



Research article

Alleviation of drought stress in grapevine by foliar-applied strigolactones

Zhuo Min^a, Runyu Li^a, Li Chen^a, Yang Zhang^a, Ziyu Li^a, Min Liu^a, Yanlun Ju^a, Yulin Fang^{a,b,c,*}^a College of Enology, Northwest A & F University, Yangling, 712100, Shaanxi, China^b Shaanxi Engineering Research Center for Viti-Viniculture, Yangling, 712100, Shaanxi, China^c Heyang Grape Station of Experimental Demonstration, Northwest A & F University, Weinan, 715300, Shaanxi, China

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ABSTRACT

Drought is one of the major abiotic stress factors that affect grape growth and yield, which in turn negatively affects the grape and wine production industry. Developing effective approaches to improve grapevine tolerance to drought stress is a priority for viticulture. Strigolactones, a newly discovered class of carotenoid-derived phytohormones, have been found to participate in various physiological processes. Herein, the effect of strigolactones (SLs) on grape seedlings under drought stress was investigated. Two-year-old grape seedlings (*Vitis vinifera* L.) were sprayed with 3 doses of *rac*-GR24 (1 μM, 3 μM and 5 μM), a synthesized strigolactone, and then were subjected to 7% (w/v) polyethylene glycol (PEG-6000) to simulate the drought conditions. Synthetic GR24 treated plants showed higher tolerance to drought stress with regard to lower electrolyte leakage, stomatal opening, reactive oxygen species (ROS), and higher relative water content, chlorophyll content, photosynthesis rate and malondialdehyde (MDA) content. GR24 application also decreased the levels of indoleacetic acid (IAA) and zeatin riboside (ZR), while increasing the level of abscisic acid (ABA), both in the roots and leaves under drought stress. These results suggested that foliar application of GR24 could ameliorate the adverse effects of drought due to its regulation of stomatal closure through ABA or ROS, and modulation of chlorophyll components and photosynthesis, as well as activation of the antioxidant defense capacity. Cross-talk with other hormones, especially ABA, was also suggested to be one of the important mechanism during this process. This study contributes to our current understanding of GR24-induced drought tolerance in grapevines.

1. Introduction

Grapevine has been recognized as one of the most important fruit crops widely cultivated around the world. Most of the world's emerging grape-growing areas, like South Australia and Northwest China, are arid or semi-arid climates and suffer from soil water scarcity at some stages during the growth cycle, causing dramatic damage to the grape industry.

It was reported that the growth and development of grapevines are severely inhibited when drought stress occurs. Photosynthesis, transpiration rate (Wang et al., 2012), and CO₂ diffusion are all limited owing to the closure of the stomata (Chen et al., 2011). Drought stress often causes oxidative damage, which is manifested in the production of reactive oxygen species (ROS), including hydrogen peroxide (H₂O₂) and

O₂^{•-}. Then a series of antioxidant enzymes, such as superoxide dismutase (SOD), peroxidases (POD), and catalase (CAT) are produced in grapevines to scavenge ROS (Salazar-Parra et al., 2012). The primary and secondary metabolism in grapevines are also altered under drought stress (Ferrandino et al., 2009). In short, under drought stress, grape leaves wilt, their margins turn yellow, the shoot and leaf development are largely suppressed, and the quality and productivity of grapes are largely affected (Fanizza and Ricciardi, 2015). The uneven distribution of regional water and frequent drought in the growing season not only have a serious impact on the growth and development of grapevines, but also reduce the yield and quality of grapes, which severely restricts the development of the grape industry. Furthermore, as a consequence of global warming, the area of drylands would expand more rapidly (Huang et al., 2016) and the problems caused by drought would

Abbreviations: ABA, abscisic acid; APX, ascorbate peroxidase; ASA, ascorbic acid; BR, brassinolide; CAT, catalase; DW, dry weight; EL, electrolyte leakage; Fv/Fm, maximum photochemical quantum yield of PSII; FW, fresh weight; GR, glutathione reductase; H₂O₂, hydrogen peroxide; IAA, indoleacetic acid; MDA, malondialdehyde; NP, net photosynthetic rate; NQ, non-photochemical quenching; PEG, polyethylene glycol; POD, peroxidases; qPCR, quantitative real-time PCR; ROS, reactive oxygen species; RWC, relative water content; SLs, Strigolactones; SOD, superoxide dismutase; Tr, transpiration rate; TW, turgid weight; ZR, zeatin riboside; ΦPSII, the effective photochemical quantum yield of PSII

* Corresponding author. College of Enology, Northwest A & F University, Yangling, 712100, Shaanxi, China.

E-mail address: fangyulin@nwsuaf.edu.cn (Y. Fang).

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become more and more prominent, which needs to be addressed urgently, as such.

Given that the growth and quality of wine grapes are threatened by drought stress, it is imperative to find approaches to address this problem, and research has progressed remarkably in this field during the last few decades. Plant hormones like melatonin (Meng et al., 2014), salicylic acid (LovinBabaei et al., 2014), and epi-brassinosteroid (Wang et al., 2015) have been applied to grape leaves or berries to protect them against damage caused by drought stress, all of which were proved to be effective to alleviate the stress by regulating the physiological processes and gene expression, providing solid foundation for their use in practical production, so more efforts could be paid in this area.

Strigolactones (SLs), a newly discovered class of carotenoid-derived phytohormones (Dun et al., 2009), were found to play important roles in different processes throughout plant development. Recent studies suggested that, in addition to the roles in inhibiting shoot branching, modulating root architecture, and promoting leaf senescence (reviewed by Ruyterspira et al., 2013), SLs could also act as positive regulators in plant responses to abiotic stress, such as drought (Ha et al., 2014), salinity (Ma et al., 2017), and dark chilling stress (Cooper et al., 2018). Ha et al. (2014) and Liu et al. (2015) found that SL-depleted *Arabidopsis thaliana* and *Lotus japonicas* exhibited hypersensitivity to drought and salt stress when compared with their wild-type (WT) counterparts, and the drought-sensitive phenotype of SL-deficient mutants like *max3* and *max4* could be rescued by exogenous application of GR24, a synthetic SL analogue. Furthermore, pretreatment with GR24, could enhance the drought and salt tolerance of WT plants (Ha et al., 2014; Kapulnik and Koltai, 2014). The same phenomena were also observed in tomato (*Solanum lycopersicum*) by Visentin et al. (2016). Liu et al. (2015) and Visentin et al. (2016) found there was complex crosstalk between SLs and ABA during drought acclimatization. A recent report suggested that *D27* (*DWARF 27*), the gene encoding β -carotene isomerase, seems to be a linker regulating SL and ABA homeostasis and hence determining drought tolerance in plants (Haider et al., 2018).

Based on the functions documented in model plants, this study was designed to explore the function of exogenously-applied SLs on wine grape seedlings under water-deficient status, hoping to alleviate the adverse effects of drought stress using a simple and environment-friendly method, and to provide a theoretical basis for improving the quality of wine grape in northwest China. To our knowledge, this is the first study to unravel the effects of SLs on grapevine under drought stress, from the perspective of morphological, physiological, biochemical characteristics, leaf anatomy and transcript levels.

2. Materials and methods

2.1. Plant materials and treatments

This study was conducted in the greenhouse of the College of Enology, Northwest A & F University, Yangling, China. Two-year-old cuttings of Cabernet Sauvignon (*Vitis vinifera* L.) were cultivated in 2.0 L plastic pots with a mixture of garden soil/vermiculite/humus (1/1/1, v/v/v) until they reached 10–12 leaves. Then, 300 healthy and morphologically uniformed (by size and number of leaves) plants were selected and transferred into the growth vessels filled with half-strength Hoagland nutrient solution (Hoagland, 1950) after gently washing the soil away from the roots, then fixed with a foam cap, and pre-cultured for 7 days in a growth chamber under the following controlled conditions: light period of 16 h light/8 h dark; 25 °C/19 °C day–night temperature cycle; photosynthetic photon flux density of 160 $\mu\text{mol m}^{-2} \text{s}^{-1}$; and relative humidity of about 50%.

Then, the cuttings were divided into five groups as follows: (1) normal control, grown with only half-strength nutrient solution (CK); (2) drought stress, half-strength nutrient solution plus 7% polyethylene glycol (PEG)-6000 (w/v) (D0); (3) 1 μM *rac*-GR24 pretreatment

combined with 7% PEG-6000 (D1); (4) 3 μM *rac*-GR24 pretreatment combined with 7% PEG-6000 (D3); (5) 5 μM *rac*-GR24 pretreatment combined with 7% PEG-6000 (D5). *Rac*-GR24 (Chiralix, Netherland) was dissolved with 3% acetone and 0.1% Tween 20, and diluted into three different concentrations (1, 3 and 5 μM). They were applied respectively with a hand sprayer until the solution began to drip off the leaves. CK and D0 group were treated with the equivalent amount of mock solution (distilled water with the same amount of acetone and Tween 20). To ensure the uptake of GR24 by leaves, they were applied for 7 consecutive days, at 24 h intervals. Then, plants of D0, D1, D3 and D5 group were subjected to drought treatment. In our preliminary experiment, different concentrations of PEG-6000 were employed and 7% (w/v) PEG-6000 was chosen as the appropriate concentration to simulate drought stress. Each treatment group contained 45 plants of three biological replicates. Leaf samples were collected at 2 h, 12 h, 24 h, 72 h, 96 h, and 120 h post PEG treatment. All samples were immediately frozen in liquid nitrogen and stored at -80 °C for further analysis.

2.2. Measurement of electrolyte leakage and relative water content

For the measurement of electrolyte leakage (EL) and relative water content (RWC), mature leaves, approximately leaves at the node of 8–10th from the base were used. The DDS-307 electrical conductivity (EC) meter (Leici, China) was used to measure the electrolyte leakage. 10 leaf discs (1 cm) derived from 3 leaves were rinsed and immersed in 10 mL de-ionized water, and then transferred into the shaking incubator (25 °C, 150 rpm) for 3 h. After that, the initial electrolyte leakage (E1) was recorded. Then, the same samples were boiled at 100 °C for 30 min and cooled to 25 °C to determine the total EC (E2). The EL was calculated according to the formula: $\text{EL} (\%) = \text{E1}/\text{E2} \times 100\%$.

