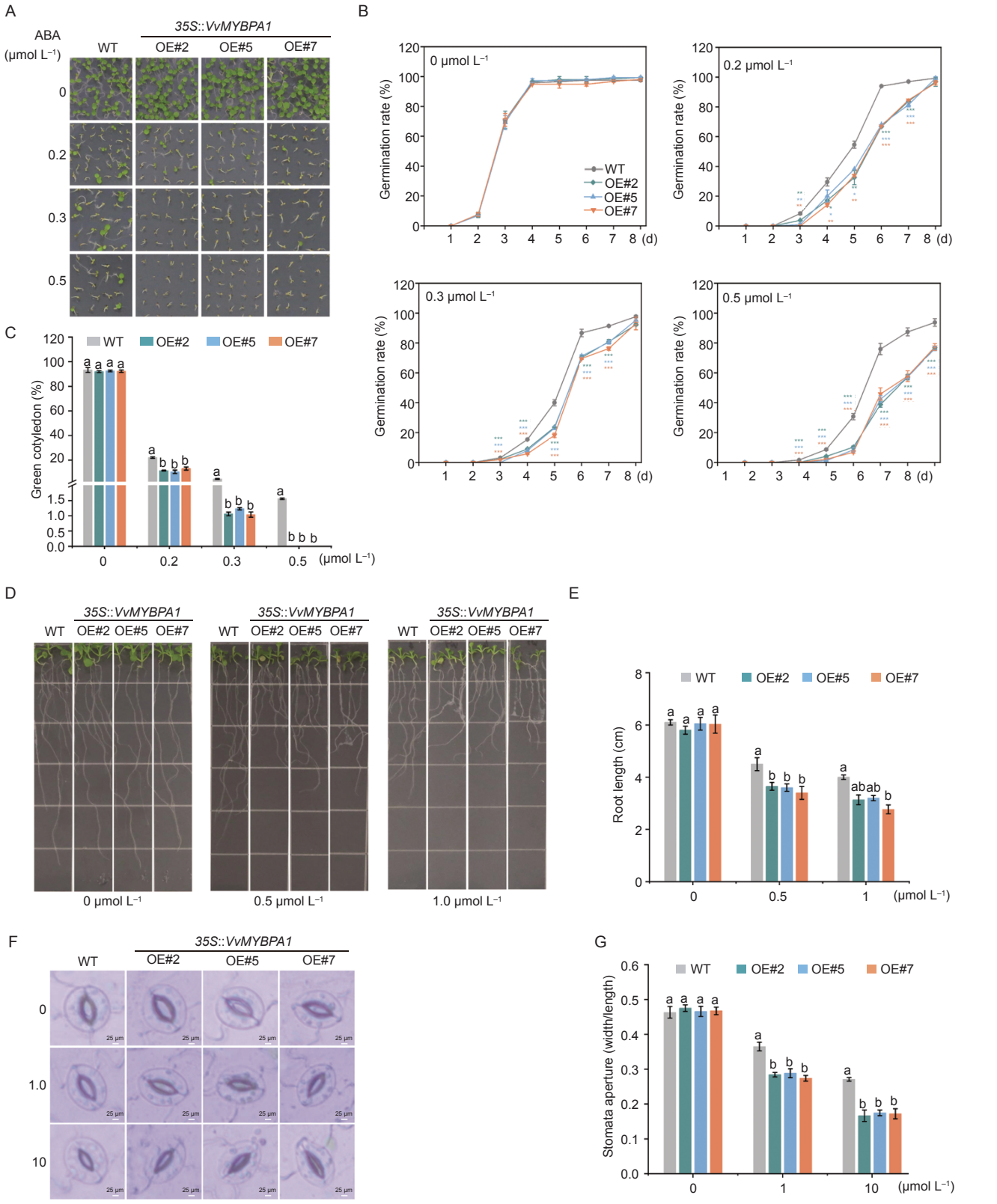


Fig. 6 ***VvMYBPA1*** **increased abscisic acid (ABA) sensitivity in** ***Arabidopsis***. A, photographs of seeds from wild-type (WT) and transgenic lines cultivated on MS medium containing different concentrations of ABA (0, 0.2, 0.3, 0.5 μmol L^{-1}). B, germination rate. C, statistics of green cotyledons from transgenic and WT seedlings in 10 d. D, photographs of seeds from WT and transgenic lines grown for 5 d on MS medium and transferred to MS medium containing different concentrations of ABA (0, 0.5, 1.0 μmol L^{-1}). E, statistical analysis of root length of seedlings. F, stomatal phenotype of WT and *VvMYBPA1*-overexpressing *Arabidopsis thaliana*. The rosette leaves of *Arabidopsis* were collected and immersed in stomatal opening solution for 4 h and in 0, 1.0, 10 μmol L^{-1} ABA solution for another 2 h, observed and photographed under an optical microscope. Bars=25 μm. G, measurement of stomatal aperture. Stomatal apertures of WT and transgenic leaves were measured as the ratio of width to length by ImageJ software. Asterisks (B) and lowercase letters (C, E, G) denote significant differences between overexpression lines and wild-type based on Duncan's test (P<0.05).

determine the effects of *VvMYBPA1* on ROS accumulation under drought stress, we determined H_2O_2, $O_2^{\cdot-}$, antioxidant enzyme activity and GSH content in WT, EV and transgenic *Arabidopsis*. The results showed that *VvMYBPA1*-OE plants exhibited