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Anatomical physiological and biochemical processes involved in grapevine rootstock drought tolerance

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Summary

In order to explore the drought resistance mechanism of grape rootstocks, two grape rootstock species, '1103P' (a drought-tolerant rootstock) and '101-14M' (drought-sensitive), were treated with moderate water deficit (field capacity of 45-50 %). Throughout the experimental period, the leaves of '1103P' showed a higher stomatal conductance (g_s), relative water content and photosynthetic rate (P_n) than '101-14M', indicating '1103P' was more resistant to tolerant than '101-14M'. We propose that '1103P' could prevent water loss from leaves under drought conditions based on the discoveries that '1103P' had higher leaf phytohormone abscisic acid (ABA) content and leaf cuticular wax content, and smaller stomata aperture than those of '101-14M'. Additionally, the activities of H_2O_2-scavenging enzymes in leaves of '1103P' were higher than those of '101-14M' under drought conditions, indicating the lipid peroxidation induced by H_2O_2 of '1103P' was less serious than that of '101-14M'. Therefore, better water-saving and higher reactive oxygen species (ROS) scavenging abilities contributed together to stronger drought resistance of '1103P' than '101-14M'.

Key words: ABA; drought; stomata; H_2O_2; chlorophyll fluorescence; *Vitis*; cuticular wax.

Introduction

Grapevine (*Vitis vinifera*) is one of the most economically important fruits worldwide. However, this crop often encounters drought stress in production. Severe water deficit is the main environmental factor limiting plant growth and yield, especially in semi-arid regions (Chaves *et al*. 2003). Plants have developed various mechanisms to enhance their drought tolerance, including changing morphological and physiological traits such as plant structure, growth rate, water-use efficiency (WUE), tissue osmotic potential, and stomatal conductance (Escalona *et al*. 2003, Sharp *et al*. 2004, Nemeskéri *et al*. 2010, Liu *et al.* 2013).

On the one hand, reducing water loss in plants could improve drought resistance. There are two main ways to prevent water loss in plants. One is stomatal transpiration, and the other is cuticular transpiration (Escalona *et al*. 2013). Stomata regulation is one of the key mechanisms allowing plants to regulate and optimize evaporative water loss (Tombesi *et al*. 2015). Under drought stress, plants partially or completely close stomata to maintain a favorable water balance and limit the carbon gain (Ciais *et al*. 2005, Franks 2013). The phytohormone abscisic acid (ABA) is considered a chemical signal to mediate stomata behavior during water deficit (Malladi and Burns 2007, Seki *et al*. 2007, Speirs *et al*. 2013, Li *et al*. 2014). Biochemical and genetic studies have suggested that 9-cis-epoxycarotenoid dioxygenase (NCED) is the key enzyme in the ABA biosynthetic pathway in plants (Iuchi *et al*. 2001, Zhang *et al*. 2009). Also, among the five NCED family members, *NCED3* plays a crucial role in drought-induced ABA biosynthesis (Guo *et al*. 2015). Leaf cuticular wax content (CWC) is an important factor avoiding non-stomatal transpiration, which protects the plants against abiotic and biotic stresses (Xue *et al*. 2017, Li *et al*. 2019). Under drought stress, cuticular wax is accumulated to reduce water losses from non-stomatal evaporation (Bi *et al*. 2017, Luo *et al*. 2019). Many studies indicated that drought-tolerant cultivars usually have higher leaf CWC than drought-sensitive cultivars (Guo *et al*. 2016).

On the other hand, plants enhance drought tolerance mainly *via* improving the ability of removing harmful substances, such as reactive oxygen species (ROS) (Mittler *et al*. 2015, Wang *et al*. 2018), which could induce membrane lipid peroxidation, ultimately leading to membrane dysfunction (Li *et al*. 2011). Drought-stressed plants over-produce ROS, and excessive ROS can lead to lipid peroxidation, protein degradation, and nucleotide damage further inhibiting a wide range of plant cellular processes (Xu *et al*. 2016). Therefore, suppressing ROS production or enhancing the capacity for ROS scavenging can weaken drought-induced oxidative damage (Hossain *et al*. 2015). H_2O_2, one of the most important ROS, participates in a series of processes for plant development, stress responses, and programmed cell death (Choudhury *et al*. 2017), and the enzymes of catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX) play an essential role in scavenging H_2O_2. Although responding behavior to drought has been investigated in several plant species, the systematic drought tolerance mechanism to drought is not yet fully understood in *Vitis*. Here, we used two genotypes of grape rootstocks with contrasting responses to drought stress, and compared their photosynthetic characteristics, stoma-

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tal behavior, ABA content, the expression of *NCED3*, H_2O_2 content and activities of H_2O_2-scavenging enzymes under moderate water stress conditions. The objective of the present work is to uncover the physiological mechanism of grapevine to drought by comparing the anatomical, physiological and biochemical processes of two *Vitis* rootstocks.