The grapevine leaves at the node of 8–10th from the base were collected, rinsed with de-ionized water, wiped with absorbent paper, and the fresh weight (FW) was recorded. The samples were soaked in distilled water for 24 h at 4 °C in darkness and the turgid weight (TW) was recorded. Then the dry weight (DW) was determined after drying at 105 °C for 30 min and 48 h at 80 °C. RWC was calculated according to the formula: $\text{RWC} (\%) = (\text{FW} - \text{DW})/(\text{TW} - \text{DW}) \times 100\%$.

2.3. Measurement of chlorophyll content and chlorophyll fluorescence

Chlorophyll content was determined according to the method of Gao (2006), with some slight modifications. For each sample, grapevine leaves were cut into small pieces and 0.05 g were added into 25 mL test tubes with 10 mL acetone/ethanol (1/1). The extracting reaction was performed under dark conditions and terminated by adding 80% acetone to the tubes until most of the leaves had turned white. After centrifugation, the supernatant was separated and its absorbance was recorded spectrophotometrically at 663, 645, and 470 nm, respectively. There were 3 replicates for each treatment. The chlorophyll content was calculated using the formula of Gao (2006).

The chlorophyll fluorescence parameters were measured using the FMS-2 Pulse Modulated Fluorometer (Hansatech, England). The 9th and 10th leaves from the base were chosen for dark adaptation for 30 min, and initial fluorescence (F_0) was recorded. Maximal fluorescence (F_m) was measured under saturated pulsed light ($5000 \mu\text{mol m}^{-2} \text{s}^{-1}$) for 0.7 s. Then, the leaves were illuminated by activating light at $500 \mu\text{mol m}^{-2} \text{s}^{-1}$, and the fluorescence value F_s at steady state was recorded. The saturated pulsed light ($5000 \mu\text{mol m}^{-2} \text{s}^{-1}$) was turned on for 0.7 s to measure F_m' , after turning off the activating light and turning on far-red light for 3 s, F_0' was recorded. The maximum photochemical quantum yield of PSII (F_v/F_m), the effective photochemical quantum yield of PSII (ΦPSII), and the non-photochemical quenching (NPQ) were calculated according to the formulas $F_v/F_m = (F_m - F_0)/F_m$, $\Phi\text{PSII} = (F_m' - F_0')/F_m'$, $\text{NPQ} = F_m/F_m' - 1$, respectively. Three replicates for each treatment were

performed.

2.4. Measurement of photosynthetic parameters

The photosynthetic parameters were determined using the Portable Photosynthesis System (LI-6800, United States) on the grapevine leaves between 9 a.m. and 11 a.m. The air flow speed was set at $500 \mu\text{mol}\cdot\text{s}^{-1}$ and the photosynthetically active radiation (PAR) at $1000 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. The leaf temperature was $25 \pm 1.5^\circ\text{C}$, and the air relative humidity was 50%. Using CO_2 cylinders, the CO_2 concentration of the leaf chamber was maintained at $400 \mu\text{mol}\cdot\text{mol}^{-1}$. The net photosynthetic rates (Pn), stomatal conductance (Gs), intercellular CO_2 concentration (Ci), and transpiration rate (Tr) were automatically collected every 2–3 min with 3 replicates.

2.5. Measurement of antioxidant enzyme activity and related metabolites

The activities of superoxide dismutase (SOD, EC 1.15.1.1), peroxidase (POD, EC 1.11.1.7), catalase (CAT, EC 1.11.1.6), ascorbate peroxidase (APX, EC 1.11.1.11), glutathione reductase (GR, EC 1.6.4.2), and contents of ascorbic acid (ASA) and reduced glutathione (GSH) were all determined using the reagent kits (Comin Biotechnology, Suzhou). Malondialdehyde (MDA) content was measured to reflect the extent of lipid peroxidation, and the concentration of hydrogen peroxide (H_2O_2) was determined following the method described by Gao (2006).

2.6. Scanning electron microscopy

At 120 h after drought stress, the 3rd and 8th leaves of each group were collected and subjected to the pre-processing with reference to the method of Wang et al. (2015). The leaves on the right side of the main leaf vein were cut into small segments ($5 \text{ mm} \times 5 \text{ mm}$), and immersed in the fixed solution of 4% glutaraldehyde for 2 h at room temperature. There were 3 replicates for each treatment. Then, the samples were rinsed 4 times with phosphate buffer solution (0.10 M PBS, pH 6.8) at 10-min intervals between each washing. After repeated rinsing, the leaf samples were dehydrated using a series of different concentration of ethanol (30, 50, 70, 80 and 90%), each of which was rinsed for 20 min. Then, they were washed by 100% ethanol for three times, with 30 min for each time and finally transferred into isoamyl acetate three times for 30 min. After being Hitachi HCP-2 critical point-dried (Tokyo, Japan) in CO_2 , the samples were sprayed with a thin layer of gold. Scanning electron microscopy (SEM-2700, Hitachi, Japan) was used to observe and take photographs of the adaxial leaf surface, which was analyzed for parameters such as stomatal length, width, and stomatal area, using Digimizer, version 4.5 (Schoonjans, 2012). The value of each parameter was the statistical average of 5 fields and was repeated 3 times. The abaxial surface of the leaf segments were also used to observe and take photographs of the epidermis cells.

2.7. Measurement of endogenous hormone content

Leaf and root samples of the CK, D0, and D5 group at 120 h after drought stress were sent to Zoonbio Technology Co., Ltd, Nanjing, China, to determine their endogenous hormone content, including IAA, ABA, ZR, GA3 and MeJA, using ESI-HPLC-MS/MS (Waters, Milford, United States) according to the method of You et al. (2016), with some slight modifications. Firstly, 0.5 g samples were ground in liquid nitrogen and 5 mL isopropanol/HCL were added to the powder. The extract was shaken at 4°C for 30 min. Then, 10 mL dichloromethane was added and the sample was shaken at 4°C for 30 min and centrifuged at 13,000 rpm for 5 min. The lower, organic phase was collected, dried with nitrogen blowing instrument (Laiheng Scientific Co., Ltd., Beijing, China), and dissolved with $400 \mu\text{l}$ methanol (0.1% methane acid). At last, the extract was filtered with $0.22 \mu\text{m}$ filter membrane and

subjected to HPLC-MS/MS analysis. HPLC analysis was performed using a ZORBAX SB-C18 (Agilent Technologies) column ($2.1 \text{ mm} \times 150 \text{ mm}$; $2.7 \mu\text{m}$). The binary solvent system used methanol with 0.1% (vol/vol) methanoic acid (A) and ultrapure water with 0.1% (vol/vol) methanoic acid (B) as mobile phases for all the 5 hormones mentioned above. The injection volume was $2 \mu\text{L}$. The gradient elution was achieved as follows: the percentage of A (vol/vol) was 20% from 0 to 1 min, then increased linearly to 80% over the next 8 min and maintained at 80% for the next 1 min; then it was decreased linearly to 20% in 10–10.1 min, and finally maintained at 20% in 10.1–15 min. MS conditions were as follows: the spray voltage was 4500 V; the pressure of the air curtain, nebulizer, and aux gas were 15, 65, and 70 psi, respectively; and the atomizing temperature was 400°C .

The endogenous SLs were evaluated using the germination bioassay of sunflower broomrape (*Orobancha cumana*) seeds according to the method of Yue et al. (2015), with some slight modifications. The sampled grapevine leaves and roots were dried in a vacuum freeze dryer (Labogene, Denmark) and ground into fine powder with mortar. 0.1 g of the powder was transferred into 50 ml centrifuge tube with 10 ml methanol (Sigma, US) added in. After 30 min extraction by ultrasonic and 2 min centrifugation at 10,000 rpm, the extracted supernatant was ready for test. Small round disks (diameter of 8 mm) of glass microfiber filter (Whatman, Germany) were placed in the petri dish and $20 \mu\text{L}$ of the extracted supernatant was added. After volatilization of methanol, around 50 sunflower broomrape (*Orobancha cumana*) seeds were sowed on the disks and $40 \mu\text{L}$ diluted water was added. Racemic GR24 (Chiralix, Netherland) solution at the concentration of $1 \mu\text{M}$ or diluted water were used as positive or negative control, respectively. A piece of filter paper folded-to-triangle was placed in the center of the petri dish and 3 mL of sterile water was added in to maintain the humidity. The petri dish was sealed and placed in the incubator at 25°C to induce germination. The germination rates were counted 10 d later by dissecting microscope (Olympus, Japan).

2.8. Gene expression analysis

Total RNA was isolated from grapevine roots and leaves according to the instructions of RNAout kit (Biotek, Beijing). RNA quality and concentration was evaluated using the NanoDrop ND-2000 Spectrophotometer (Thermo Fisher Scientific Inc., USA). 500 ng RNA was used for reverse-transcription using the HiScript RT SuperMix for quantitative real-time PCR (qPCR) (Vazyme, Nanjing). Real-time PCR was performed using the CFX96 Touch (Bio-Rad, US) and qPCR SYBR green as recommended by the manufacturer (Vazyme, Nanjing). Primer sequences of genes encoding the key enzymes in ABA and SLs synthesis and signaling were obtained from the recent publication (Ferrero et al., 2018) and were shown in Table S1. *VvUBI* (*Ubiquitin*, VIT_16s0098g01190) was selected as the reference gene to normalize all data and experiments were repeated three times. The relative gene expression level was estimated using threshold cycles by the $2^{-\Delta\Delta\text{CT}}$ method (Livak and Schmittgen, 2001). The leaf and root sample at 0 h was used as the reference sample, which represented $1 \times$ expression of the target gene. The relative expression amounts were normalized to values ranging from -1 to 1 using the SPSS 21.0. And MEV 4.9.0 software was used to draw the heat-map.