lower H_2O_2 and $O_2^{\cdot-}$ contents, while higher antioxidant enzyme activity and GSH contents than that of WT and EV plants (Fig. 7-J–O). These data suggested overexpression of *VvMYBPA1* reduced the accumulation of H_2O_2 and $O_2^{\cdot-}$, increased more antioxidant substances to scavenge ROS and improve the drought tolerance of plants.

We further examined the expression levels of ABA and stress-responsive genes (*ABI1*, *ABI5*, *ABF3*, *ABF4*, *DEEB2A*, *NCED3*, *RD22*, *RD29A*, *KINI1*, *COR15A*, and *RAB18*) in 3-week-old WT, EV and transgenic plants under control and drought conditions. The expression levels of most genes showed no difference among WT, EV and *VvMYBPA1*-OE plants under normal conditions (0 d), while they were rapidly induced and the expression levels in *VvMYBPA1*-OE were significantly higher than in WT and EV plants after drought stress for 14 d (Appendices V and W).

3.14. *VvMYBPA1* regulates drought resistance in grape callus

To investigate the roles of *VvMYBPA1* in response to drought stress, *VvMYBPA1*-OE grape calli were generated and treated with 4% (w/v) PEG6000. As shown in Fig. 8-A, the *VvMYBPA1*-OE calli exhibited faster growth and better phenotype than wild type after 20 d of PEG treatment. The fresh weight and the transcript levels of *VvMYBPA1*-OE calli were significantly higher than WT after PEG treatment (Fig. 8-B and C). In addition, the contents of MDA, H_2O_2 and $O_2^{\cdot-}$ in WT calli were increased sharply compared