Material and Methods

Plant material: Plants of '1103 Paulsen' (1103P) and '101-14' Millardet et de Grasset (101-14M), tolerant and highly sensitive to drought respectively, originating from different climate regions (Tab. 1), were used in this study. The former originated in dry regions, and is one of the most drought tolerant grapevine rootstocks. By comparison, 101-14M, which thrives in wet habitats, is highly resistant to water-logging and shade, but sensitive to drought (LI *et al.* 2019b). One-year-old vines of both rootstocks were grown in PVC pots (20 cm × 15 cm × 28 cm) filled with a 5:1:1 (v:v:v) mixture of forest soil:sand:organic substrate. Pots were placed in a condition-controlled greenhouse (30 ± 2 °C during the day and 25 ± 2 °C at night, with relative humidity 50 ± 5 % and natural light condition). The plants were watered once every three days with half-strength Hoagland nutrient solution before the experiments. Standard horticultural practices were followed for disease and pest control.

Experimental designs: 50 d after planting, the plants of each cultivar were divided into two groups: control plants were maintained at near field capacity throughout, and drought-stressed plants were exposed to 45-50 % field capacity (STOLF-MOREIRA *et al*. 2019). Moisture stress was initiated by withholding irrigation, until soil humidity reached 45-50 % field capacity; the control group was kept at near field capacity until end of the experiment. Pots were weighed twice per day (early in the morning and late afternoon) and water was added to maintain the soil at the desired values. The experimental design was completely randomized, with 3 replicates of each group (40 plants per replicate). When the soil of drought stressed treatment dropped to designated values (45-50 % field capacity), then we defined the day as day 0. The third to fifth leaves (generated during the experiment) from the top of a stem were sampled from both groups between 09.00 and 11.00 h on 0, 7, 14, 21, and 28 d. The collected leaves were rapidly frozen in liquid nitrogen and stored at -80 °C, which were used for measuring the content of ABA, H_2O_2 content, activities of H_2O_2 -scavenging enzymes, and relative expression of *NCED3*.

Relative water content: On day 0, 7, 14, 21, and 28 of drought treatment, the relative water content was computed according to the method described by GAXIOLA *et al*. (2001). Leaves of each rootstock were excised from each treatment group and their fresh weights were recorded immediately. After the leaves were floated in deionized water at 4 °C overnight, their rehydrated weights were determined. Finally, they were oven-dried at 70 °C for 48 h and weighed again. Relative water content was calculated as RWC = (fresh weight–dry weight)/(rehydrated weight–dry weight).

Measurements of photosynthetic characteristics: The net photosynthetic rate (P_n), transpiration (T_r), the intercellular CO_2 concentration (g_i) and stomatal conductance (g_s) were recorded between 09.00-11.00 h, with a portable system (Li-6400; LICOR, Lincoln, NE, USA). All measurements were carried out at 1,500 μmol photons·m^{-2}·s^{-1}. The cuvette CO_2 concentration was set at 400 μmol CO_2·mol^{-1} air, with a vapor pressure deficit of 2.0-3.4 kPa. For each group, measurements were done in fully expanded and fully exposed leaves on day 0, 7, 14, 21, and 28 of drought treatment.

Determination of chlorophyll fluorescence: Chlorophyll fluorescence parameters were measured with a pulse-amplitude modulated (PAM-2500) fluorometer (Walz, German). Measurement of chlorophyll fluorescence parameters was repeated once for each leaf, and five leaves of each treatment were chosen for dark adaptation for more than 30 min. After dark-adapted treatment, the minimal fluorescence (Fo) and the maximal fluorescence (Fm) were measured under a low modulated light over a 0.8 s period. The maximum fluorescence in the light-adapted state (Fm′) was recorded after a second saturation pulse. Then, the actinic light (7,000 μmol·m^{-2}·s^{-1}) turned off and the far-red light turned on for measuring the minimal fluorescence in a light-adapted state (Fo). The maximum photochemical quantum yield of PSII (Fv/Fm), the effective photochemical quantum yield of PSII (ΦPSII), and electron transport rate (ETR) were calculated according to WANG *et al*. (2012) and PEREZ-MARTIN *et al*. (2014).

Quantification of cuticular wax content: The method for cuticular wax isolation was performed as described previously by GUO *et al*. (2016), with minor modifications. Five new leaves (3rd to 5th position from the top on each plant) were collected from each treatment on day 14 of drought treatment. Wax was extracted by dipping the leaves in 30 mL $CHCl_3$ for 30 s. The wax extract was filtered using filter paper and air-dried in a desiccator at room temperature until there was no change in weight. Subsequently, the leaves were oven-dried for 24 h at 70 °C. Yield of cuticular wax and dry weight (DW) of leaves were determined on an analytical scale with an accuracy of 0.01 mg (Sartorius Quintix BP211D, Germa-

Table 1

Origin information for two *Vitis* rootstocks used in experiments of responses to drought stress

Rootstocks	Genetic origin	Origin locality	Climate	Soil type
1103P	*V. berlandieri* × *V. rupestris*	Sicily, Italy	Etesian climate	sandy and stony soil
101-14M	*V. riparia* × *V. rupestris*	Western France	Temperate marine climate	Marl loam

ny). Cuticular wax content (CWC) was calculated using the following formula: CWC ($mg \cdot g^{-1}$) = Extracted wax weight/DW.

