Plant Physiology and Biochemistry 148 (2020) 114–121

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Research article

# Negative regulation by transcription factor VvWRKY13 in drought stress of *Vitis vinifera* L

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ARTICLE INFO

*Keywords:*
VvWRKY13
*Vitis vinifera* L
Drought
Negative regulation
ROS
Osmotic regulator substance

ABSTRACT

Drought is a major environmental factor limiting crop growth and development worldwide. WRKY transcription factor, a unique transcription factor in plants, has been shown to play important roles in plant response to abiotic stress. Previously, we have cloned the *VvWRKY13* gene from resistant grape varieties and found that its expression was obviously induced by drought. Here we further explored the mechanism of VvWRKY13 in response to drought stress. After drought treatment, the expression of *VvWRKY13* in the sensitive grape varieties was significantly higher than resistant grape varieties. Moreover, phenotypic changes of *VvWRKY13* transgenic *Arabidopsis* were observed and drought-related indexes were detected under drought treatment. The results showed that *VvWRKY13* transgenic *Arabidopsis* exhibited more sensitive phenotype to drought stress compared with wild type. The water loss rate of leaves in the transgenic *Arabidopsis* was significantly higher than wild type. The content of proline, soluble sugar and the expression of related genes decreased in transgenic *Arabidopsis* leaves under drought stress. The level of endogenous hydrogen peroxide and oxygen free radicals was increased, while the activity of catalase (CAT) and superoxide dismutase enzyme (SOD) were decreased. In addition, the expression of stress response gene was significantly decreased in transgenic *Arabidopsis*. Taken together, our results suggest that VvWRKY13 negatively modulates plant drought tolerance through regulating the metabolism of intracellular osmotic substances (proline, soluble sugar), the level of ROS, and the expression of stress-related genes.

## 1. Introduction

*Vitis vinifera* L. is an important economic fruit crop in the world. At present, China has become the second cultivation area and first production region of grape worldwide. The growth of grapes is often affected by environmental factors, such as drought and other stresses, which would seriously affect the yield and quality of grapes and then restrict the development of the grape industry (Król and Weidner, 2017).

Drought stress is a major environmental factor that limits plant growth, development, and geographic distribution. During long-term evolution, plant cells have maintained the normal metabolic processes under drought stress by forming regulatory mechanisms, such as the increase of osmotic regulatory substances and antioxidant enzyme activity (Zhu, 2002). In order to maintain the water content of cells and the osmotic balance between the intracellular and extracellular spaces, the osmotic potential of the cells was improved mainly by increasing the content of soluble substances such as proline, soluble sugar, betaine and polyamines (Hochberg et al., 2013). Besides, unique reactive oxygen scavenging systems (ROS) in plants, including catalase (CAT), superoxide dismutase (SOD), peroxidase (POD) and other antioxidant enzymes, could also work together to maintain the balance of ROS level and avoid oxidative damage to plants (Suzuki et al., 2011).

In response to various stresses, plants have been protected by complex regulatory networks in which transcription factors play a central role by regulating target gene expression leading to downstream physiological level changes (Agarwal et al., 2006). WRKY transcription factor, a unique transcription factor in plants, plays a positive or negative regulatory role in response to biological and abiotic stress (Rushton et al., 2010). WRKY transcription factors have completely conserved WRKY domains and zinc finger motifs. According to the number of WRKY domains and the characteristics of zinc finger motifs, they are divided into three subfamilies. The WRKY transcription factors can bind W-box elements in the promoter of downstream genes and regulate their expression level (Eulgem et al., 2000; Chen et al., 2017). AtWRKY57 can directly regulate the expression of *RD29A*, *ABA3* and

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https://doi.org/10.1016/j.plaphy.2020.01.008
Received 5 November 2019; Received in revised form 23 December 2019; Accepted 6 January 2020
Available online 08 January 2020
0981-9428/ 

*NCED3*, thereby affecting the synthesis of ABA and increasing the resistance to drought stress of plants (Jiang et al., 2012). TaWRKY19 can also bind to the promoter of *DREB2A* and activate the expression of *RD29A*, *RD29B* and *COR6.6*, which attribute the downstream stress-related gene to participate the process in response to drought stress (Niu et al., 2012). In addition, the transcription factor ZmWRKY106 isolated from maize can improve the drought and heat resistance of transgenic *Arabidopsis* by increasing the activity of antioxidant enzymes and the expression of related genes and reducing the content of ROS (Wang et al., 2018).

Genome sequencing revealed 59 WRKY transcription factors in grape (Guo et al., 2014; Wang et al., 2014), including three *WRKY* genes that were cloned from Chinese wild *Vitis pseudoreticulata* W. T. Wang 'Baihe-35-1'. Among which, *VpWRKY1*-overexpressing *Arabidopsis* and *VpWRKY2*-overexpressing *Arabidopsis* enhanced resistance to salt, cold, and disease (Li et al., 2010). *VpWRKY3* improved salt tolerance and disease resistance of transgenic tobacco (Zhu et al., 2012). The *VlWRKY3* gene was cloned from hybrid grape variety 'Kyoho' and transformed into *Arabidopsis*. The transgenic lines were resistant to salt, drought and disease (Guo et al., 2018). The grape *VvWRKY2* gene transformed into tobacco and enhanced salt and drought tolerance (Mzid et al., 2018). Besides, we also focused on the function of grape WRKY family members under abiotic stresses, such as overexpressed *VvWRKY30* in *Arabidopsis* enhanced tolerance to drought (Zhu et al., 2018) and salt (Zhu et al., 2019). However, the negative roles of grape WRKY transcription factors in response to drought stress have not been studied yet.