2.9. Statistical analysis

Data were statistically analyzed with SPSS 21.0. One-way analysis of variance (ANOVA) and Duncan's multiple range test were used to determine the significance of the differences among samples ($p < 0.05$).

3. Results

GR24 improved the resistance of grape cuttings to water deficit

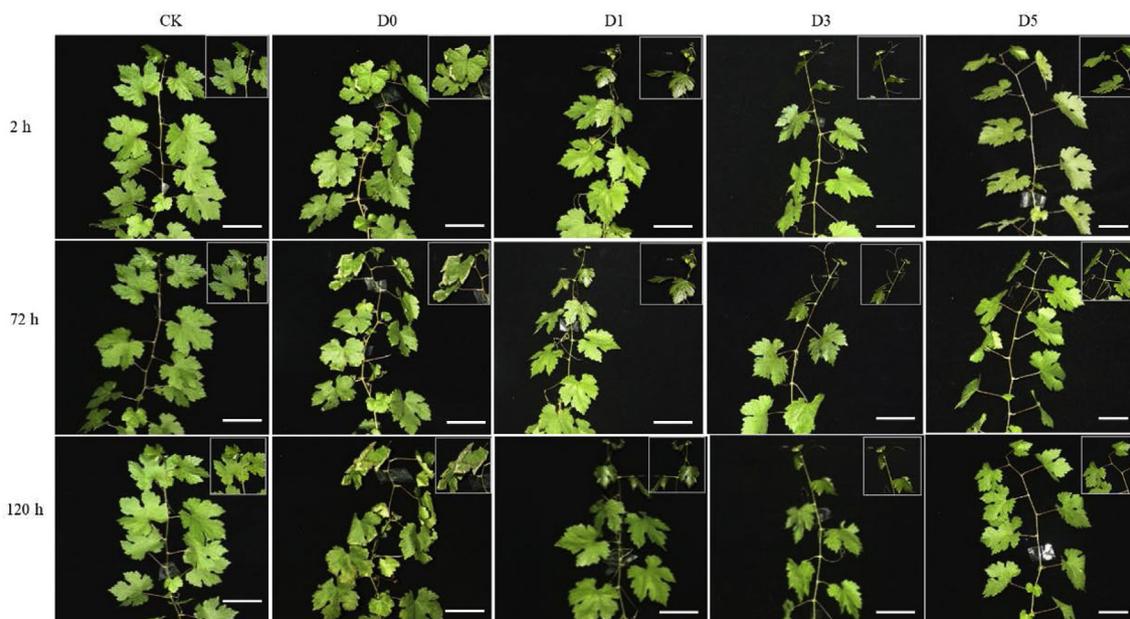


Fig. 1. Growth status of grapevines under control and drought conditions with and without GR24 application. Highlighted in grey boxes were close-up of leaves with obvious signs of drought stress. CK: normal control, grown with only half-strength nutrient solution; D0: drought stress, half-strength nutrient solution plus 7% PEG-6000 (w/v); D1: half-strength nutrient solution plus 1 μM *rac*-GR24 pretreatment combined with 7% PEG-6000; D3: half-strength nutrient solution plus 3 μM *rac*-GR24 pretreatment combined with 7% PEG-6000; D5: half-strength nutrient solution plus 5 μM *rac*-GR24 pretreatment combined with 7% PEG-6000.

stress induced by PEG. As was shown in Fig. 1, at 72 h after treatment, most of the leaves, especially the top ones in D0 group (drought treatment without GR24 application) turned yellow and slightly curled. At 120 h after treatment, the areas that turned yellow became larger and the leaves appeared blighted. The application of GR24 mitigated the stress damage to varying degrees, especially in the D5 group, which appeared to be almost unaffected by drought stress, just like CK group.

3.1. Electrolyte leakage and relative water content

The effects of GR24 on the EL and RWC of grapevine seedlings under drought stress were shown in Fig. 2. Fig. 2A shows that EL of D0 group increased rapidly, and it continued to increase with the progression of the drought treatment. While exogenous application of GR24 greatly reduced EL under drought stress, especially at 120 h after treatment, the values presented significant differences ($p < 0.05$). Drought stress significantly reduced the RWC ($p < 0.05$), especially at 120 h post PEG treatment, whereas the drought-induced decrease in RWC was less evident in groups with GR24 application (D1, D3, and D5), and showed an inverse correlation with the concentration of GR24

applied (Fig. 2B), indicating that GR24 could alleviate the negative effect of drought stress on water availability.

3.2. Chlorophyll content and chlorophyll fluorescence

Chlorophyll is the main pigment involved in photosynthesis. As shown in Fig. 3, the content of chlorophyll *a*, *b*, and total chlorophyll all decreased significantly in the D0 group, compared with that in the CK group ($p < 0.05$). With the prolongation of treatment time, the chlorophyll content of the control and treatment group both showed a decreasing trend. The decrease in D0 group was obviously more pronounced than that in the groups with GR24 application. For example, from 2 h to 72 h after drought treatment, the total chlorophyll content decreased by 47.94% in the D0 group, while it decreased by 41.43%, 30.98%, and 18.79% in the D1, D3, and D5 group, respectively. At 120 h, the total chlorophyll content in group D1, D3, and D5 was 36.90%, 71.78%, and 93.31% higher, respectively, than that in the D0 group, suggesting that spraying of GR24 could largely alleviate the decrease of chlorophyll content caused by drought; and the effect was more obvious with increasing GR24 concentration.

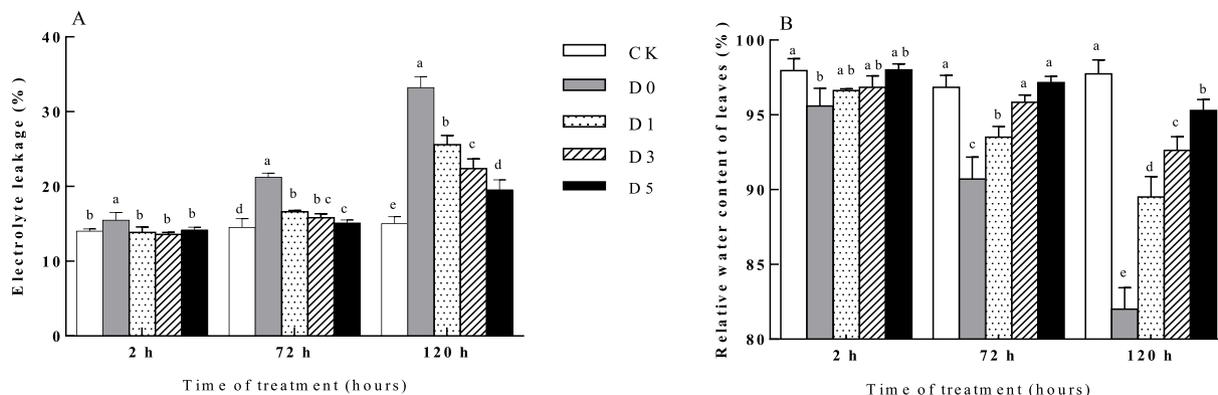


Fig. 2. Effects of GR24 treatment on the electrolyte leakage (EL) (A) and relative water content (RWC) (B) of grape leaves under drought stress. Each value represents the mean \pm SE ($n = 3$). Different letters indicate significant differences among different treatments at the same period.

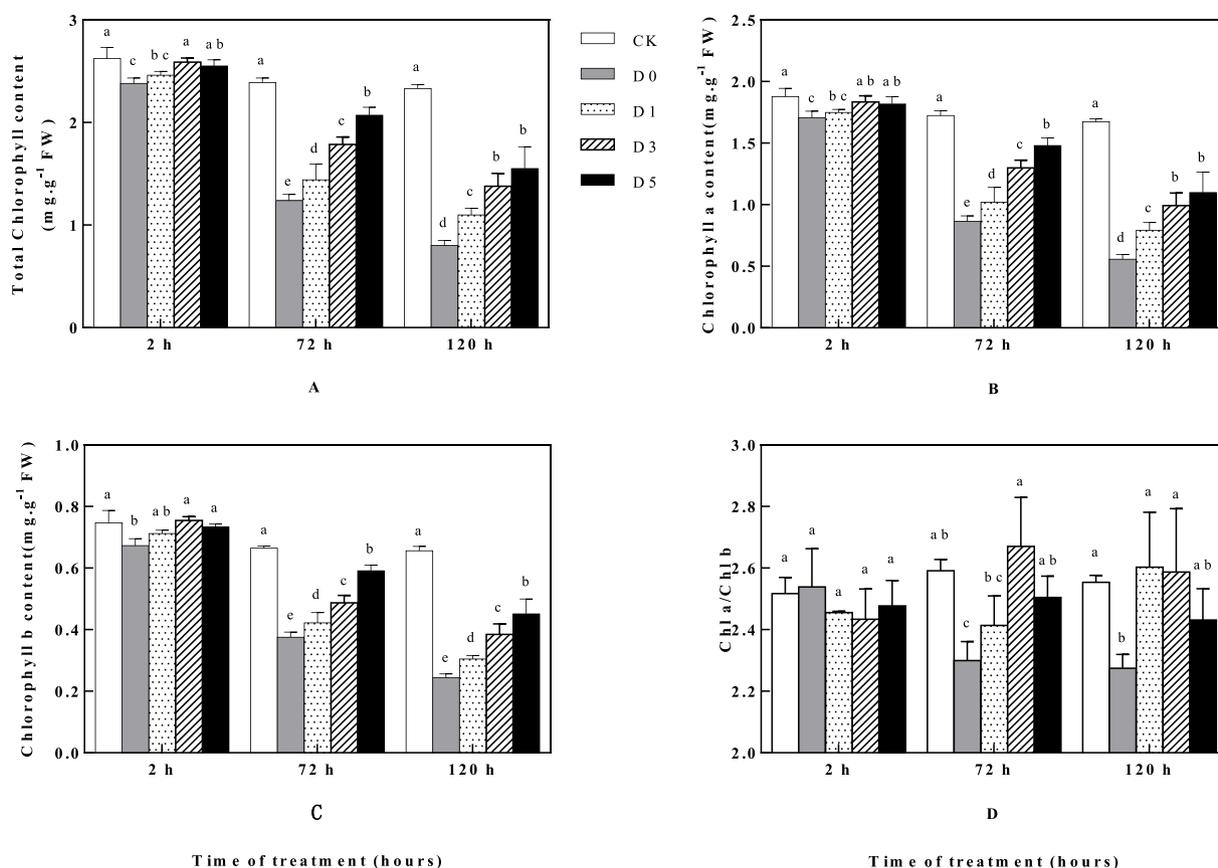


Fig. 3. Effects of GR24 on the content of chlorophyll in grape seedlings under drought stress. A, Total chlorophyll content; B, chlorophyll a content; C, chlorophyll b content; D, chl a/chl b. Each value represents the mean \pm SE ($n = 3$). Different letters indicate significant differences among different treatments at the same period.