Measurements of leaf stomatal density: Five new leaves (third to fifth position from the top of each plant) were sampled from the control and drought-stressed plants of '101-14M' and '1103P' on a 14 d of drought treatment. A leaf surface imprint method was used (Yu *et al*. 2008). Briefly, a drop of nail varnish was applied to a glass slide, and the adaxial side of a sampled leaf was pressed on the glue for about 30 s. The leaf was removed and the imprint on the glass slide was observed with a light microscope (Olympus BX53F; Tokyo, Japan). Five plants per treatment, three leaves per plant and three areas per leaf, were examined.

Observations of leaf stomata by scanning electron microscopy (SEM): Four new leaves (3rd to 5th position from the top on each plant) were collected per treatment group for each rootstock on day 14 of drought treatment. The samples were immediately fixed with a 4 % glutaraldehyde solution in 0.1 M phosphate-buffered saline (PBS; pH 6.8) to avoid any alterations during sample preparation. After being rinsed five times with PBS (for 5, 10, 15, 20, and 30 min), they were dehydrated in a graded ethanol series, vacuum dried, and gold-coated. Samples were scanned on a SU8010 scanning electron microscope (JHITACHI Ltd., Tokyo, Japan). Stomata were counted at random in 20 visual sections on the abaxial epidermis, and final tallies were used to compute their densities. Lengths, widths, and apertures were measured randomly from 20 stomata on the same specimens, using Image J software.

Determination of ABA content: BA in frozen levels, collected on day 0, 7, 14, 21, and 28 of drought treatment, were measured as described by Zhang *et al*. (2008). Briefly, analyses were performed using an Agilent 1290 HPLC system (Agilent Technologies, Böblingen, Germany) (Autosampler, Binary Pump and diode array detector) equipped with a reverse phase column (Inertsil ODS-3, 250 × 4.6 mm, 5 μm); the injection volume was 20 μL and the detection was made at 254 nm. The mobile phase was methanol with 0.6 % acetic acid (dissolved in re-distilled water) (45:55, v: v); and flow rate, 0.8 $mL \cdot min^{-1}$. The compound was identified by comparing the retention times with ABA external standards (Sigma, St. Louis, MO, USA). The peaks were quantified by an external standard method, using the measurements of the peak areas and a calibration curve.

H_2O_2 content and activities of H_2O_2-scavenging enzymes: H_2O_2 was extracted with 5 % (w/v) trichloroacetic acid and measured as described by Patterson *et al.* (1984). For H_2O_2-scavenging enzymes, 0.1 gram of frozen leaf samples were ground in a chilled mortar with 1 % PVP, then homogenized with 1.2 mL of 50 mM potassium phosphate buffer (pH 7.8) containing 1 mM EDTA-Na_2 and 0.3 % Triton X-100. For the assay of ascorbate peroxidase (APX), 1 mM ascorbate was added to this mixture. Each homogenate was centrifuged at 13 000 g for 20 min at 4 °C. The supernatant was used for analysis of SOD, POD, and APX. Catalase (CAT) activity was determined by monitoring the disappearance of H_2O_2 by measuring the decrease in absorbance at 240 nm (extinction coefficient of 39.4 $mM^{-1} \cdot cm^{-1}$) (Chance and Maehly 1955). Peroxidase (POD) was assayed at 470 nm (extinction coefficient 25.2 $mM^{-1} \cdot cm^{-1}$) by using H_2O_2 and guaiacol as the reaction substrates (Chance and Maehly 1955). APX activity was monitored as the decrease in absorbance at 290 nm when reduced ascorbate was oxidized (extinction coefficient of 2.8 $mM^{-1} \cdot cm^{-1}$) (Nakano and Asada 1981).

RT-PCR analysis: Total RNA was extracted from frozen leaves, collected on day 0, 7, 14, 21, and 28 of drought treatment, according to the method described by Pou *et al*. (2013). Sequence for primers of *VvNCED3* was determined according to Zheng *et al.* (2015). All primers have been used in this study are listed in Tab. 2. Poly(A)$^+$ RNA was purified with a poly(A)$^+$ PrimeScript™ RT reagent Kit with gDNA Eraser (TaKaRa, Japan) according to the manufacturer's instructions. Real-time PCR was performed on an ABI7500 instrument (ABI, USA) using SYBR Green qPCR kits (TaKaRa) according to the manufacturer's instructions. To test the suitability of these primers, the specificity and identity of the reverse transcription (RT)-PCR products were monitored after each reaction by conducting melting-curve analysis of the products.

Ubiquitin was used as the reference gene, and relative expression data of *VvNCED3* gene was calculated as Pou *et al*. (2013). Three independent biological replications were performed for each experiment.

Statistical analysis: Data were expressed as means ± standard deviation (SD). The data were analysed by Tukey's tests. A p-value of < 0.05 indicated a significant difference.