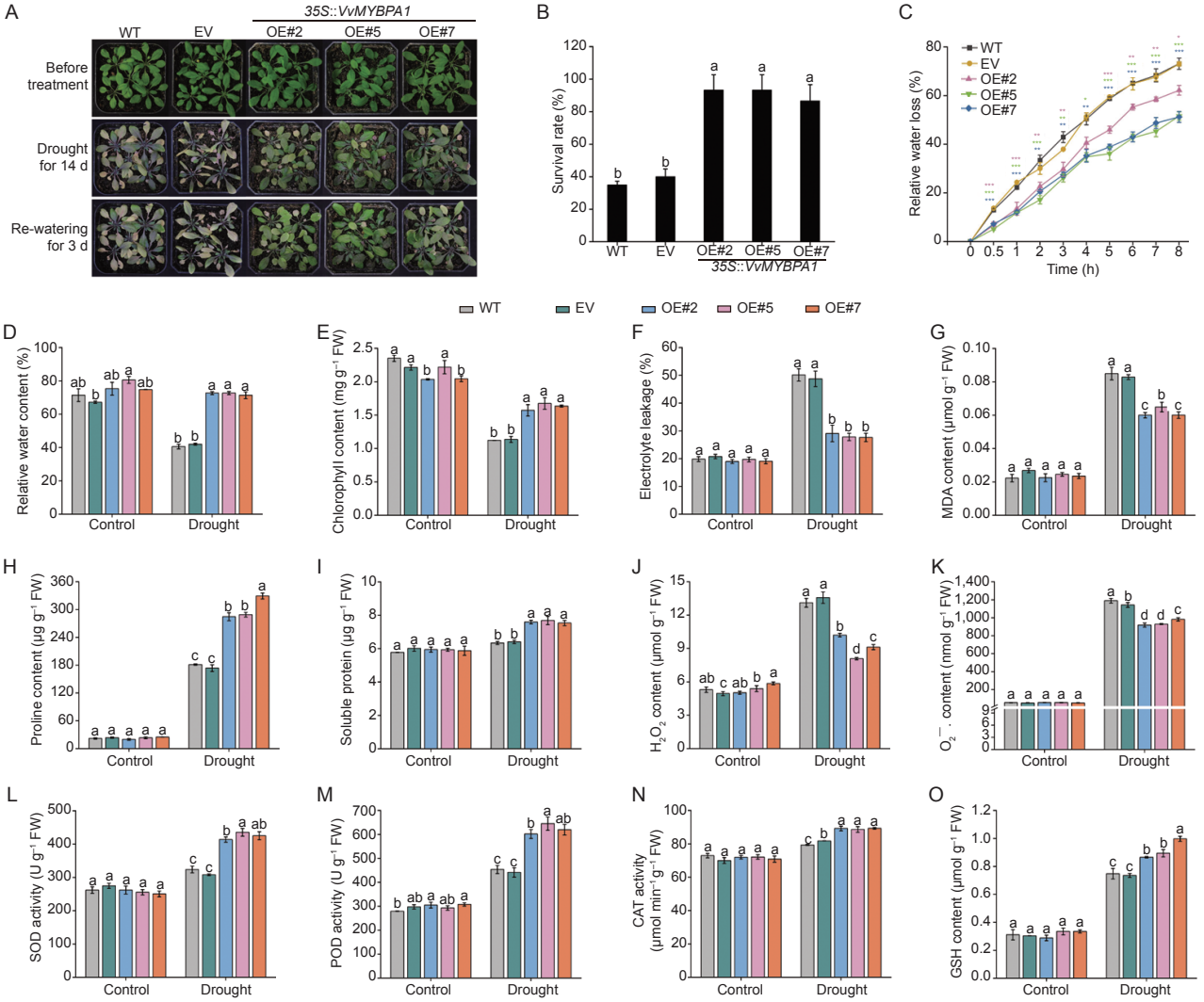


Fig. 7 *VvMYBPA1*-overexpressing in *Arabidopsis thaliana* enhanced drought resistance. A, phenotype of wild-type control (WT), pCAMBIA2300 empty vector transgenic lines (EV) and three *VvMYBPA1*-overexpressing lines (OE#2, OE#5, and OE#7) before and after of drought stress treatment. B, survival rate. C, leaf water loss rate. D, leaf relative water content. E, chlorophyll content of leaves. F, electrolyte leakage. G, malondialdehyde (MDA) content. H, proline content. I, soluble protein content. J, H_2O_2 content. K, $O_2^{\cdot-}$ content. L, superoxide dismutase (SOD) activity. M, peroxidase (POD) activity. N, catalase (CAT) activity. O, glutathione (GSH) content. In (B), the values are the means±SD (n=60), in (C–O) the values are the means±SD (n=3). Asterisks and lowercase letters denote significant differences between overexpression lines and WT based on Duncan's test (P<0.05).