In a previous study, the *VvWRKY13* gene was cloned from the resistant grape variety 'Zuoyouhong'. Experimental results showed that *VvWRKY13* was located in the nucleus and functioned as transcription factor, which could be induced by drought, salt, abscisic acid (ABA) and ethylene (Ma et al., 2015; Hao et al., 2017). On the basis of these progress, we further detected the different expression of *VvWRKY13* among resistant and sensitive grape varieties with or without drought stress. *VvWRKY13* transgenic *Arabidopsis* was used to analyze the drought phenotype, the content of osmotic adjustment substances, the activity of antioxidant enzymes and the expression of drought-related marker genes under drought treatment to explore the function and mechanism of VvWRKY13 involved in drought stress.

## 2. Materials and methods

### 2.1. Plant material cultivation and treatment

Grape tissue culture seedling: 'Cabernet Sauvignon', 'Chardonnay', 'Zuoyouhong' and 'Vidal Blanc' shoots with buds were used as explants. They were washed with water overnight and then treated with 75% ethanol for 30 s, 0.1% $HgCl_2$ for 8 min, and rinsed with sterile water 6 times. The appropriate length (about 2–3 cm) of stem segment was cut with scissors and the apical bud was retained. They were inoculated on 1/2 MS + 0.1 $mgL^{-1}$ IAA root media under light intensity of 200 μmol $m^{-2}{\cdot}s^{-1}$ (25 ± 1 °C, 12 h/12 h photoperiod). The tissue culture seedlings were used in experiments after 45–55 d.

Grape treatment: Grape tissue culture plantlets at the 45–55 d phase were treated with 300 mmol $L^{-1}$ mannitol treatment for 9 h. The leaves of the treated plants were collected and stored in liquid nitrogen in order to detect the *VvWRKY13* expression level.

*Arabidopsis thaliana* cultivation: The seeds of *Arabidopsis thaliana* were placed in a 1.5 mL centrifuge tube, and sterilized for 8–10 min with 1 mL 10% NaClO, then rinsed with sterile water 5 times. After being treated at 4 °C for 3–4 days, the seeds were sown on sterile MS solid medium and cultured in a light incubator (22 °C ± 2 °C, 16 h/8 h photoperiod) with the light intensity of 120 μmol $m^{-2}{\cdot}s^{-1}$.

*Arabidopsis thaliana* treatment: After 1 week of vertical culture on sterile MS solid medium, the seedlings of *VvWRKY13* transgenic *Arabidopsis* and wild type were transferred into the soil pots in a light incubator (22 °C ± 2 °C, 16 h/8 h photoperiod) with the light intensity of 120 μmol $m^{-2}{\cdot}s^{-1}$. The small pots of different seedlings were placed randomly and the position of small pots was adjusted frequently. After 2 weeks growth, the seedlings were treated with natural drought for about 20 days, then the phenotypes were observed, photographed and sampled in order to test the changes in proline content, superoxide dismutase (SOD), catalase (CAT) activities, soluble sugar content and expression levels of drought-related gene.

### 2.2. Determination of water loss rate of isolated leaves

Rosette leaves of 4-week-old *Arabidopsis* were cut and placed on dry filter paper in constant temperature incubator (25 °C). The weight was measured after 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 h and the water loss rate was calculated. The experiment was repeated three times.

### 2.3. Quantitative real-time PCR

Total RNA was extracted from the leaves of the treated plants using the CTAB methods (Iandolino et al., 2004). The first chain of cDNA was synthesized via the M-MLV RT Kit (Promega, USA). Real-time PCR was performed using the MyiQ Real-Time PCR Detection System (Bio-Rad, USA) in the presence of SYBR green I (BioWhittaker Molecular Applications) in the amplification mixture. The qRT-PCR program was as follows: 60 s at 95 °C, 10 s at 95 °C, 20 s at 56 °C, and 15 s at 72 °C in 40 cycles. The Melt curve is maintained for 45 s at the first step from 72 °C to 99 °C, and for 5 s for every subsequent increase of 1 °C. Three independent experiments are done, each with three replicates. Calculation referring to the method of $2^{-\Delta\Delta CT}$ (Livak and Schmittgen, 2001). Oligonucleotides used in real-time PCR experiments are listed in Table 1.

### 2.4. Determination of proline content

Proline content was measured by ninhydrin reaction. The wild type and *VvWRKY13* transgenic *Arabidopsis* were treated with drought stress according to the above methods; 0.5 g leaves were treated with 5 mL 3% sulfosalicylic acid at 100 °C for 10 min. Then the extract of 2 mL was added to 2 mL glacial acetic acid and 3 mL 2.5% acid ninhydrin reagent, with heating at 100 °C for 30 min. After cooling at room temperature, 4 mL methyl benzene was added and oscillated for 30 s and incubated for 10 min. The absorbance test at 520 nm wavelength