Results

Photosynthetic and RWC responses to drought stress: In response to drought stress, P_n, g_s, g_i, T_r were reduced in the stressed plants of both rootstocks throughout the drought stress period (Fig. 1). In the first 7 d, the decline of P_n, g_s, g_i, T_r on '101-14M' were much more rapid than that on '1103P'. More specifically, the reduction of P_n, g_s, g_i, T_r on '1103P' were 53.1, 61.7, 27.8, and 47.3 %. While, for '101-14M', the counterparts were

Table 2

Primers used for quantitative real-time RT-PCR

Gene	Forward primer (5′ - 3′)	Reverse primer (5′ - 3′)
VvNCED3	TTCCCTCACGAGTTCCCTATG	TCCTCTGCAATCTGACACCAAG
Ubiquitin	GTGCTGTCAACTGCAGGAAA	GTAGCCATGGCACATCCAAT

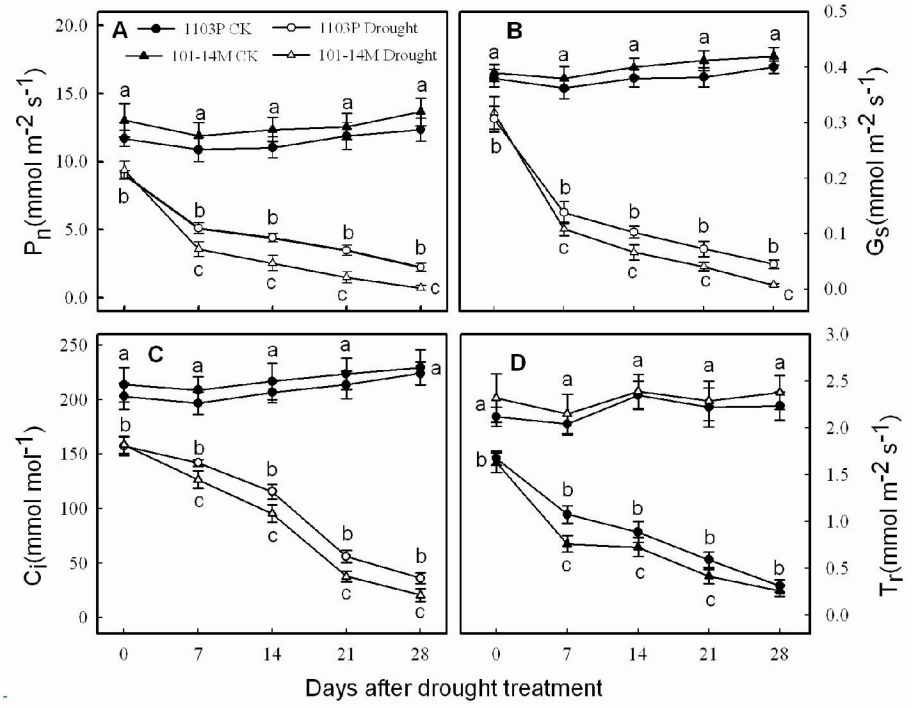

Fig. 1: Changes in net photosynthesis (P_n), transpiration rate (T_r), stomatal conductance (g_s), and intercellular CO_2 concentration (g_i) of '1103 P' and '101-14M' under control (CK) and drought conditions. Data are means of 5 replicates ± SD. Different letters denote statistically significant differences by Tukey's test ($P < 0.05$).

70.0, 71.5, 39.4 and 64.6 %, respectively. Nevertheless, throughout the drought stress period, except day 0, '1103P' had high reading for all four photosynthetic characteristics.

LRWC on '1103P' was higher than '101-14M' under non-stressed conditions (Fig. 2). Values for this parameter on both rootstocks decreased under water stress, while the decline rate of LRWC on '101-14M' was faster than that on '1103P'. Moreover, '1103P' had higher LRWC than '101-14M' under drought stress conditions.

Chlorophyll fluorescence responses to drought stress: The response of Fo, Fv/Fm, ΦPSII, and ETR to drought conditions for '1103P' and '101-14M' plants was shown in Fig. 3. Although, Fo increased, Fv/Fm, ΦPSII, and ETR decreased with continuing drought, and changes of '101-14M' were much more rapid than '1103P'. More specifically, compared to control (c.f., day 0), Fo on '1103P' increased by 102.0 % on day 28 and Fv/Fm, ΦPSII, and ETR on '1103P' diminished by 31.3, 62.4, and 78.0 %, respectively. However, Fo on '101-14M' increased by 138.0 % and Fv/Fm, ΦPSII, and ETR on '101-14M' decreased by 40.9, 81.6 and 89.7 %, respectively. . On the other hand, those changes began at different times. For example, the Fo on '101-14M' increased rapidly on day 7, while '1103P' began on day 14. Besides, throughout the drought stress, the Fo on '101-14M' was higher and the Fv/Fm, ΦPSII, ETR were lower than those on '1103P'.