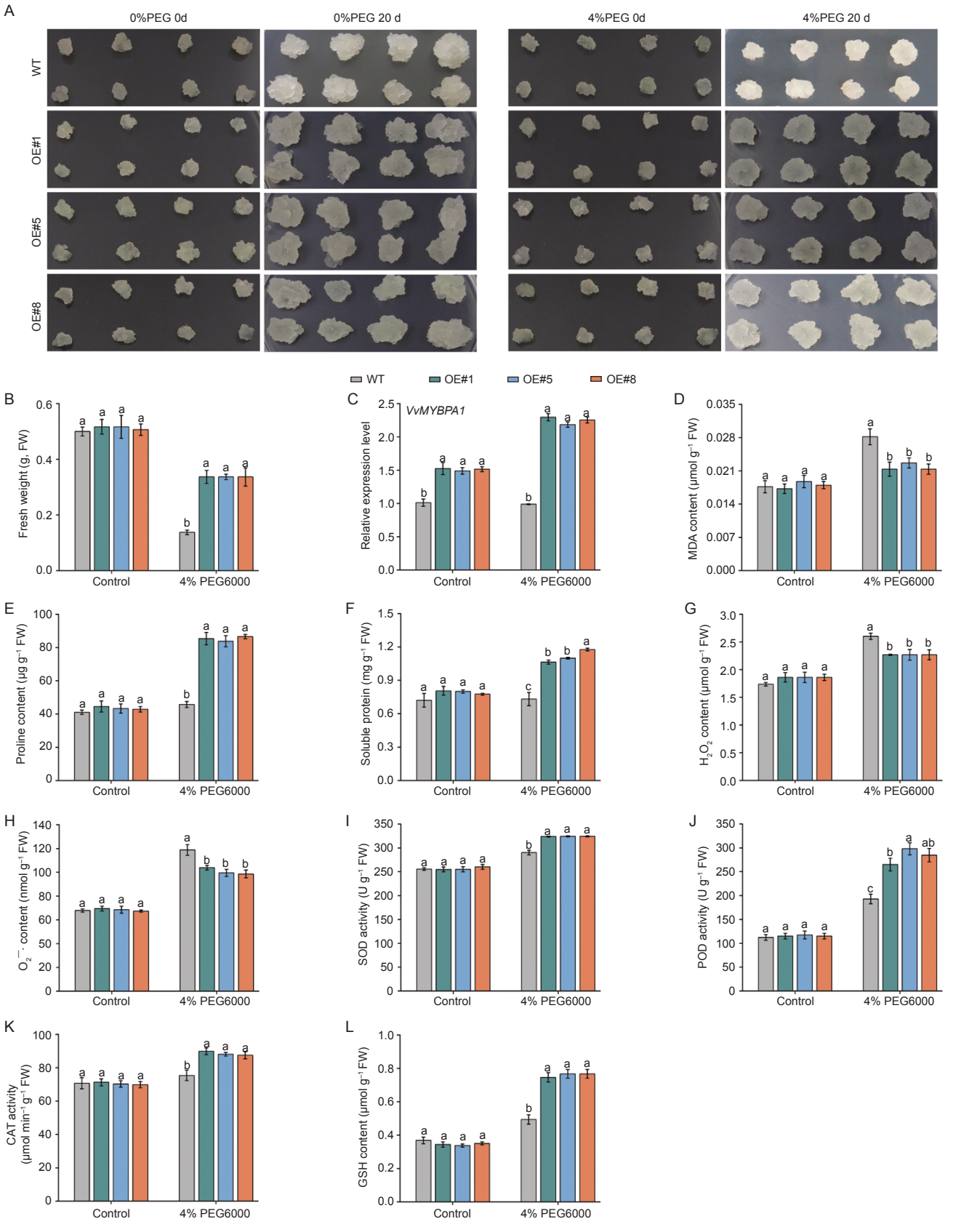


Fig. 8 *VvMYBPA1*-overexpressing in grape calli enhanced drought resistance. A, phenotypic changes of wild-type (WT) and *VvMYBPA1* OE grape 'Pinot Noir' calli (OE#1, OE#5 and OE#8) before and after 4% PEG treatment. B, the fresh weight. C, the expression levels of *VvMYBPA1*. D, malondialdehyde (MDA) content. E, proline content. F, soluble protein content. G, H_2O_2 content. H, $O_2^{\cdot-}$ content. I, superoxide dismutase (SOD) activity. J, peroxidase (POD) activity. K, catalase (CAT) activity. L, glutathione (GSH) content. Lowercase letters denote significant differences between overexpression lines and wild-type based on Duncan's test (P<0.05).