Drought treatment diminished F_v/F_m and q_N , especially after 24 h, when compared with the CK group, the difference reached a significant level, while the decrease was alleviated by the pretreatment of GR24. And all the groups presented a concentration-dependent effect. Drought treatment increased both q_P and Φ_{PSII} , but no significant difference was detected among the groups with GR24 application (Fig. 4).

3.3. Photosynthetic parameters

As illustrated in Fig. 5, upon drought treatment, net photosynthetic rate (NP), stomatal conductance (g_s), and transpiration rate (Tr) displayed a decreasing trend, and GR24 application dampened the decrease of these photosynthetic parameters caused by drought; but unlike the parameters described above, NP, g_s , Tr did not display a simple inverse correlation with GR24 concentration during the whole period of drought treatment. Taking the changing trend of g_s as an example, at 2 h after drought stress, no significant change in g_s was identified in the D0 group when compared with CK, while significant differences were identified in the D3 and D5 group ($p < 0.05$), with a decrease of 63.1% and 57.1%, respectively, indicating that this change was caused by the pretreatment of GR24, rather than a response of stomata to the drought treatment. At 24 h, exogenous GR24 concentrations were inversely correlated with g_s . It was noteworthy that during the later period of drought treatment, g_s seemed to decrease and then increase at 96 h, and basically stabilized at $30 \mu\text{mol m}^{-2}\text{s}^{-1}$. The NP was inhibited in all the groups by exposure of grapevine to drought stress, but was higher in D1, D3, and D5 than in D0 group. In the later drought period, water use efficiency was higher in D1, D3, and D5 than in D0, indicating that the decrease in transpiration rate was less than that of photosynthesis.

3.4. Measurement of antioxidant enzyme activity and related metabolites

The effects of GR24 application on antioxidant enzyme and antioxidant metabolites were shown in Fig. 6 and Fig. 7. No significant difference was detected in SOD enzyme activity among different groups until 72 h after drought treatment. Compared with the CK group, SOD activity increased significantly in the drought-stressed groups with or without GR24 application at 72 h. At 120 h, compared with CK, SOD activity in D0 and D1 group increased by 105.6% and 90.7%, respectively, and increased by 72.7% and 70.0% in D3 and D5, respectively, both reaching a significant level ($p < 0.05$) (Fig. 6A). Similar trends were also observed in the content of AsA. Other antioxidant enzyme activities (i.e., POD, CAT, APX and GR) (Fig. 6B–E) and the amount of antioxidant metabolites (i.e., GSH, AsA) (Fig. 6D and E) all reached a significant higher level at 72 h after drought treatment and then decreased, while GR24 application decreased their increasing amount caused by drought stress and presented a concentration-dependent effect.

The content of H_2O_2 and MDA both increased significantly after 72 h of drought treatment, while decreased in groups with GR24 pretreatment. Although no significant difference was detected between D1 and D3 group, 5 μM GR24 treatment significantly decreased both the contents.

3.5. Microscopic structure of leaf tissue

Stomata, comprised of a pair of guard cells, are portals of gas exchange between the interior of plant leaves and the atmosphere, controlling CO_2 entry into the leaf for photosynthesis and diffusive water vapor loss out of the leaf during transpiration. Stomatal morphology of mature leaves were observed using scanning electron microscopy, and

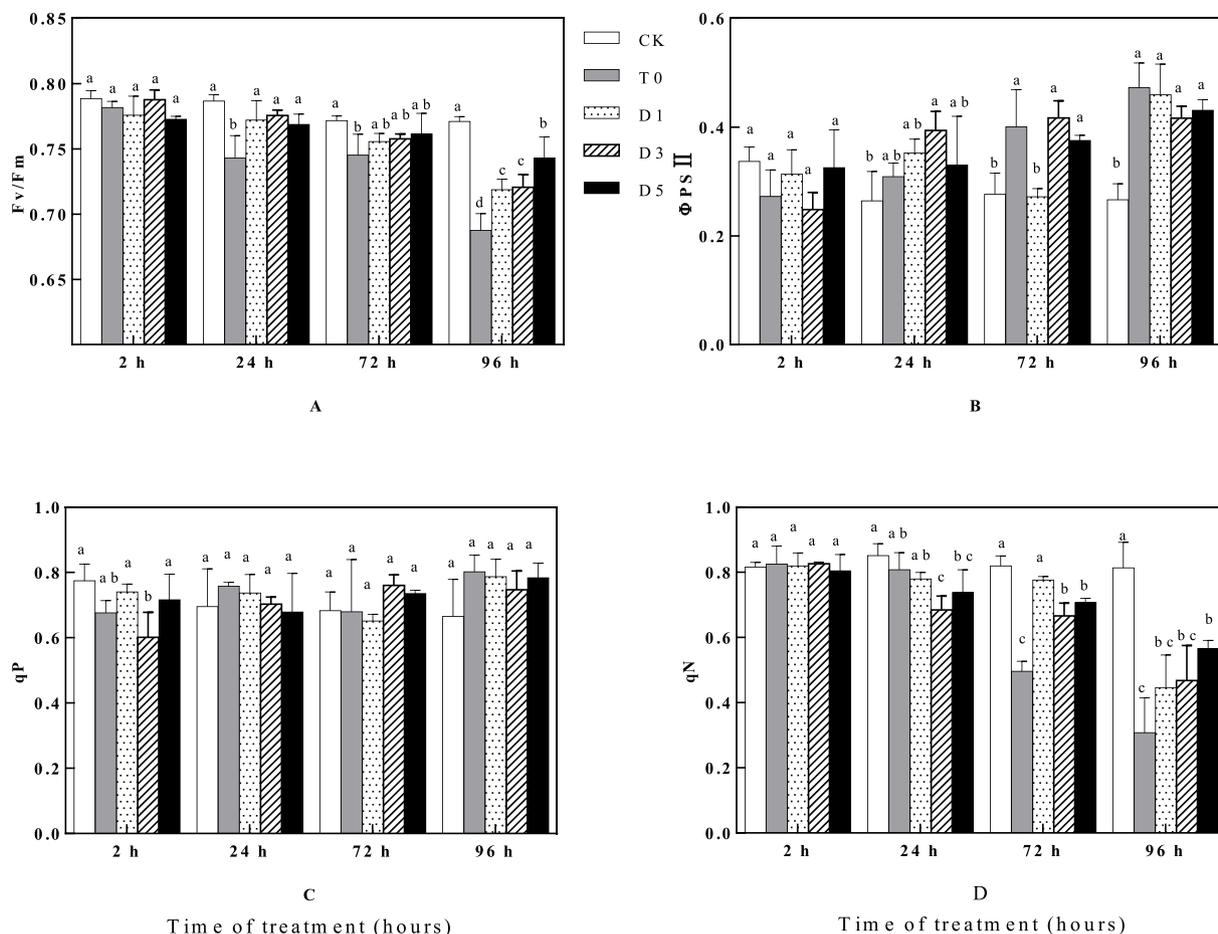


Fig. 4. Effects of GR24 on the chlorophyll fluorescence in grape seedlings under control and drought conditions with or without GR24 application. A, Fv/Fm; B, ΦPSII; C, qP; D, qN. Each value represents the mean ± SE (n = 3). Different letters indicate significant differences among different treatments at the same period.

stomatal length, width, and area were all found to be decreased at 120 h in the D0 group; the application of GR24 significantly intensified the decrease of each parameter, generally (Fig. 8, Table 1), indicating that GR24 could better induce the closure of stomata. As for the stoma density, GR24 could significantly dampened its decrease caused by drought.

Epidermal cells were also observed. At 120 h after drought stress, epidermal cells in the D0 group were significantly depressed and shrunk. Dehydration of leaves caused the epidermal cells to become unevenly shaped: epidermal cells in the D1 group were slightly depressed, whereas epidermal cells in the D3 and D5 groups were smooth and plump, just like that in CK group (Fig. 8 A3 - E3).

3.6. Measurement of endogenous hormone content

The endogenous hormone contents were also determined both in grapevine leaves and roots of CK, D0, and D5. In the leaves, drought treatment caused a decrease of ZR and IAA, and an increase of ABA (Table 2). However, with the addition of GR24, the IAA and ABA levels were partially restored and became closer to that of CK. In the roots, drought treatment increased the ZR content. The ABA content were also increased dramatically, reaching almost twice that in CK, and the levels of IAA and MeJA were detected to be reduced under drought stress, while in the groups with GR24 application, these hormone levels more closely approached the CK group.

From the seed germination assays of *O. cumana*, it was found that the tissue extracts from the leaves and roots of grape could effectively stimulate seed germination (Fig. 9), indicating that certain amount of SLs existed in both. As shown in Fig. 9A, drought treatment increased

the content of SLs in grape leaves. The D5 group also had a greater level of SLs content in the leaves, when compared with the D0 group, indicating that the SLs were effectively absorbed by the leaves. In the root system, the SLs content decreased dramatically under drought stress without GR24 (D0) application, especially at 72 h, while no significant difference was observed between the D5 and CK group.

3.7. Gene expression analysis

To understand whether the observed changes of grapevine in groups with exogenous SLs under drought stress are a result of differential gene regulation, real-time qPCR was used to quantify the transcript levels of putative SL and ABA related genes, at 0 h, 72 h, and 120 h, both in root and leaves.