Leaf cuticular wax content: Drought stress caused an increase in leaf cuticular wax content on both rootstocks (Fig. 4). Whether under non-stressed or drought stressed conditions, the leaf cuticular wax content of '1103P' was higher than for '101-14M'. In addition, under drought conditions, cuticular wax content rose by 38.4 % and 21.2 % in the drought-tolerant and -sensitive rootstocks, respectively.

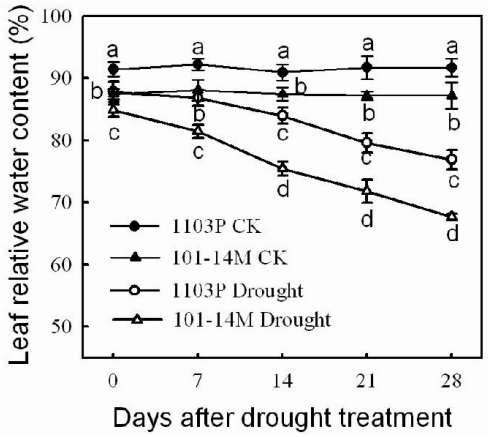

Fig. 2: Changes in leaf relative water contents of '1103 P' and '101-14 M' under control (CK) and drought conditions. Data are means of 5 replicates ± SD. *Different letters* denote statistically significant differences by Tukey's test ($P < 0.05$).

Stomatal density and behavior: Leaf lower surfaces were scanned at ×3000 magnification. The drought stress caused stomata to close in both rootstocks (Fig. 5) Under well-watered conditions, '101-14M' had significantly higher stomatal density (Fig. 5A, D), whereas the stomata length, width and aperture were almost similar on both rootstocks (Fig. 5B, C). Stomatal apertures on both rootstocks significantly decreased under water stress (Fig. 5D). Compared with control, stomatal apertures were 24.3 % on '1103P' and 40.5 % on '101-14M' (Fig. 5D, Fig. 6). Besides, stomata length and width were not significantly affected by the water deficit, except stomata width of '1103P' became remarkably smaller compared with the control (Fig. 5C).

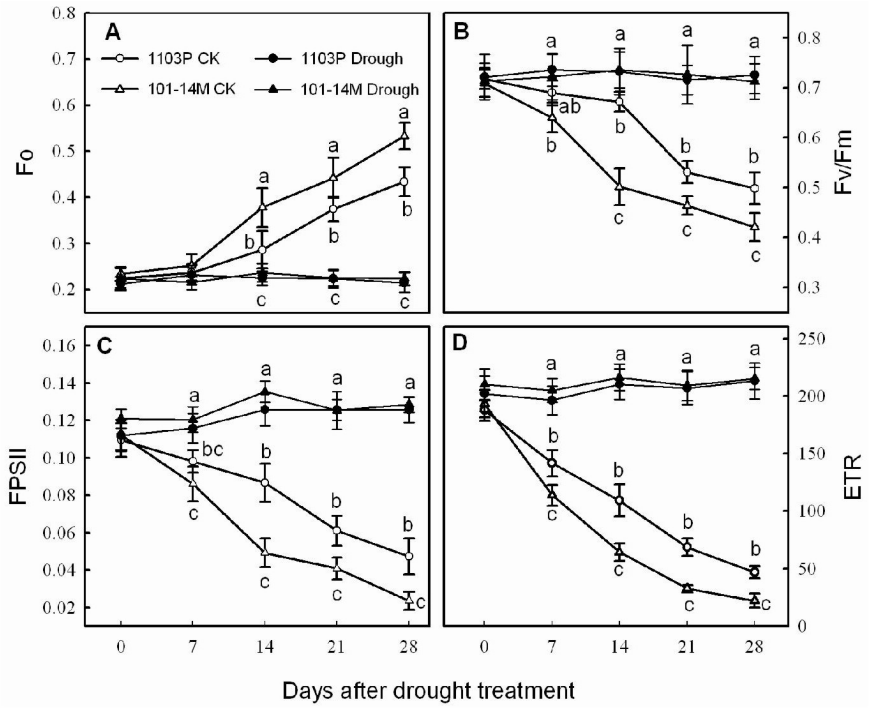

Fig. 3: Changes in parameters of chlorophyll fluorescence of '1103 P' and '101-14M' under control (CK) and drought conditions. Data are means of 5 replicates ± SD. Different letters denote statistically significant differences by Tukey's test ($P < 0.05$).

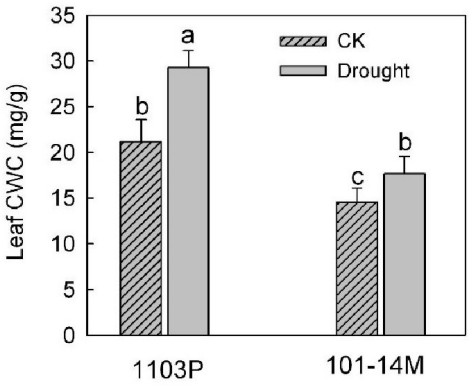

Fig. 4: Leaf cuticular wax content (CWC) comparison between '1103 P' and '101-14 M' under control (CK) and drought conditions. Data are means of five replicates ± SD. *Different letters* denote statistically significant differences by Tukey's test ($P < 0.05$).