with *VvMYBPA1*-OE after PEG treatment. Meanwhile, the contents of proline, soluble protein, GSH and three antioxidant enzyme activity (SOD, POD and CAT) exhibited higher level in *VvMYBPA1*-OE calli compared with WT calli (Fig. 8-D–L).

Next, we tested whether overexpression of *VvMYBPA1* in calli affected ABA and stress-related genes expression. The results showed that the levels of these genes were also much higher in *VvMYBPA1*-OE calli than in WT after PEG treatment (Appendices X and Y). Our findings suggests that *VvMYBPA1* stimulated the expression of ABA and drought-stress genes, which is speculated that *VvMYBPA1* enhance drought resistance in grape calli by participating in ABA signal pathway.

4. Discussion

Gafting in grapevine can improve environmental adaptation and protect against abiotic stress. According to our observations, grafting of the SM scion onto the 1103P rootstock had a positive effect on drought tolerance. However, there are relatively few studies evaluating the effects of rootstock on grafted vines and its possible mechanism under drought conditions. In this research, leaf samples from self-rooted SM and grafted SM/1103P plants, along with root samples from self-rooted 1103P and grafted SM/1103P plants, were used for transcriptomic analysis to investigate the response of self-rooted and grafted grapevines to drought stress.

We obtained a total of 23,052 DEGs from 24 cDNA libraries, including 10,886 upregulated and 12,166 downregulated genes.

4.1. The roles of ABA and IAA in mediating grapevine drought tolerance

Phytohormones, a diverse group of signaling molecules produced in very low concentrations in cell, play key roles in signal transduction pathways in response to various internal and external stimuli (Wani *et al.* 2016; Pandey *et al.* 2017). ABA, plays a significant role in many physiological processes of plants, including maintenance of seed dormancy, stomatal closure, embryo morphogenesis, fruit ripening and mediating abiotic and biotic stress responses (Li *et al.* 2025). ABA is considered an essential messenger for orchestrating the drought stress response. This is supported by the facts that endogenous ABA levels increase rapidly in response to drought stress (Yoshida *et al.* 2014). Both in roots and leaves shown higher ABA levels after drought stress in our research, indicating that accumulation of ABA increased drought tolerance of grape (Fig. 1-A). Drought stress enhances the expression of numerous genes in ABA signaling pathway (Zareen *et al.* 2024). Consistent with the physiological data of ABA levels in response to drought stress conditions, 31 DEGs involved in ABA biosynthesis and signaling pathways were identified in leaves and roots (Appendix N). Several ABA biosynthesis genes, including *ZEP*, *NCED*, *PYR/PYL*, *PP2C*, *SnRK2* and *ABF* genes were altered both in leaves and roots under drought stress (Appendix M). These genes may regulate downstream transcription factors or functional genes, and play critical roles in drought stress response in grape. IAA is a vital phytohormones of plant growth and development throughout the life cycle of plants, which coordinated plant growth under stress conditions (Zhuang *et al.* 2025). Interestingly, there was evidence showed that IAA levels increased root and shoot growth under abiotic stress, and the IAA levels were dramatically altered in plants under drought condition (Li *et al.* 2020). We found that IAA content was increased in self-rooted and grafted grapevines after drought stress, and the IAA content accumulated more in roots compared with leaves, which indicated that IAA as a regulator of root water transport played crucial roles under drought stress (Fig. 1-B) (Zhao *et al.* 2012). Auxin induces rapid and efficient expression of specific genes, such as *Auxin/Indole-3-Acetic Acid* (*Aux/IAA*), *Gretchen Hagen 3* (*GH3*), *small auxin-up RNA* (*SAUR*) (Bao *et al.* 2024). In general, *Aux/IAAs* are auxin-sensitive repressors that bind to the *ARFs* to mediate the plant development (Salehin *et al.* 2015; Weijers and Wagner 2016). *AUX1*, *AUX/IAA*, *ARF* and *SUAR* genes were down-regulated in grafted grape leaves, indicating that these genes showed the similar regulated processes under drought stress (Appendix M). In a word, these results indicated that the dynamic changes of IAA content was important in regulating stress-responsive genes expression in grape.