VvNCED1, the rate-limiting gene involved in ABA biosynthesis, was up-regulated by drought stress both in leaves and roots, and the increase was more obvious when GR24 was applied (Fig. 10). Two genes encoding ABA 8'-hydroxylases, *VvHYD1* and *VvHYD2*, were down-regulated in both leaves and roots (Fig. 10), while GR24 treatment dampened their decrease. Transcription of *VvCCD7* and *VvCCD8* were up-regulated in leaves while down-regulated in roots under drought stress. The three putative paralogues of branching gene *TB1* (Min et al., 2017), especially *VvBRC1* was up-regulated in leaves, and GR24 application increased its expression (Fig. 10). Compared with the CK group, D0 group had higher level of *VvD27* both in roots and leaves and its expression level in leaves was much higher than that in roots. In leaves, *VvD27* was up-regulated first and then down-regulated in D0 and D5 group.

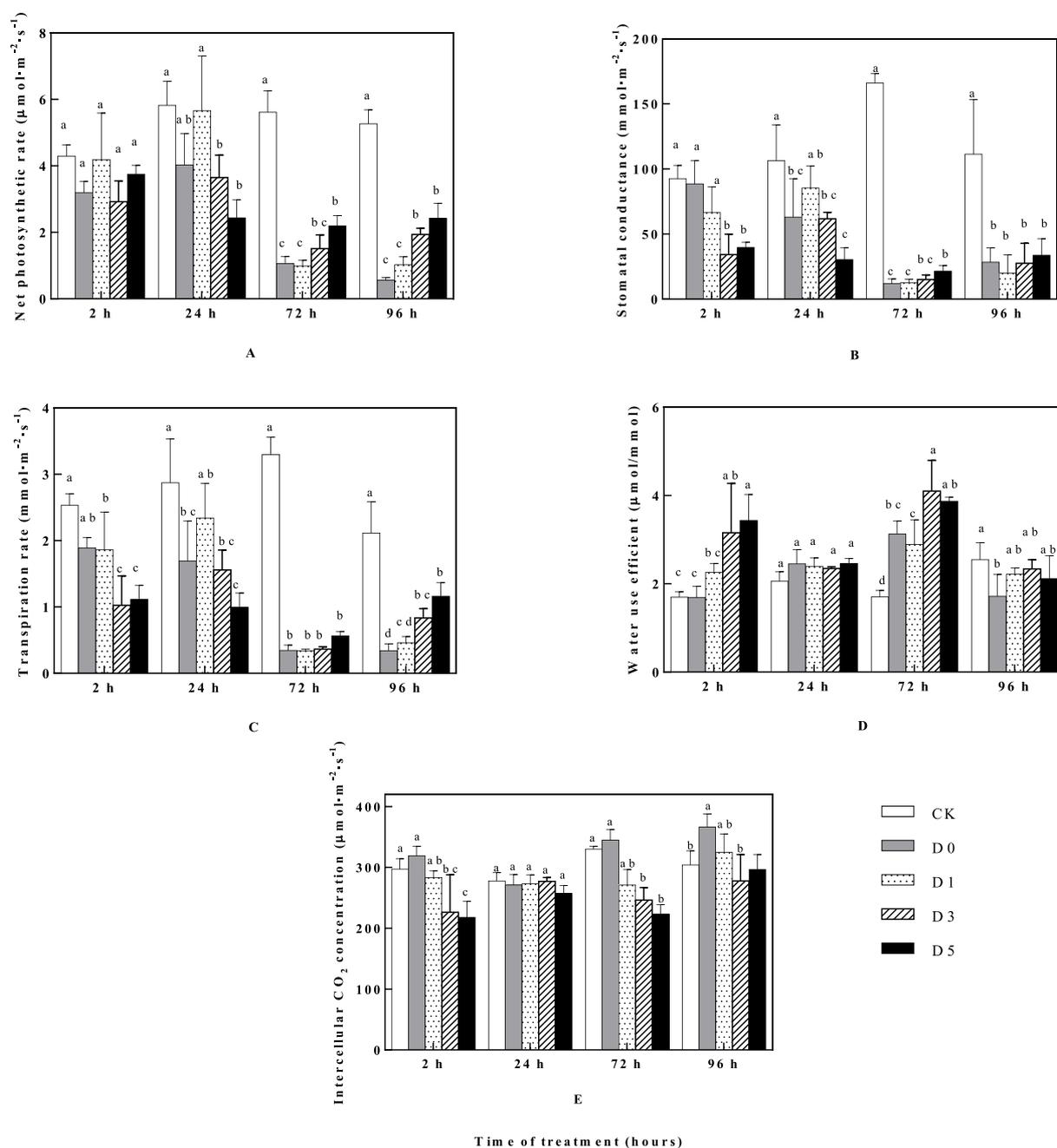


Fig. 5. Effects of GR24 on photosynthetic parameters in grapevine leaves under control and drought conditions with or without GR24 application. A, Net photosynthetic rate; B, stomatal conductance; C, transpiration rate; D, water use efficiency. Each value is the mean \pm SD of 3 replicates. Different letters indicate significant differences among different treatments at the same period.

4. Discussion

In general, grapevine is considered to be a species that is relatively well acclimatized to drought stress. However, stress caused by water availability is having progressively greater impact, due to its significant effects on grapevine cultivation and grape quality. Under drought conditions, the cutting rooting, growth and development, and longevity of the grapevines were largely inhibited, and the yield and quality of grapes were unfavorably affected (Fanizza and Ricciardi, 2015). In this study, *rac*-GR24 was applied to ‘Cabernet Sauvignon’ grapevine leaves for 7 days prior to 7% PEG treatment, which was used to simulate drought stress, and the effect of GR24 was evaluated from the perspective of morphological, physiological, anatomical, and transcriptional levels.

In view of the results presented herein, it is quite clear that some of the grape leaves turned yellow and wilted 72 h after treatment, while GR24 treatment relieved the symptoms of drought, especially in the D5 group (Fig. 1). The RWC of leaves can effectively reflect the whole water deficit situation of plants under drought stress, and thus can indirectly reflect the strength of drought resistance (Kadioglu et al., 2011). As shown in Fig. 2, the drought-induced decrease of RWC was less evident in groups with GR24 application and was concentration-dependent with the amount of GR24 applied (Fig. 2B), indicating that GR24 could help mitigate the loss of water in the drought conditions; this may largely be caused by the decrease of transpiration resulting from the closure of stomata, as was shown in Figs. 5C and 8. In the study of Ha et al. (2014), SL-depleted *Arabidopsis* was found to disperse more water than the wild-type, and this phenomenon was caused by

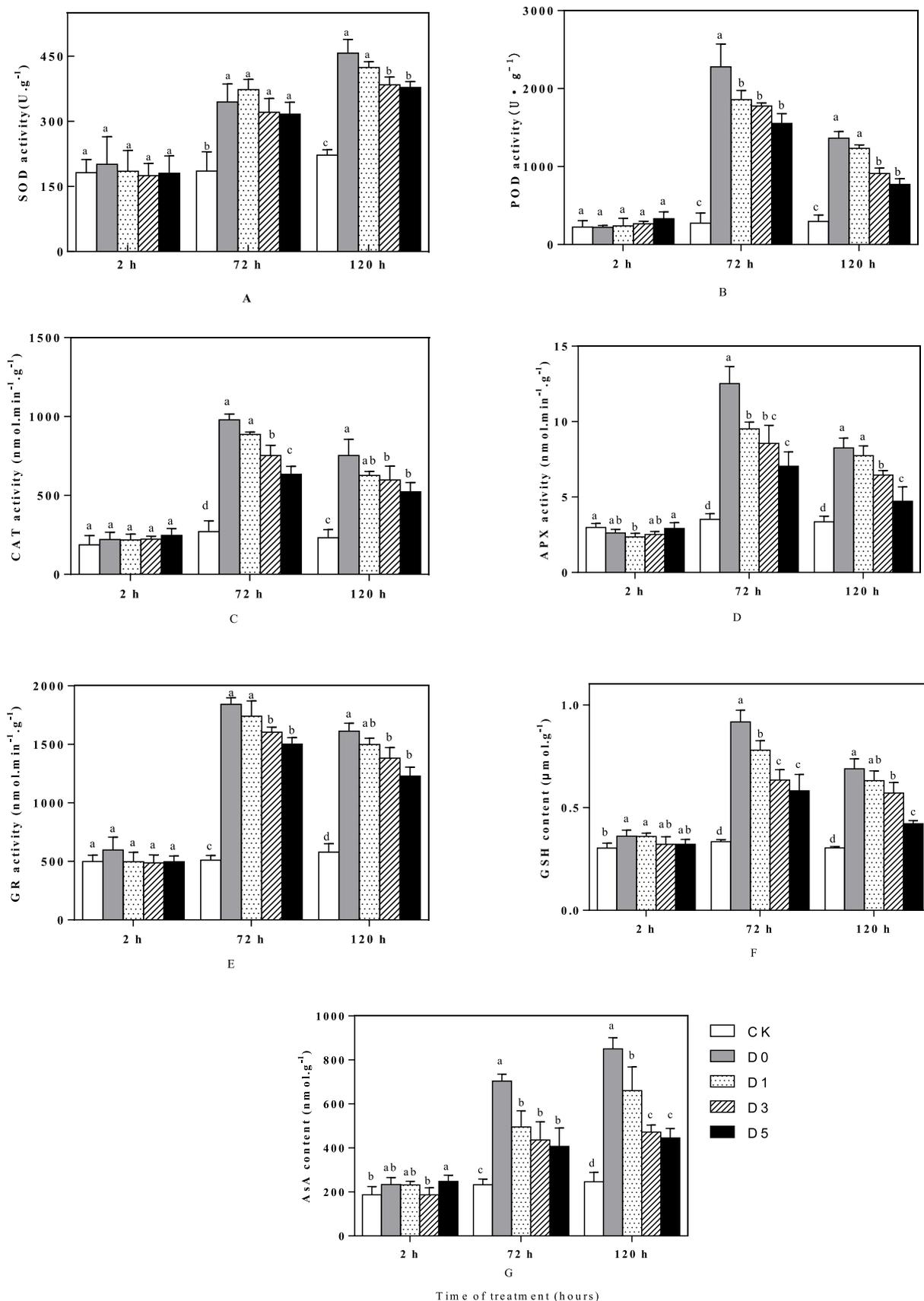


Fig. 6. Effects of GR24 application on the activities of antioxidant enzymes and on the contents of related metabolites. Each value is the mean ± SD of 3 replicates. A, SOD activity; B, POD activity; C, CAT activity; D, APX activity; E, GR activity; F, GSH content; G, AsA content. Different letters indicate significant differences among different treatments at the same period.