ABA content and relative expression of NCED3: The ABA contents and relative expression of *NCED3* in leaves of both rootstocks increased, and then decreased under the drought treatment, peaking at around day 14. Meanwhile, the ABA contents and expression of *NCED3* in leaves of '1103P' were higher than that of '101-14M' under water stressed conditions (except Day 0) (Fig. 7A, B).

H_2O_2 content and activities of antioxidant enzymes: Drought stress caused a rapid increase of H_2O_2 contents in both rootstocks leaves, while the content on '101-14M' was higher than that on '1103P' throughout almost the whole drought period (Fig. 8A). Both rootstocks significantly enhanced activities of CAT (Fig. 8B), POD (Fig. 8C), and APX (Fig. 8D) under stress. The peaking time of CAT and POD activities was at around day 14, and the peaking time of APX activity was at around day 21. However, APX activity was significantly elevated throughout the whole drought period compared with the control. In addition, the activities of CAT, POD, and APX on '1103P' were higher than those on '101-14M' under drought stress.

Discussion

There were a variety of mechanisms in plants in response to water stress, which might maintain plant function in multiple ways (Hoekstra *et al*. 2001). The adaptive responses to water deficit include mechanisms to avoid water loss, protect cellular components, and repair damages (scavengers of toxic oxygen species). However, the mechanisms of how grapevine rootstock coped with drought have not been fully clarified. Here we focus on the physiological and biochemical processes of two specific rootstocks (tolerant and sensitive to drought) to determine the mechanisms of grapevine rootstock under drought stress.

(1) '1103P' had higher ability of managing stomata behavior in comparison to '101-14M': Stomatal transpiration is one of the main ways of plants losing internal water. Their opening and closing are controlled by environmental and internal parameters (Kolbe *et al*. 2018), including water deficit. Under drought stress, partial or complete stomatal closure allows plants to maintain a favorable water balance (Liu *et al*. 2013). Therefore, it is proved that stomatal density and behavior have an essential role in determining drought tolerance. In this study, '1103P' had smaller stomatal density and width than '101-14M' under both well-watered and drought conditions. Especially, under drought conditions, the closing degree of stomata on '1103P' (75.7 %)

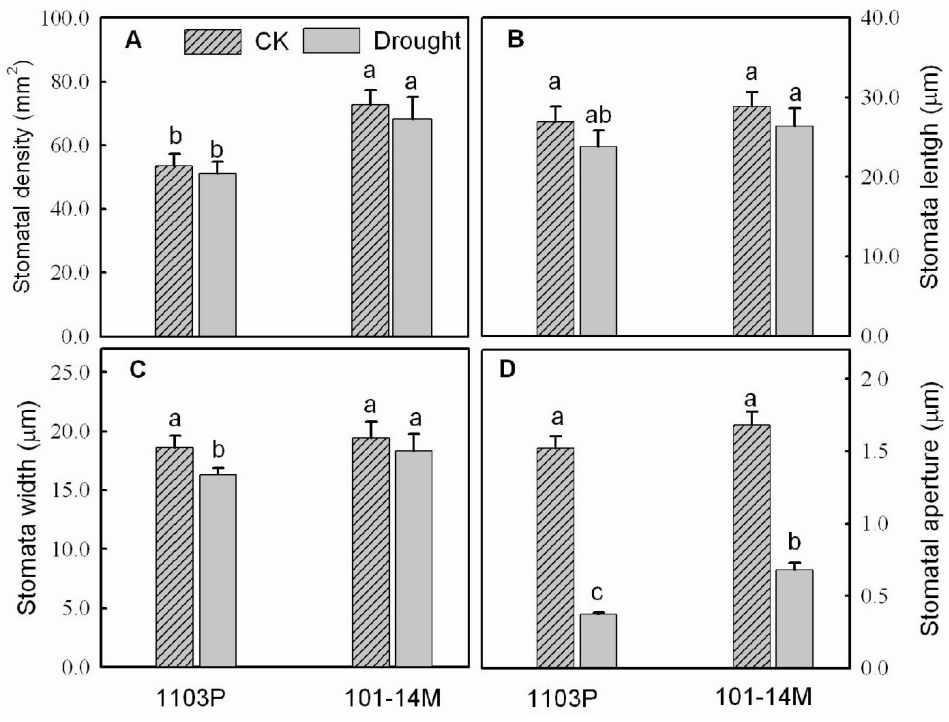

Fig. 5: Effects of drought on stomatal properties of leaves from '1103P' and '101-14M' under control and drought conditions: density (**A**), length (**B**), width (**C**), and aperture size (**D**), Data are means of values from 15 images ± SD. Different letters denote statistically significant differences by Tukey's test ($P < 0.05$).