4.2. Transcriptional regulation of drought response in grapevine: Signaling pathways and key transcription factors

For DEGs, We obtained a total of 23,052 DEGs from 24 cDNA libraries, including 10,886 up-regulated and 12,166 down-regulated genes (Fig. 2-B). Many previous studies have found that DEGs involved in drought stress related to plant hormone signal transduction and MAPK signaling pathway (Dudhate *et al.* 2018; Li *et al.* 2020). In our study, KEGG analysis revealed that 1,697 DEGs were enriched in hormone signal transduction, MAPK signaling pathway-plant and phenylpropanoid biosynthesis both in leaves and roots (Appendix I), indicating that these processes were important for grape in response to drought stress. TFs, broadly categorized as early-induced genes, play important roles in response to drought stress (Wang *et al.* 2016).We identified 916 TFs that were differentially expressed in grape leaves and roots (Fig. 4). Amongst the genes of the TFs, the *AP2/ERF* were the most abundant genes, indicating that they play important roles in regulating plant growth and responses to drought stress. For instance, *DREB2* subfamily of the *AP2/ERF* TFs exhibited drought tolerance in transgenic *Arabidopsis* (Sakuma *et al.* 2006). In our study, the expression levels of most *AP2/ERF* were up-regulated in SCK-L *vs*. GCK-L and SDS-L *vs*. GDS-L, this is consistent with the previous study (Sakuma *et al.* 2006). Most *bHLH* genes exhibited down-regulated expression levels in grape leaves. Similarly, recent studies shown that *bHLH6*, *bHLH65*, *bHLH152*, and *bHLH185* in rice were significantly down-regulated after drought treatment (Wei and Chen 2018). Additionally, NAC factors are known to play crucial roles in diverse development processes and stress responses (Wang L *et al.* 2017). In particular, ectopic expression of maize *ZmNAC55* in transgenic *Arabidopsis* improved drought

tolerance (Mao *et al.* 2016). Similarly, WAKY, C2H2 and HSF TFs were strongly induced by drought stress, revealing their role in drought response mechanisms (Kielbowicz-Matuk 2012; Guo *et al.* 2016; Zhang L *et al.* 2018).

4.3. *VvMYBPA1* confers drought resistance by regulating ABA signaling, osmoprotection, and ROS scavenging

The MYB protein is a group of large transcription factors, and widely present in plants. Recent reports show that MYB proteins have been found to play important roles in response to drought stress (Zhang *et al.* 2025). There are 138 MYB Transcription factors in grapes (data from Plant Transcription Factor Database), yet the function of MYBPA1 in response to drought stress is little known. Here, the *VvMYBPA1* gene was screened based on transcriptomic data and qRT-PCR analysis. Overexpression of *VvMYBPA1* in *Arabidopsis* displayed a higher seed germination rate, cotyledon greening and root length than wild type lines under mannitol treatment, indicating that transgenic plants enhanced drought resistance (Fig. 5). The ABA sensitivity test showed that transgenic lines was more sensitive to ABA compared to WT, which was speculated that *VvMYBPA1* promoted stomatal closure by participating in ABA signal pathway, reducing water loss, and thus enhancing drought tolerance (Fig. 6). Phenotypic changes directly reflect the strength of drought resistance of plants under drought stress. In this study, overexpression of *VvMYBPA1* in *Arabidopsis* and grape 'Pinot Noir' calli showed a better phenotype than wild type after drought treatment, indicating that *VvMYBPA1* positively regulated the drought tolerance (Figs. 7-A; 8-A). Leaf water content and chlorophyll can effectively reflect the drought resistance of plants (Anjum *et al.* 2011; Meng *et al.* 2014). Transgenic *Arabidopsis* exhibited the higher leaf relative water content and chlorophyll content in our work, indicating that *VvMYBPA1* increased drought resistance (Fig. 7-D and E).