**Table 1**
Real-time PCR primers.

| Gene name | Primer sequence (5′ to 3′) |
|---|---|
| *VvWRKY13* | FP: GTTGCCAACAATCCCT |
| | RP: TCATCTCCACCGATACTTC |
| *VvACTIN* | FP: ATAGAAGCAGCAAGGGA |
| | RP: TGAGGCTCTTACTAATG |
| *AtBAM4* | FP: GCTTCCGTCTTGTCTCGTCA |
| | RP:TGCCTTAGTCCCATCTCATC |
| *AtBAM1* | FP:TGTGATTCTGTGCCTGTC |
| | RP: CAATAACTCCTCCTATGTAGC |
| *AtP5CS1* | FP: AGGGAAAGTTCCAGAAAG |
| | RP: CATAACTAAGCGAGCCAC |
| *AtSS1* | FP: AAATCAGCTATACGCAATG |
| | RP: ATCTTTCGACAAGGGAGT |
| *AtRD29A* | FP: GGTTGGGAGGATTAAAGGATG |
| | RP: AACAGTGGAGCCAAGTGATTG |
| *AtRD29B* | FP: TGGTGGGGAAAGTTAAAGGAG |
| | RP: TTCCCAGAATCTTGAACTCCC |
| *AtRD22* | FP: GACCCTACACTCAACACTAC |
| | RP: TTCATTGCCTCCACGAATGC |
| *AtDREB2A* | FP: TGACCTAAATGGCGACGATGT |
| | RP: TCCAAGTAACTCAAGTCGTCG |
| *AtACTIN* | FP: GGTAACATTGTGCTCAGTGGTGG |
| | RP: CACGACCTTAATCTTCATGCTGC |

was measured with methyl benzene solution as control.

### 2.5. Determination of soluble sugar content

The content of soluble sugar was detected by anthrone colorimetry. The leaves of *Arabidopsis* were ground with 10 mL distilled water, then boiled at 100 °C for 10 min, then diluted to 100 mL after cooling to room temperature. To an extract of 1 mL, 1 mL distilled water and 0.5 mL anthrone were added, then slow addition of 5 mL concentrated sulfuric acid, gently shaken, placed boiling water bath for 10 min. The absorbance was detected at 620 nm.

### 2.6. Detection of reactive oxygen (ROS)

1 g leaves were extracted with 5 mL acetone. 1 mL extract was added to 0.1 mL TiCl–$H_2SO_4$ solution and 0.2 mL ammonia solution, then centrifuged for 10 min. The precipitation was dissolved in 3 mL sulfuric acid and the absorbance was detected at 410 nm. At the same time, rosette leaves of *Arabidopsis* were used for DAB histochemical staining in 3,3′-diaminobenzidine (DAB) solution (Sigma-Aldrich; 1 g $L^{-1}$ DAB-HCl, pH = 3.8) according to previous publication (Zhang et al., 2011). 0.5 g leaves were added to 5 mL phosphate buffer (pH = 7.8), ground to homogenate, and centrifuged. 0.5 mL supernatant was added to 0.5 mL phosphate buffer (pH = 7.8) and 1 mL hydroxylamine hydrochloride, kept at 25 °C for 1 h, then 1 mL p-aminobenzenesulfonic acid and 1 mL α-naphthylamine were added, and kept at 2 °C for 20 min. The absorbance was detected at 530 nm. Rosette leaves were used for nitroblue tetrazole (NBT) histochemical staining (Zhang et al., 2011).

### 2.7. Measurement of antioxidant enzyme activity

The activity of superoxide dismutase (SOD) and catalase (CAT) were detected according to the following method (Nourimand and Todd, 2016). *Arabidopsis* leaves were homogenized and centrifuged, then the ability of the supernatant to inhibit photochemical reduction of NBT chloride by 50% was considered as one unit of SOD activity. CAT activity was assayed using ultraviolet spectrophotometry.

### 2.8. Statistical analysis

Statistical analysis for all experiments were carried out using SAS, and the statistical significance evaluated by one-way ANOVA. Tukey's post hoc test was applied. A difference was considered significant at $P < 0.05$. Data are the means ± SE of three independent experiments.

## 3. Results

### 3.1. Expression pattern of VvWRKY13 in different grape varieties leaves under drought stress

In order to study VvWRKY13 response to drought stress in grapevine, the expression pattern of *VvWRKY13* in leaves was detected by analysis of the tissue culture seedlings of different varieties of resistance after drought treatment. The expression of *VvWRKY13* in the leaves of sensitive grape varieties 'Cabernet Sauvignon' and 'Chardonnay' were much higher than that of the other four resistant varieties 'Zuoyouhong', 'Vidal Blanc', 'Kyoho' and 'Beta' under normal condition. After mannitol treatment to simulate drought stress, the *VvWRKY13* expression of different resistant grape varieties increased significantly, and the high expression of *VvWRKY13* was most significant in the sensitive grape variety 'Cabernet Sauvignon' and 'Chardonnay' (Fig. 1). This indicated that the expression of *VvWRKY13* was significantly induced by drought stress and *VvWRKY13* might play a negative regulatory role in this response.

### 3.2. Phenotypic analysis of VvWRKY13-overexpressing Arabidopsis under drought stress

To further test if *VvWRKY13* is involved in response to drought stress. Three weeks old wild type and *VvWRKY13*-overexpressing *Arabidopsis* plants were used as materials for natural drought treatment. In normal condition, there was almost no difference between the growth of transgenic *Arabidopsis* and wild type. However, after 21 days of natural drought treatment, the drought stress of *VvWRKY13*-overexpressing *Arabidopsis* was significantly higher than that of wild type, followed with dark green leaves, lack of luster and different degrees of wilting (Fig. 2A). It was also found that the fresh weight of transgenic *Arabidopsis* plants was significantly lower than wild type (Fig. 2B). These results showed that *VvWRKY13* was indeed involved in drought stress response as a negative regulatory factor.