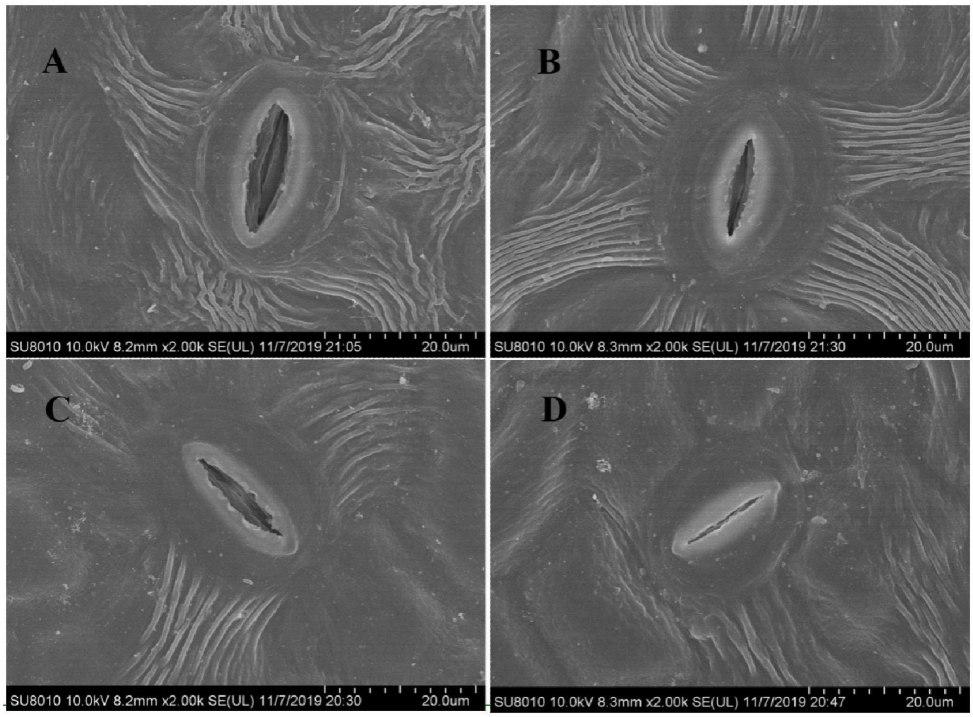

Fig. 6: SEM images of stomata from leaves of '1103P' and '101-14M': control leaves with open stomata (**A**, '101-14M' and **C**, '1103P'); closed stomata of leaves exposed to drought stress for 7 d (**B**, '101-14M' and **D**, '1103P'). Magnification × 3000, scale bars = 20 μm.

was much higher than that on '101-14M' (59.5 %). And this might be the reason why '1103P' maintained higher leaf RWC under drought conditions. Although the stomata aperture of '1103P' was smaller than that of '101-14M', the photosynthetic rate of '1103P' was higher. This might be caused by the differences of mesophyll conductance between '1103P' and '101-14M' (TOMÁS *et al*. 2014).

Many studies have confirmed that abscisic acid mediates the stomatal behavior in response to drought by activating activity of guard cell membrane-localized channels and transporters, which decreased guard cell turgor and ultimately closing the stomata (MALCHESKA *et al*. 2017, QUAN *et al*. 2018, DONG *et al*. 2018). Under drought conditions, expression of Vv*NCED3* (ABA biosynthesis gene) was

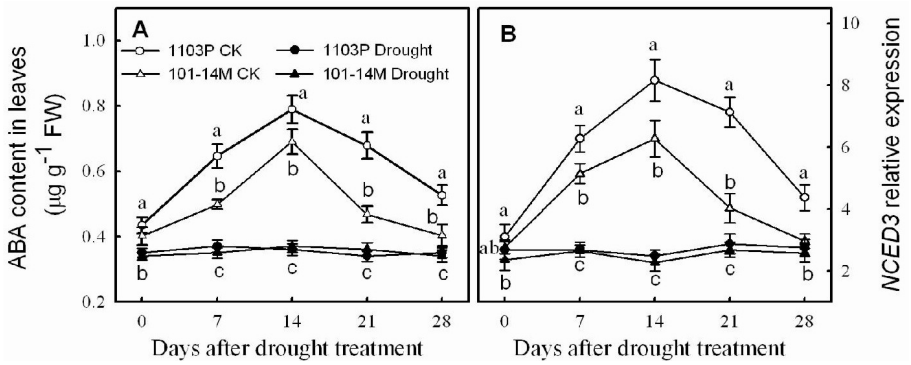

Fig. 7: Changes of ABA contents and expression of *NCED3* gene in leaves of '1103 P' and '101-14M' under control (CK) and drought conditions. Data are means of 5 replicates ± SD. Different letters denote statistically significant differences by Tukey's test ($P < 0.05$).