The parameters of H_2O_2 and MDA are regarded as indicators of environmental injury severity in plants (Mittler 2002; Liu *et al.* 2014; Chen *et al.* 2018). Drought stress result in the accumulation of reactive oxygen species (ROS), which produce H_2O_2 and MDA, lead to cell membranes and even cell death. In this study, drought treatment significantly increased the contents of H_2O_2 and MDA, and a lower H_2O_2 and MDA contents were observed in *VvMYBPA1*-overexpressing plants compared to WT, indicating that transgenic plants reduced oxidative stress. In addition, plants reduce the osmotic pressure of cytoplasm by accumulating osmoregulatory substances such as proline or soluble protein in response to drought stress (Strange 1994). Under drought stress, overexpression of *VvMYBPA1* in *Arabidopsis* and grape calli produced higher content of proline and soluble protein than those of WT, indicating that *VvMYBPA1* positively regulates drought stress by enhancing osmoregulatory substances. To overcome the effects of oxidative stress, the antioxidant defense system, such as SOD, POD, CAT were activated, while when compared with WT, higher levels of SOD, POD , CAT activities and GSH contents were measured in transgenic plants, indicating that overexpression of *VvMYBPA1* could promote the scavenging of ROS and reduce the lipid peroxidation under drought treatment (Figs. 7 and 8). The similar phenomenon was also reported in other plants under drought stress (Rahimi *et al.* 2016; Zhao *et al.* 2019). Many genes and signaling pathways are involved in drought stress in plants. ABFs, the basic leucine zip-type (bZIP) transcription factors, which are involved in ABA signal transduction, among them ABF2, ABF3, ABF4 play important roles in ABA-dependent stress signaling pathways (Jakoby *et al.* 2002; Yoshida *et al.* 2014). ABI1 and ABI5 are regulators of ABA signaling pathways and play key regulatory roles in abiotic stress (Finkelstein and Lynch 2000; Chen *et al.* 2020). Up-regulation of *ABI5* induces the expression of downstream genes, including *RD22*, *RD29B*, *COR15A*, *KINI*, and *RAB18*. In this study, the expression levels of ABA signaling pathway (*ABI1/5*, *ABF2/3/4*, *RD22*, *RD29A/B*, *KINI1/2*, *COR15A* and *RAB18*) were higher in *VvMYBPA1*-OE plants than WT after drought stress, suggesting that *VvMYBPA1* induced the expression of these genes and enhanced drought resistance (Appendices V and X). Moreover, *NCED1/3*, *DREB2A*, *ERD1/14* and *CBF4* genes also play important roles in response to stress (Yoshida *et al.* 2014). We found that the expression levels of these genes were up-regulated after drought stress and exhibited a significantly higher levels in *VvMYBPA1* overexpressed materials (Appendices V and X). These results indicated that *VvMYBPA1* enhanced plant drought resistance by regulating the expression of ABA signaling pathway genes.

5. Conclusion

In summary, we analyzed hormone levels, contents of H_2O_2 and MDA, and activity of antioxidant enzymes, identified DEGs, TFs and signaling pathways in leaves and roots of self-rooted and grafted grapevines under drought stress condition**s** through RNA-seq analysis. Our findings shed light on the role of hormone and MAPK signaling pathways as the major gene modules that control grape growth and development under drought stress. Then, the function of *VvMYBPA1* was verified by transforming *Arabidopsis thaliana* and grape calli, which is conducive to drought resistance. The transcriptional changes in grapes and functional validation of *VvMYBPA1* provide a theoretical basis for future mechanistic and functional studies on the response of plants to drought stress.

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Declarations of competing interest

The authors declare that they have no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that they did not use AI in the preparation

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