### 3.3. VvWRKY13-overexpressing Arabidopsis is sensitive to drought stress due to decrease of water in leaves

To address how *VvWRKY13* participated in the response of drought stress, the rate of water loss in leaves of wild type and *VvWRKY13*-overexpressing *Arabidopsis* plants was analyzed. The results showed that the rate of water loss in leaves of transgenic *Arabidopsis* was much faster than that of wild type (Fig. 3A and B), which suggested that *VvWRKY13*-overexpressing lines showed more sensitive to drought stress might be related to the increased rate of water loss compared with wild type.

### 3.4. VvWRKY13 participates in drought stress by reducing content of proline and soluble sugar

Proline and soluble sugar, which are important osmotic regulators, participate in various stress responses of plants has been reported. Under drought stress, the proline and soluble sugar content of wild type and *VvWRKY13*-overexpressing *Arabidopsis* were significantly increased, but the change range of wild type was significantly higher than transgenic lines (Fig. 4A and C). Drought stress could also significantly induce the expression of proline biosynthesis key enzyme gene *P5CS1*, β-amylase gene *BAM1*, *BAM4,* and sucrose synthase gene *SS1*. The expression of above genes in wild type was significantly higher than that in transgenic *Arabidopsis* lines (Fig. 4B, D, E and F). These results prove that *VvWRKY13* is involved in the response to drought stress by reducing metabolism of proline and soluble sugar.

### 3.5. VvWRKY13 participates in drought stress by increasing reactive oxygen species accumulation

Reactive oxygen species (ROS) are significantly accumulated under abiotic stress conditions. Thus, the effects of drought stress on the content of $H_2O_2$, $O_2^{\cdot-}$ and the activity of related protective enzymes (CAT, SOD) were detected in the leaves of wild type and *VvWRKY13*-overexpressing *Arabidopsis*. NBT and DAB staining were used to further analyze the content of ROS. The results showed that the content of $O_2^{\cdot-}$ and $H_2O_2$ in transgenic *Arabidopsis* were significantly higher than those in wild-type plants under drought stress (Fig. 5A–D). In normal condition, the enzyme activity of CAT and SOD in *VvWRKY13*-overexpressing *Arabidopsis* was not significantly different with wide type, but the activity of these antioxidative enzyme in wild type was significantly higher than that of *VvWRKY13*-overexpressing *Arabidopsis* under drought stress (Fig. 5E and F). Therefore, *VvWRKY13* could negatively regulate the response to drought stress by enhancing content of ROS.

### 3.6. VvWRKY13 is involved in response to drought stress by reducing the expression of drought-related gene

In order to explore whether VvWRKY13 regulates the expression of

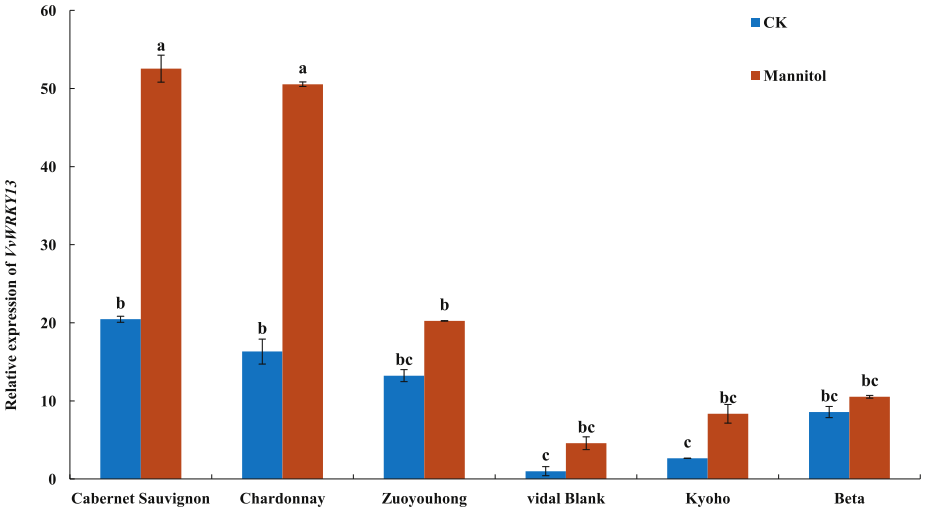


**Fig. 1. Relative expression level of VvWRKY13 in different grape varieties**. Difference in *VvWRKY13* expression between the sensitive varieties 'Cabernet Sauvignon', 'Chardonnay' and the resistant varieties 'Zuoyouhong','Vidal Blanc', 'Kyoho' and 'Beta' with 300 mmol·L$^{-1}$ mannitol treatment after 9 h. Three independent experimental replications were conducted. Values are the means ± SE of three independent experiments ($P < 0.05$).