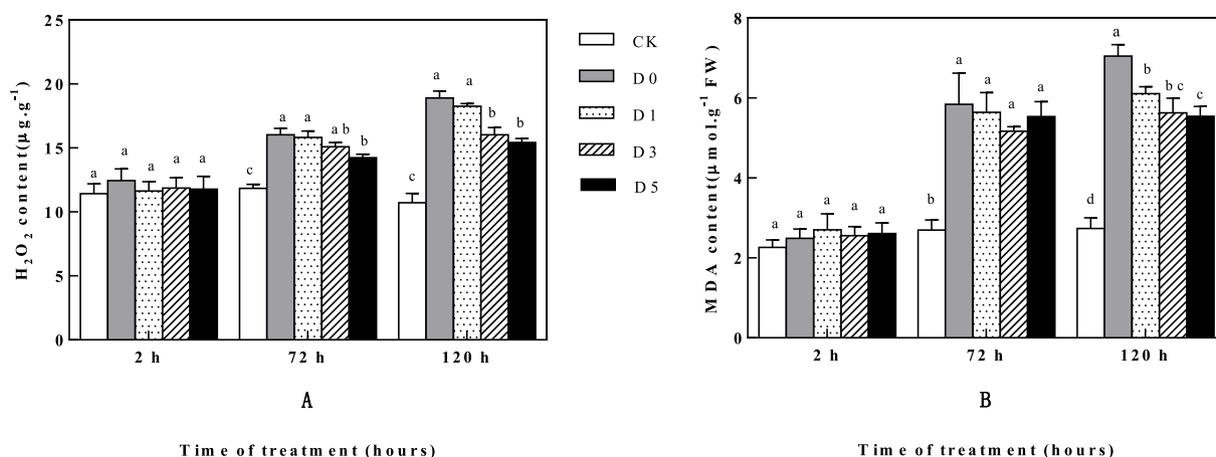


Fig. 7. Effects of GR24 application on the contents of H₂O₂ and MDA. Each value is the mean ± SD of 3 replicates. A, H₂O₂ content; B, MDA content. Different letters indicate significant differences among different treatments at the same period.

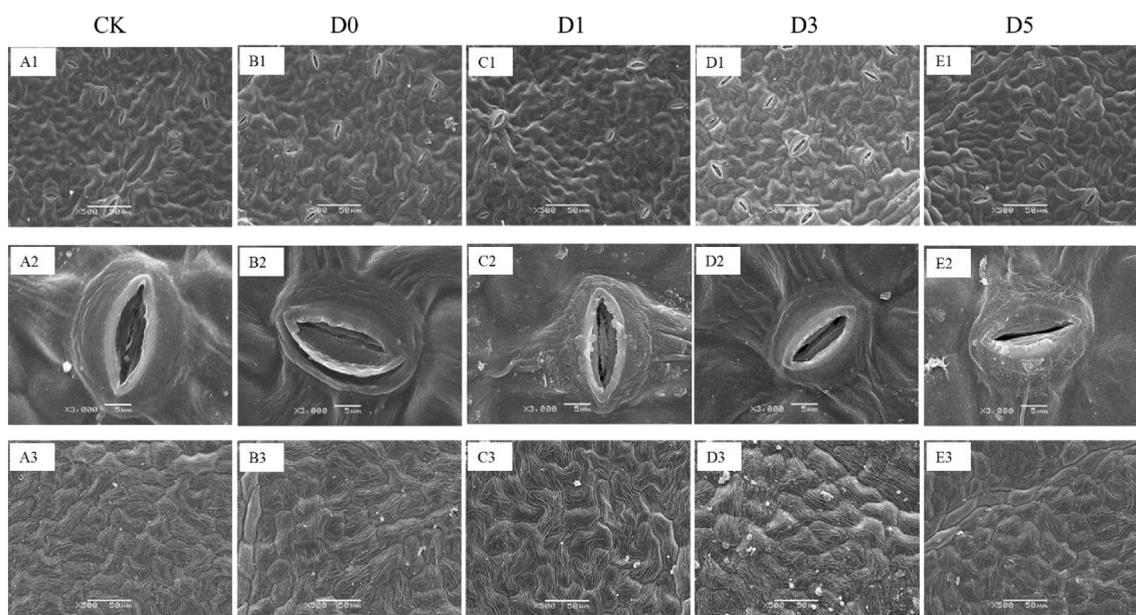


Fig. 8. Effects of GR24 on stomatal morphology and epidermis cells of leaves of 'Cabernet Sauvignon' grapevines under drought stress. A₁, B₁, C₁, D₁ and E₁ represent leaf stomatal distribution of grape leaves in CK, D0, D1, D3 and D5, respectively, 500x, scale bars = 50.0 μm; A₂-E₂ represent leaf stomatal characteristics in CK-D5, 3000x, scale bars = 5.0 μm; A₃-E₃ represent leaf surface characteristics in CK-D5, 500x, scale bars = 50.0 μm.

Table 1

Effects of GR24 on the stomatal parameters in mature leaves under drought stress (determined at 120 h post PEG-treatment).

Treatment	Stomatal length (μm)	Stomatal width (μm)	Opening of stomata (μm)	Stoma density (numbers mm ⁻²)
CK	19.41 ± 0.89a	5.48 ± 1.19a	66.72 ± 5.20a	263.94 ± 10.50a
D0	18.74 ± 0.61a	5.02 ± 0.34a	58.74 ± 1.42b	140.38 ± 3.46c
D1	18.25 ± 0.92a	5.33 ± 0.37a	57.90 ± 2.41b	191.31 ± 11.86b
D3	15.86 ± 0.85b	4.35 ± 0.70a	55.94 ± 3.51c	225.59 ± 20.50ab
D5	14.17 ± 0.38c	2.85 ± 0.20b	51.77 ± 5.70d	240.44 ± 9.06a

changes in transpiration. These results also add evidence for the reports that SLs could act as common regulators in inducing stomatal closure (Zhang et al., 2018). Hence, it was speculated that reducing transpiration through regulation by SLs on the stomatal apparatus could be one of the major mechanisms in alleviating drought stress.

Owing to the lack of water necessary for maintaining growth, grapevine plants always have a lower chlorophyll content when subjected to drought stress (Meng et al., 2014; Wang et al., 2015). GR24 application restored the level of chlorophyll in drought stress (Fig. 3).

Such preserving effects of SL on chlorophyll contents were also observed in rice under drought stress (Sedaghat et al., 2017), and rapeseed under salinity stress (Ma et al., 2017). The chlorophyll *a/b* ratio, an important indicator used to characterize the plant's ability to use light energy of different wavelength (Dale and Causton, 1992), was reduced in SL-biosynthesis mutant *max*, and the value of electron transport rate through PSII, Y(II), QNP was lower than that in WT, indicating that SLs could modulate the capacity of leaves for capturing light energy by altering the components of photosynthetic pigments (Li et al., 2017).

Table 2

Effects of GR24 on endogenous hormone contents of leaves of ‘Cabernet Sauvignon’ grapevines under drought stress (determined at 120 h post PEG-treatment).

organ	hormone	CK	D0	D5
leaves	ZR content (ng·g ⁻¹ FW)	8.12 ± 0.11b	7.38 ± 0.05c	6.30 ± 0.22d
	IAA content (ng·g ⁻¹ FW)	49.85 ± 0.47b	40.74 ± 1.22c	40.75 ± 0.54c
	ABA content (ng·g ⁻¹ FW)	158.96 ± 2.37b	169.47 ± 2.48a	162.58 ± 1.24b
	MeJA content (ng·g ⁻¹ FW)	14.93 ± 0.55a	14.93 ± 0.38a	13.01 ± 0.34b
roots	ZR content (ng·g ⁻¹ FW)	3.44 ± 0.07c	4.34 ± 0.06b	3.63 ± 0.08c
	IAA content (ng·g ⁻¹ FW)	20.30 ± 0.88b	17.37 ± 0.53c	18.45 ± 0.35c
	ABA content (ng·g ⁻¹ FW)	49.34 ± 1.71c	82.16 ± 3.97a	66.00 ± 1.46b
	MeJA content (ng·g ⁻¹ FW)	10.54 ± 0.14c	10.09 ± 0.19d	12.38 ± 0.30b

This may be caused by the induction of light-harvesting gene expression, as reported in tomato (Mayzlish-Gati et al., 2010). As for the photosynthetic efficiency, GR24 treatment also dampened the loss of these photosynthetic parameters caused by drought (Fig. 4). Therefore, the regulation of chlorophyll components and photosynthesis may be another strategy for SLs to help plants adapt to different stress conditions.