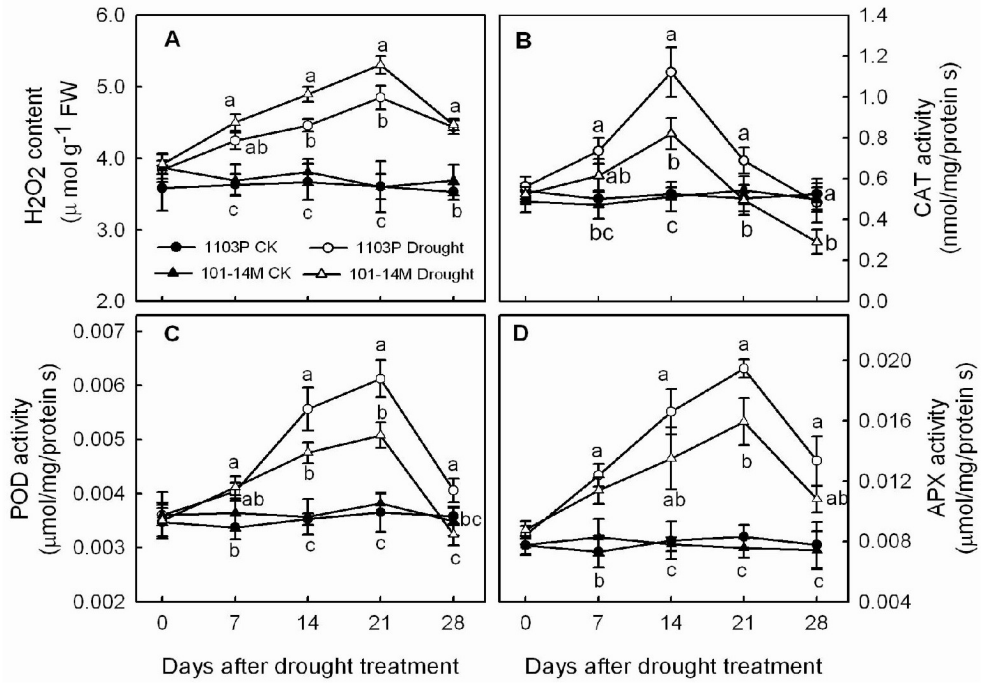

Fig. 8: Changes in H_2O_2 accumulations and activities of antioxidant enzymes in leaves under control (CK) and drought conditions: H_2O_2 content (**A**), CAT activity (**B**), POD activity (**C**), and APX activity (**D**). Data are means of 5 replicates ± SD. Different letters denote statistically significant differences by Tukey's test ($P < 0.05$).

significantly up-regulated on both rootstocks, and the ABA content also elevated. However, the leaves of '1103P' had higher ABA contents and *NCED3* expression level than that of '101-14M', which led to the faster stomata closing on '1103P' in response to drought.

(2) The leaves of '1103P' had higher cuticular wax content: The aerial parts of plants are covered with cuticular wax, which controls non-stomatal water loss and gas exchange, and protects plants from UV irradiation, thus contributing to drought tolerance (Kunst and Samuels 2009). Cuticular wax accumulated under drought stress in plants, such as wheat, rice, and maize (Bi *et al*. 2017, Xue *et al*. 2017, Guo *et al*. 2018, Li *et al*. 2019a). Guo *et al*. (2016) suggested that drought-tolerant wheat cultivars usually have higher leaf cuticular wax content (CWC). In this study, '1103P' (drought-tolerant cultivar), had higher leaf CWC than '101-14M' (the drought-sensitive cultivar) under both well-watered and drought conditions, and this helped avoid non-stomatal water loss on '1103P' under drought stress.

(3) '1103P' had more active antioxidant enzymes: Reactive oxygen species (ROS) significantly accumulated under abiotic stress conditions, which caused oxidative damage and cell death (You *et al*. 2015, Nxele *et al*. 2017). Increasing evidence showed that accumulation of ROS damages photosystem II proteins by lipid peroxidation (Pospíšil *et al*. 2017, Guo *et al*. 2018). Therefore, manipulating ROS levels provides an opportunity to enhance plants tolerances to unfavorable environmental conditions (Nxele *et al*. 2017). Hydrogen peroxide (H_2O_2), one of the most important ROS, participates in a series of processes under abiotic stress (Bae *et al*. 2016, Li *et al*. 2017, Zhou *et al*. 2018). Drought induced the H_2O_2 accumulation in leaves of both '1103P' and '101-14M'. The activities of H_2O_2-scavenging enzymes, including CAT, POD, and APX, were also up-regulated. However, the activities of those enzymes on '1103P' were higher than those on '101-14M', leading to lower leaf H_2O_2 content on '1103P'. Many studies suggested that H_2O_2 directly involved in the regulation of stomatal movement (An *et al*.

2016, SUN *et al*. 2017). LI *et al*. (2014) reported the correlation between elevated H_2O_2 level and ABA-induced stomatal closure. Additionally, photosystem II proteins on '1103P' were less damaged under drought stress, and thus Fv/Fm on '1103P' was higher. In conclusion, higher ABA content, ROS scavenging enzymes activity, and leaf cuticular wax content, and smaller stomatal aperture on '1103P' contribute to stronger drought resistance.

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