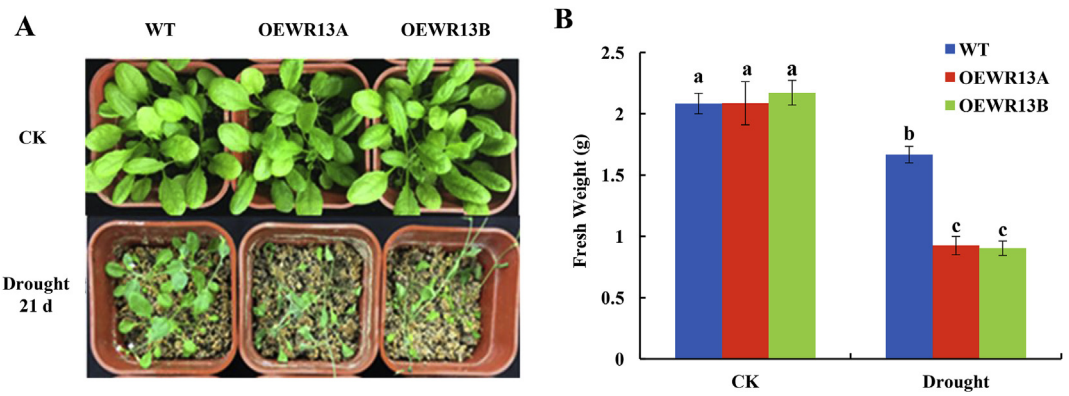


**Fig. 2. Phenotypic of *VvWRKY13*-overexpressing *Arabidopsis* plants responses to drought stress**. *VvWRKY13* transgenic *Arabidopsis* at the three-week phase was treated with natural drought for 20 d. **A.** Phenotypic differences between WT and transgenic *Arabidopsis* plants under natural drought. These experiments were repeated three times at least and 30 plants for each individual line were used in each repeated experiment. **B.** Fresh weight of aboveground part of WT and transgenic *Arabidopsis* plants. Three independent experimental replications were conducted. Values are the means ± SE of three independent experiments ($P < 0.05$).

stress-related genes under drought stress, qRT-PCR technique was used to analyze the expression of drought-related marker genes *RD29A* (*Responsive to Dehydration 29A*), *RD29B* (*Responsive to Dehydration 29B*), *RD22* (*Responsive to Dehydration 22*), *DREB2A* (*Dehydration-Responsive Element Binding protein 2A*) in different transgenic lines with or without drought treatment (Fig. 6). After drought treatment, the expression of *RD29A*, *RD29B*, *RD22* and *DREB2A* was up-regulated in both wild type and overexpressing lines, but the rangeability in overexpressing lines was lower than that in wild type. It is believed that VvWRKY13 may negatively regulate drought response of plants through inhibiting the upregulation of *RD29A*, *RD29B*, *RD22*, and *DREB2A*.

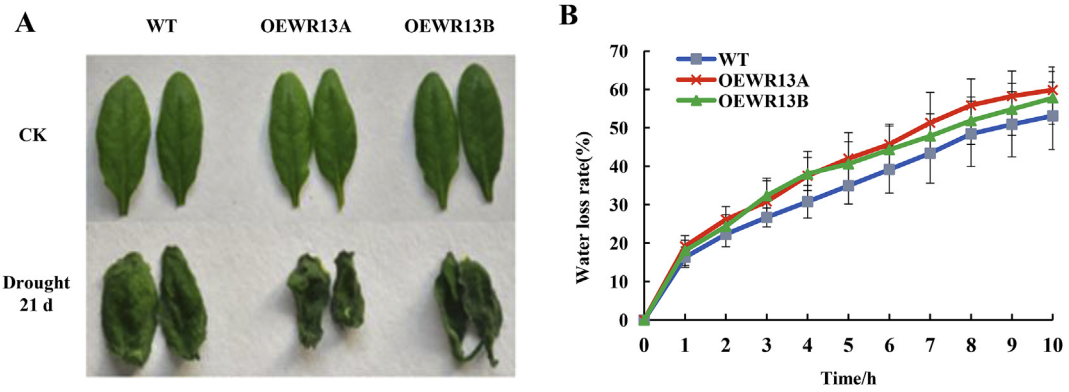


**Fig. 3. Leaves phenotype and water loss rate of *VvWRKY13* transgenic *Arabidopsis* under drought stress**. The rosette leaves of *Arabidopsis* at four-week phase were cut off and placed on a dry filter paper. The leaves were weighed after 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 h in an incubator (25 °C) and the water loss rate was calculated. **A.** Phenotypic differences between WT and transgenic *Arabidopsis* plants after drought stress for 10 h. These experiments were repeated three times at least and 30 plants for each individual line were used in each repeated experiment. **B.** The water loss rate of WT and transgenic *Arabidopsis* plants. Three independent experimental replications were conducted.