When subjected to drought conditions, the plant cell electron transport chain will always be disrupted, resulting in oxidative stress and the accumulation of ROS, which causes damage to some organelles, including the chloroplast, mitochondrion, and peroxisome (Meng et al., 2014). Similar phenomena have also been found in our study, and the production rate of H₂O₂ increased significantly after PEG treatment. Levels of MDA were also detected to increase at an early stage, indicating that the antioxidant enzyme defense system was weakened and lipid peroxidation was enhanced under drought stress. To overcome the effects of oxidative stress, the antioxidant system was activated (Fig. 6), which was the internal physiological regulation in response to environmental stimuli (Salazar-Parra et al., 2012). While when compared with D0 group, lower levels of SOD and POD activities were detected in the groups with GR24 application, accompanied by the decrease of H₂O₂ and MDA content, indicating that GR24 could promote the scavenging of ROS and reduce the lipid peroxidation induced by drought stress in grapevine, consistent with the reports by Ma et al. (2017) and Sedaghat et al. (2017). In addition, H₂O₂ was previously reported to induce stomatal closure through an ABA-dependent (Yao et al., 2013) signaling pathway. SLs were demonstrated, at least in part, to positively regulate drought stress response through ABA signaling (Ha et al., 2014), and ABA, in turn, could induce the antioxidant enzyme activity (Lu et al., 2014). The observed changing trends in H₂O₂ content, stomatal closure, and ABA content in our study indicated some complex crosstalk may exist in the regulatory pathway of GR24 on grapevine drought responses.

Hormonal cross talk has been increasingly spotlighted in response to

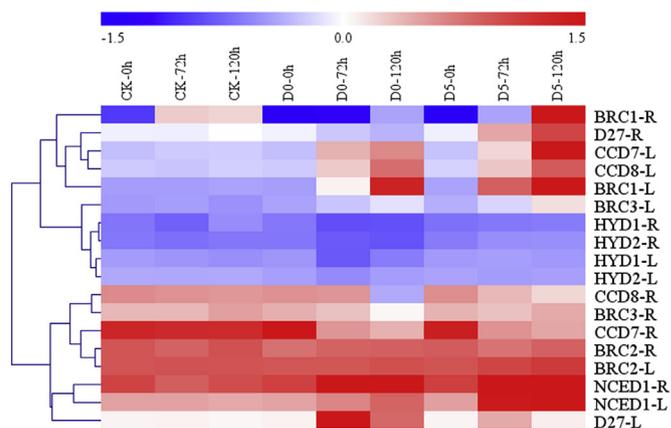


Fig. 10. Transcript levels of genes involved in SLs and ABA biosynthesis and signaling in grapevine leaves (L) and roots (R).

environmental stimuli, as well as changes in architecture and acclimatization under challenging conditions, such as nutrient starvation and heat/cold/salinity/light stress, by mediating growth, development, nutrient allocation, and source/sink transitions (Munné-Bosch and Müller, 2013). The content of total strigolactones were evaluated in grape roots and leaves based on the findings that only strigolactones could promote the germination of *Orobanchae* seeds (Yoneyama et al., 2008). Grapevine leaves' SLs were increased when compared with the D0 and CK group, indicating that GR24 was effectively absorbed, and further confirming that all the effects could be attributed to GR24 application. Large number of researchers found that SLs could act as negative regulators of shoot branching (Dun et al., 2009; Gomez-Roldan et al., 2008; Umehara et al., 2008). And the negative effects of SLs on shoot branching via a *TBI*-mediated pathway in rice was recently reported to be one of the major mechanisms for Drought Escape (DE) (Du

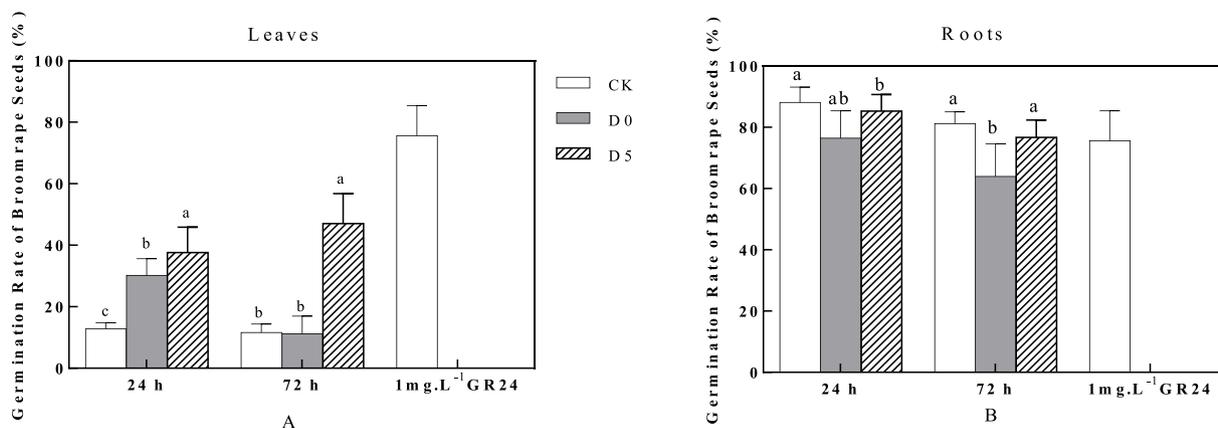


Fig. 9. Germination rate of broomrape seeds stimulated by grapevine leaf and root extract solution at different times after drought treatment. A, The germination rate of broomrape seeds stimulated by leaf extracting solution at different time after drought treatment; B, The germination rate of broomrape seeds stimulated by root extracting solution at different time after drought treatment.

et al., 2018). In conjunction with these reports, the SLs levels were strongly increased in leaves under drought stress, accompanied by the up-regulation of genes involved in SLs biosynthesis, such as *VvCCD7* and *VvCCD8* (Fig. 9A). While in roots they were largely decreased (Fig. 9B), in line with the findings in Lotus that water deficit caused by osmotic stress, resulting from a treatment with polyethylene glycol (PEG), inhibited SL accumulation in roots (Liu et al., 2015). Visentin et al. (2016) demonstrated in tomato that the decrease of SLs in roots might be a systemic signal of drought stress and may prime shoots for drought stress avoidance. This result seems to be inconsistent with the results of Haider et al. (2018), which may be due to the fact that the forms of drought stress are not comparable in terms of drought severity and adaptation time. It is well known that different types of drought provoke a different repertoire of plant responses inherent to the different strategies that plants use for their survival during these conditions (Pinheiro and Chaves, 2011). Haider et al. (2018) also found that the content of strigolactone produced under severe drought conditions is much lower than that under mild drought treatment (about 1/5 of the latter), which is an energy-saving strategy for plants to cope with adverse stress. The decreasing of the production of SLs was suggested to be a prerequisite to allow rapid accumulation of ABA in the shoot (Visentin et al., 2016). ABA, the well-known stress-response hormone, was found to accumulate both in roots and leaves (Table 2), accompanied by the up-regulation of *NCED1*, the gene encoding key enzyme involved in ABA biosynthesis, and the down-regulation of *HYD1* and *HYD2*, the ABA catabolic genes. Recent studies showed that ABA was mainly biosynthesized in leaf in response to water stress in tomato and poplar (Mcadam et al., 2016) and was transported basipetally to the roots where it promotes root growth to absorb more water. *VvD27*, the gene reported to be linker integrating ABA and SLs, was up-regulated first, which may be a response to drought, and then down-regulated in roots. As Haider et al. (2018) suggested, its down-regulation may be a negative feedback resulting from increased ABA content (Fig. 10). Organ-specific SL-ABA interplay was previously reported (Ha et al., 2014; Liu et al., 2015; Visentin et al., 2016). Contrary changes were also observed between them in our study, especially in the D0 group. While, what we are seeing now is only the appearance, and how they interact with each other requires more in-depth research. As for MeJA, there were some reports on its interaction with SLs in the process of hypocotyl elongation (Jia et al., 2014), though whether it is conserved in drought stress still requires further exploration.

5. Conclusions

In conclusion, foliar application of GR24 prior to PEG-induced drought treatment could mitigate the adverse effects, including the decrease of RWC and oxidase-induced damage to the photosystems, and could also modulate the chlorophyll component and levels of different hormones. The application of GR24 may be an effective strategy to improve the drought tolerance of grapevine seedlings.

Conflicts of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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CRediT authorship contribution statement

Zhuo Min: Formal analysis, Writing – original draft. **Runyu Li:** Formal analysis, Writing – original draft. **Li Chen:** Formal analysis. **Yang Zhang:** Formal analysis, Writing – original draft. **Ziyu Li:** Formal analysis. **Min Liu:** Formal analysis. **Yanlun Ju:** Formal analysis. **Yulin Fang:** Writing – review & editing.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.plaphy.2018.11.037>.