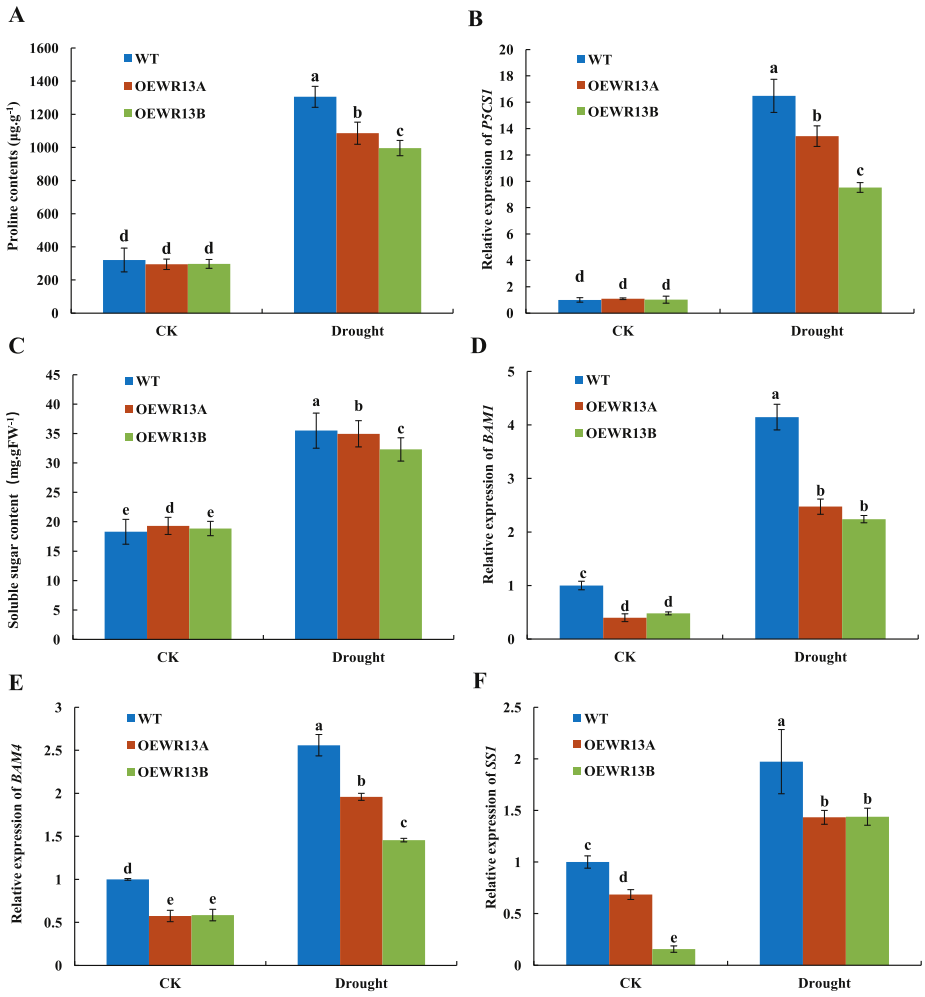


**Fig. 4. Effects of drought stress on proline and soluble sugar metabolism of *VvWRKY13*-overexpressing *Arabidopsis* leaves.** The proline content (**A**) and *P5CS1* expression (**B**), soluble sugar content (**C**) and *BAM1* expression (**D**), *BAM4* expression (**E**), *SS1* expression (**F**) in the leaves of overexpressing lines under drought stress. Three independent experimental replications were conducted. Values are the means ± SE of three independent experiments ($P < 0.05$).

## 4. Discussion

Drought severely restricts the growth and development of plants. Genetic engineering can be used to improve drought resistance of plant effectively. In this study, we identified a negative regulated transcription factor VvWRKY13 under drought stress, which was expressed differently among sensitive and resistant grape varieties. The results showed that the expression of *VvWRKY13* in leaves of sensitive grape varieties 'Cabernet Sauvignon' and 'Chardonnay' was significantly higher than that of resistant grape varieties with or without drought treatment (Fig. 1). It is speculated that transcription factor VvWRKY13 might play a negative role in response to drought resistance of grape.

WRKY transcription factors, such as OsWRKY13 (Xiao et al., 2013), AtWRKY13 (Li et al., 2016), GmWRKY13 (Zhou et al., 2008), are closely related to VvWRKY13, belong to WRKY13 transcription factor Group II C subfamily. WRKY13 transcription factors in different species play a major role in negative regulation. For example, OsWRKY13, a transcription suppressor in rice, can specifically bind to the W-box region of *SNAC1* and *WRKY45-1* gene promoter, thereby weakening the drought resistant (Xiao et al., 2013). Moreover, the AtWRKY13 of *Arabidopsis* inhibited the binding of AtWRKY12 to the promoter of *FUL* gene and acted as a blooming inhibitor (Li et al., 2016). The GmWRKY13 of soybean had the same function as VvWRKY13 and enhanced the drought sensitivity of transgenic lines (Zhou et al., 2008). Duan et al. (2014) isolated *MdWRKY13* gene from apple (*Malus domestica*) and found that overexpression of this gene in *Arabidopsis* resulted in drought tolerance decrease when compared to wild type plants, indicated that MdWRKY13 seems to be a negative regulator in drought stress response. In this study, *VvWRKY13*-overexpressing *Arabidopsis* showed a sensitive phenotype to drought stress (Fig. 2A and B) and accelerated water loss rate in leaves (Fig. 3A and B). It is suggested that VvWRKY13, as a transcription suppressor, is involved in the response to drought stress.

Under drought stress, plants usually alleviates damage by increasing the content of proline, soluble sugar and other osmotic regulators (Jin et al., 2013). The heterologous overexpression of wheat TaWRKY10 could increase the content of soluble sugar and proline, decrease the content of ROS and MDA, and improve the drought resistance of transgenic tobacco (Wang et al., 2013). In this experiment, the contentof proline, soluble sugar and other osmotic substances in *VvWRKY13*-overexpressing *Arabidopsis* were decreased (Fig. 4A and C), and the expression of genes related to proline synthesis and soluble sugar metabolism was significantly decreased (Fig. 4B, D, 4E and 4F). It is inferred that *VvWRKY13*-overexpressing *Arabidopsis* was sensitive to osmotic stress by reducing osmotic regulators such as proline and soluble sugar. As VvWRKY13 is a typical transcription factor, what is the relationship between VvWRKY13 and proline synthesis gene *P5CS1*, amylase related genes *BAM1*, *BAM4*, or sucrose synthase related gene *SS1*? Is there a direct interaction of each other? Further research will be performed.