References

- Chen, L., Jun, A.I., Wang, Z.X., Zhao, Y., 2011. Research Progress on effect of drought stress on the physiological property and microstructure in grapevine. *Northern Hortic.* 06, 205–209.
- Cooper, J.W., Hu, Y., Beyyoudh, L., Yildiz, D.H., Kunert, K., Beveridge, C.A., et al., 2018. Strigolactones positively regulate chilling tolerance in pea and in Arabidopsis. *Plant Cell Environ.* 41, 1298–1310. <https://doi.org/10.1111/pce.13147>.
- Dale, M.P., Causton, D.R., 1992. Use of the chlorophyll a/b ratio as a bioassay for the light environment of a plant. *Funct. Ecol.* 6, 190–196. <https://doi.org/10.2307/2389754>.
- Du, H., Huang, F., Wu, N., Li, X., Hu, H., Xiong, L., 2018. Integrative regulation of drought escape through ABA-dependent and -independent pathways in rice. *Mol. Plant* 11, 584–597. <https://doi.org/10.1016/j.molp.2018.01.004>.
- Dun, E.A., Brewer, P.B., Beveridge, C.A., 2009. Strigolactones: discovery of the elusive shoot branching hormone. *Trends Plant Sci.* 14, 364–372. <https://doi.org/10.1016/j.tplants.2009.04.003>.
- Fanizza, G., Ricciardi, L., 2015. Influence of drought stress on shoot, leaf growth, leaf water potential, stomatal resistance in wine grape genotypes (*Vitis vinifera* L.). *Vitis* 29, 371–381.
- Ferrandino, A., Perrone, I., Tramontini, S., Lovisolo, C., 2009. Physiological and molecular mechanisms of resistance to drought in *Vitis vinifera* L.: aspects of primary and secondary metabolisms and adaptation of genotypes. *Italus Hortus.* 16, 23–44.
- Ferrero, M., Pagliarini, C., Novák, O., Ferrandino, A., Cardinale, F., Visentin, I., et al., 2018. Exogenous strigolactone interacts with abscisic acid-mediated accumulation of anthocyanins in grapevine berries. *J. Exp. Bot.* 69, 2391–2401. <https://doi.org/10.1093/jxb/ery033>.
- Gao, J., 2006. *Experimental Guidance for Plant Physiology*. Higher education press, Beijing.
- Gomez-Roldan, V., Feras, S., Brewer, P.B., Puech-Pages, V., Dun, E.A., Pillot, J.P., et al., 2008. Strigolactone inhibition of shoot branching. *Nature* 455, 189–194. <https://doi.org/10.1038/nature07271>.
- Ha, C., Leyva-González, M., Osakabe, Y., Tran, U., Nishiyama, R., Watanabe, Y., et al., 2014. Positive regulatory role of strigolactone in plant responses to drought and salt stress. *Proc. Natl. Acad. Sci. U.S.A.* 111, 851–856. <https://doi.org/10.1073/pnas.1322135111>.
- Haider, I., Andrejimejnez, B., Bruno, M., Bimbo, A., Floková, K., Abuauf, H., et al., 2018. The interaction of strigolactones with abscisic acid during the drought response in rice. *J. Exp. Bot.* 69, 2403–2414. <https://doi.org/10.1093/jxb/ery089/4925813>.
- Hoagland, R., 1950. *The water culture methods for growing plants without soil*. *Cal. Agric. Exp. St. Circ.* 347.
- Huang, J.P., Yu, H.P., Guan, X.D., Wang, G.Y., Guo, R.X., 2016. Accelerated dryland expansion under climate change. *Nat. Clim. Change* 6, 166–171. <https://doi.org/10.1038/nclimate2837>.
- Jia, K.P., Luo, Q., He, S.B., Lu, X.D., Yang, H.Q., 2014. Strigolactone-regulated hypocotyl elongation is dependent on cryptochrome and phytochrome signaling pathways in Arabidopsis. *Mol. Plant* 7, 528–540. <https://doi.org/10.1093/mp/sst093>.
- Kadioglu, A., Saruhan, N., Sağlam, A., Terzi, R., Acet, T., 2011. Exogenous salicylic acid alleviates effects of long term drought stress and delays leaf rolling by inducing antioxidant system. *Plant Growth Regul.* 64, 27–37. <https://doi.org/10.1007/s10725-010-9532-3>.
- Kapulnik, Y., Koltai, H., 2014. Strigolactone involvement in root development, response to abiotic stress, and interactions with the biotic soil environment. *Plant Physiol.* 166, 560–569. <https://doi.org/10.1104/pp.114.244939>.
- Li, G., Tian, M., Shen, R., 2017. Analysis of chlorophyll fluorescence parameters in leaves of strigolactone mutants of Arabidopsis thaliana. *J. Zhejiang A & F Univ.* 34, 36–41.
- Liu, J.W., He, H.Z., Vitali, M., Visentin, I., Charnikhova, T., Haider, I., et al., 2015. Osmotic stress represses strigolactone biosynthesis in Lotus japonicus roots: exploring the interaction between strigolactones and ABA under abiotic stress. *Planta* 241, 1435–1451. <https://doi.org/10.1007/s00425-015-2266-8>.
- Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-

- time quantitative PCR and the 2^{-ΔΔC_T} method. *Methods* 25, 402–408. <https://doi.org/10.1006/meth.2001.1262>.
- LovinBabaei, Marandi, R.J., Abbaspour, N., 2014. The effect of salicylic acid on photosynthetic system of two varieties of grapes (*Vitis vinifera* L.) under drought stress conditions. *Int. J. Plant Anim. Envir. Sci.* 4, 630–640.
- Lu, S., Zhuo, C., Wang, X., Guo, Z., 2014. Nitrate reductase (NR)-dependent NO production mediates ABA- and H₂O₂-induced antioxidant enzymes. *Plant Physiol. Bioch.* 74, 9–15. <https://doi.org/10.1016/j.plaphy.2013.10.030>.
- Ma, N., Hu, C., Wan, L., Hu, Q., Xiong, J., Zhang, C., 2017. Strigolactones improve plant growth, photosynthesis, and alleviate oxidative stress under salinity in rapeseed (*Brassica napus* L.) by regulating gene expression. *Front. Plant Sci.* 8, 1671. <https://doi.org/10.3389/fpls.2017.01671>.
- Mayzlish-Gati, E., LekKala, S.P., Resnick, N., Winger, S., Bhattacharya, C., Lemcoff, J.H., et al., 2010. Strigolactones are positive regulators of light-harvesting genes in tomato. *J. Exp. Bot.* 61, 3129–3136. <https://doi.org/10.1093/jxb/erq138>.
- Mcadam, S.A.M., Manzi, M., Ross, J.J., Brodribb, T.J., Gómez-Cadenas, A., 2016. Uprooting an abscisic acid paradigm: shoots are the primary source. *Plant Signal. Behav.* 11 (6), 652–659. <https://doi.org/10.1080/15592324.2016.1169359>.
- Meng, J.F., Xu, T.F., Wang, Z.Z., Fang, Y.L., Xi, Z.M., Zhang, Z.W., 2014. The ameliorative effects of exogenous melatonin on grape cuttings under water-deficient stress: antioxidant metabolites, leaf anatomy, and chloroplast morphology. *J. Pineal Res.* 57, 200–212. <https://doi.org/10.1111/jpi.12159>.
- Min, Z., Zhao, X., Li, R., Yang, B., Liu, M., Fang, Y., 2017. Comparative transcriptome analysis provides insight into differentially expressed genes related to bud dormancy in grapevine (*Vitis vinifera*). *Sci. Hortic.* 225, 213–220. <https://doi.org/10.1016/j.scienta.2017.06.033>.
- Munné-Bosch, S., Müller, M., 2013. Hormonal cross-talk in plant development and stress responses. *Front. Plant Sci.* 4, 529. <https://doi.org/10.3389/fpls.2013.00529>.
- Pinheiro, C., Chaves, M.M., 2011. Photosynthesis and drought: can we make metabolic connections from available data? *J. Exp. Bot.* 62, 869–882. <https://doi.org/10.1093/jxb/erq340>.
- Ruyterspira, C., Albabili, S., Krol, S.V.D., Bouwmeester, H., 2013. The biology of strigolactones. *Trends Plant Sci.* 18, 72–83. <https://doi.org/10.1016/j.tplants.2012.10.003>.
- Salazar-Parra, C., Aguirreolea, J., Sánchez-Díaz, M., Irigoyen, J.J., Morales, F., 2012. Climate change (elevated CO₂, elevated temperature and moderate drought) triggers the antioxidant enzymes' response of grapevine cv. Tempranillo, avoiding oxidative damage. *Physiol. Plantarum* 144, 99–110. <https://doi.org/10.1111/j.1399-3054.2011.01524.x>.
- Schoonjans, F., 2012. *Digitizer Manual*.
- Sedaghat, M., Tahmasebisarvestani, Z., Emam, Y., Mokhtassibidgoli, A., 2017. Physiological and antioxidant responses of winter wheat cultivars to strigolactone and salicylic acid in drought. *Plant. Physiol. Bioch.* 119, 59–69. <https://doi.org/10.1016/j.plaphy.2017.08.015>.
- Umehara, M., Hanada, A., Yoshida, S., Akiyama, K., Arite, T., TakedaKamiya, N., et al., 2008. Inhibition of shoot branching by new terpenoid plant hormones. *Nature* 455, 195–200. <https://doi.org/10.1038/nature07272>.
- Visentin, I., Vitali, M., Ferrero, M., Zhang, Y., Ruyter-Spira, C., Novák, O., et al., 2016. Low levels of strigolactones in roots as a component of the systemic signal of drought stress in tomato. *New Phytol.* 212, 954–963. <https://doi.org/10.1111/nph.14190>.
- Wang, Z., Zheng, P., Meng, J., Xi, Z., 2015. Effect of exogenous 24-epibrassinolide on chlorophyll fluorescence, leaf surface morphology and cellular ultrastructure of grape seedlings (*Vitis vinifera* L.) under water stress. *Acta Physiol. Plant.* 37, 1–12. <https://doi.org/10.1007/s11738-014-1729-z>.
- Wang, Z.X., Chen, L., Ai, J., Qin, H.Y., Liu, Y.X., Xu, P.L., et al., 2012. Photosynthesis and activity of photosystem II in response to drought stress in Amur Grape (*Vitis amurensis* Rupr.). *Photosynthetica* 50, 189–196. <https://doi.org/10.1007/s11099-012-0023-9>.
- Yao, Y., Liu, X., Li, Z., Ma, X., Wang, X., 2013. Drought-induced H₂O₂ accumulation in subsidiary cells is involved in regulatory signaling of stomatal closure in maize leaves. *Planta* 238, 217–227. <https://doi.org/10.1007/s00425-013-1886-0>.
- Yoneyama, K., Xie, X., Sekimoto, H., Takeuchi, Y., Ogasawara, S., Akiyama, K., et al., 2008. Strigolactones, host recognition signals for root parasitic plants and arbuscular mycorrhizal fungi, from Fabaceae plants. *New Phytol.* 179, 484–494. <https://doi.org/10.1111/j.1469-8137.2008.02462.x>.
- You, C., Zhu, H., Xu, B., Huang, W., Wang, S., Ding, Y., et al., 2016. Effect of removing superior spikelets on grain filling of inferior spikelets in rice. *Front. Plant Sci.* 7, 1161. <https://doi.org/10.3389/fpls.2016.01161>.
- Yue, Z., Liu, H., Ma, F., 2015. The Malus carotenoid cleavage dioxygenase 7 is involved in stress response and regulated by basic pentacysteine 1. *Sci. Hortic.* 192, 264–270. <https://doi.org/10.1016/j.scienta.2015.06.027>.
- Zhang, Y., Lv, S., Wang, G., 2018. Strigolactones are common regulators in induction of stomatal closure in planta. *Plant Signal. Behav.* 13, 01–12. <https://doi.org/10.1080/15592324.2018.1444322>.