Under stress, the content of ROS in plant is often accumulated in large quantities, which results into serious membrane lipid

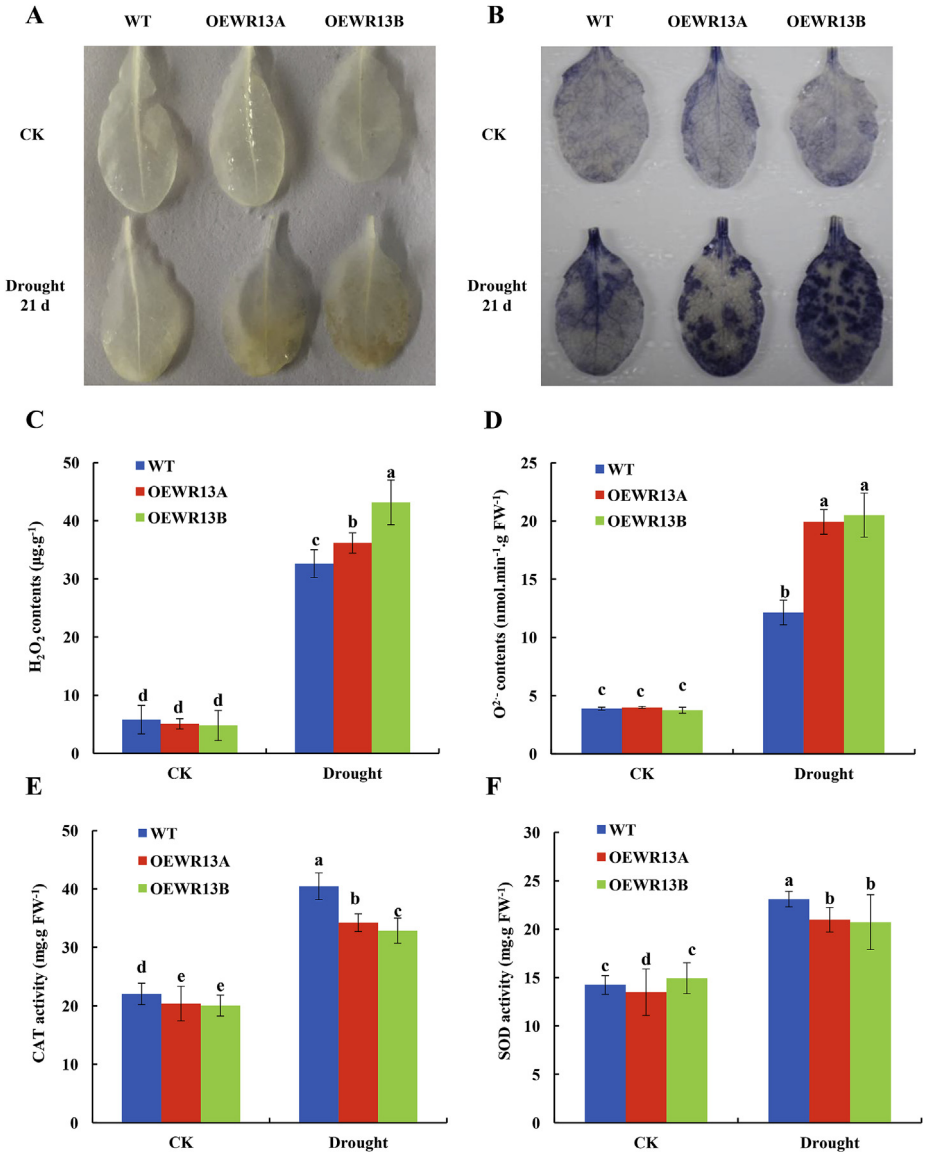


**Fig. 5. Effects of drought stress on ROS accumulation of *VvWRKY13*-overexpressing *Arabidopsis* leaves**. The DAB histochemical staining (**A**) and the NBT histochemical staining (**B**), $H_2O_2$ content (**C**) and $O_2^{\cdot-}$ content (**D**), activity of CAT (**E**) and SOD (**F**) in the leaves of overexpressing lines under drought stress. Three independent experimental replications were conducted. Values are the means ± SE of three independent experiments ($P < 0.05$).

peroxidation, loss of cell membrane function and destruction of intracellular environment, thus affecting the normal growth of plant (Zheng et al., 2006). ROS scavenging mechanisms have been developed in plants, mainly including some antioxidant enzymes and strong reductive small molecules. The activity of antioxidant enzymes in plants can often reflect their resistance to stress. It was reported that the SOD, POD, CAT activity of grape leaves increased significantly under drought stress (Haider et al., 2017). In this experiment, the content of endogenous hydrogen peroxide and oxygen free radical in *Arabidopsis* were increased (Fig. 5A–D) and the activities of SOD and CAT were decreased (Fig. 5E and F), indicating that the ability of VvWRKY13-overexpressing *Arabidopsis* to resist drought stress was weakened.

Plants respond to drought stress relying on the regulation of gene expression in ABA-dependent pathway and ABA-independent pathway. The expression of marker genes *RD22*, *RD29B* in ABA-dependent pathway and marker genes *RD29A*, *DREB2A* in ABA-independent pathway in *Arabidopsis* was detected under drought stress (Shinozaki and Yamaguchi-Shinozaki, 1997). Consistently, it was found that the expression of *RD22*, *RD29B*, *RD29A* and *DREB2A* in *VvWRKY13*-overexpressing *Arabidopsis* was also lower than that in wild type (Fig. 6A–D). Above data speculated that VvWRKY13 may inhibit the expression of genes related to ABA-dependent pathway and ABA-independent pathway, and then decreased the plant resistance to drought stress. It has been reported that *VvWRKY11* gene transformed into *Arabidopsis* would induce the expression of *RD29A* and *RD29B* genes to enhance the drought tolerance in plants (Liu et al., 2011). Analysis of the promoter regions of *AtRD29B* and *AtRD22* revealed that they contained W-box and ABRE cis-elements. Can VvWRKY13 directly bind to their promoters and inhibit their expression? If so, whether these interactions are drought-specific or not? These questions may be solved in nearly future.

In our previous work, VvWRKY13 were involved in ethylene biosynthesis (Ma et al., 2015) and ABA biosynthesis (Hao et al., 2017). They detected ethylene and ABA content in *VvWRKY13* over-expressing *Arabidopsis* leaves under normal condition not under drought stress. In this study, our results showed that *VvWRKY13* transgenic *Arabidopsis* exhibited more sensitive phenotype to drought stress compared with wild type. There were many factors influencing plant response to drought, ABA was only one of them, and there could be other factors. VvWRKY13 might also affect other physical changes such as reactive

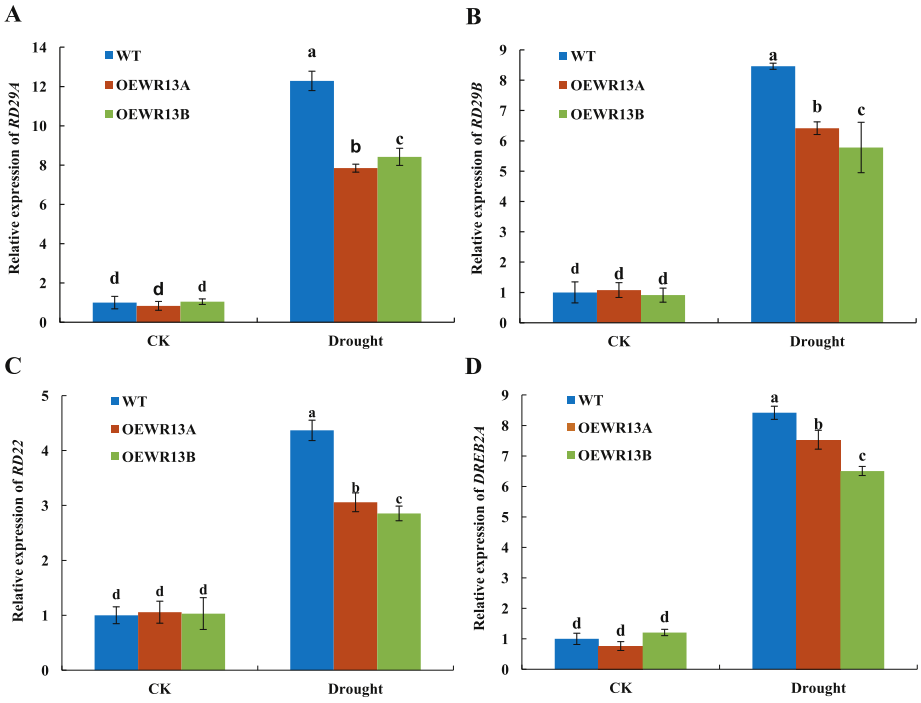


**Fig. 6. Effects of drought stress on the expression of stress drought-related genes in *VvWRKY13*-overexpressing *Arabidopsis* leaves.** The expression of *RD29A* (**A**), *RD29B* (**B**), *RD22* (**C**) and *DREB2A* (**D**) in the leaves of overexpressing lines under drought stress. Three independent experimental replications were conducted. Values are the means ± SE of three independent experiments ($P < 0.05$).

oxygen species accumulation, osmotic regulators reduction. These changes caused sensitive to drought of transgenic lines. In further, the expression of the marker genes in ABA-dependent pathway and ABA-independent pathway was detected. The expression of these marker genes was more lower in *VvWRKY13* transgenic *Arabidopsis* than that in wild type. What is the relationship among ethylene biosynthesis, ABA biosynthesis, stress tolerance and VvWRKY13 function? It will be studied in nearly future.

## Contributions

Lixia Hou performed experiments, interpreted data, and wrote the article. Xinxin Fan performed experiments. Xin Liu conceived and designed experiments, and edited the article. Jie Hao performed all the qRT-PCR and the analysis of statistics. Guangchao Liu and Zhen Zhang performed stress experiments. All authors read and approved the manuscript.

## Funding

This work was supported by “National Key Research and Development Program of China (Grant No. 2018YFD1000302)” and “National Natural Science Foundation of China (Grant No. 31572107, 31872082, 31770275 and 31401844)”.

## Declaration of competing interest

The authors declare that there is no conflict of interest in this work.

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