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RESEARCH ARTICLE

Stilbene production as part of drought adaptation mechanisms in cultivated grapevine (*Vitis vinifera* L.) roots modulates antioxidant status

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ABSTRACT

- Stilbenes, naturally occurring polyphenolic secondary metabolites, play a pivotal role in adaptation of various plant species to biotic and abiotic factors. Recently, increased attention has been directed toward their potential to enhance plant stress tolerance.
- We evaluated drought tolerance of three grapevine varieties grown with different levels of water deficit. Throughout, we studied physiological mechanisms associated with drought stress tolerance, particularly stilbene accumulation in root tissues, using HPLC. Additionally, we explored the possible relationship between antioxidant potential and stilbene accumulation in response to water deficit.
- The results underscore the detrimental impact of water deficit on grapevine growth, water status, and membrane stability index, while revealing varying tolerance among the studied genotypes. Notably, Syrah variety had superior drought tolerance, compared to Razegui and Muscat d'Italie grapes. Under severe water deficit, Syrah exhibited a substantial increase in levels of stilbenic compounds, such as *t*-resveratrol, *t*-piceatannol, *t*-ε-viniferin, and *t*-piceid, in root tissues compared to other genotypes. This increase was positively correlated with total antioxidant activity (TAA), emphasizing the active role of resveratrol and its derivatives in total antioxidant potential. This demonstratres the potential involvement of resveratrol and its derivatives in enhancing antioxidant status of the drought-tolerant Syrah grape variety.
- Our findings suggest that these stilbenes may function as valuable markers in grapevine breeding programs, offering novel insights for the sustainable cultivation of grapevines in water-limited environments.

INTRODUCTION

Grapevine (*Vitis vinifera* L.) has been recognized for its adaptability to environmental changes, particularly water deficit stress (Zhang *et al.* 2016; Ollat *et al.* 2019). However, climate change is expected to increase the frequency and duration of drought periods. The water availability will decline by 50%, while crop water demand is expected to double by 2050 (Gupta *et al.* 2020). Future climate projections indicate insufficient water availability to sustain agricultural production worldwide, especially in the Mediterranean region (Hannah *et al.* 2013; Santillán *et al.* 2019). Insufficient water supply is linearly associated with increased accumulation of reactive oxygen species (ROS) in plant cells, leading to oxidative stress. Oxidative stress can affect the integrity of cellular components, such as membrane lipids, proteins, and nucleic acids, leading to metabolic dysfunction and cell death (Zhu 2016; Antoniou *et al.* 2017; Hasanuzzaman *et al.* 2017; Batista *et al.* 2018; Guo *et al.* 2018). To ensure survival under such stressful conditions, plant cells have developed a sophisticated antioxidant defence system (Boubakri *et al.* 2021). In this context, some stress-related secondary metabolites were found to be a part of the antioxdant system through their ability in scavenging ROS (Nakabayashi *et al.* 2014). Recent reports have described drought stress modulation of biosynthetic pathways for both phenolic acids and flavonoids, which act as important antioxidants, thereby protecting plants from the deleterious effects of water deficit stress (Gharibi *et al.* 2019; Kumar *et al.* 2020, 2023). Under water deficit, grapevine plants adjust their cellular homeostasis by reprogramming their metabolism to ensure efficient removal of ROS (Cramer *et al.* 2007; Gambetta *et al.* 2020). Among phenolic compounds, stilbenes are known to be involved in adaptation of plants to hostile environments (Valletta *et al.* 2021). Their biological activity is significantly influenced by structural modifications, such as hydroxylation, glycosylation, methylation, and oligomerization, which enhance antioxidant properties and overall effectiveness in protecting plants from biotic and abiotic stresses (Jeandet *et al.* 2021). Previous studies analysing the impact of water deficit on stilbene accumulation in grape berry tissues are inconsistent, indicating potential variety-specific responses. For instance, Deluc *et al.* (2011) found an increase in

accumulation of *t*-piceid, a glycosylated form of resveratrol, in Cabernet Sauvignon berries but not in Chardonnay under water deficit. Similarly, Sun *et al.* (2023) reported that content of resveratrol and its derivatives increased during berry ripening, in particular, resveratrol amount increased with increasing water stress severity. In contrast, Vezzulli *et al.* (2007) reported unchanged accumulation of resveratrol under water deficit in *Vitis vinifera* cv. Barbera berries. Moreover, a decrease in stilbene biosynthesis was also noted in grape berries under water deficit, probably related to competition for some precursors between the stilbene and flavonoid pathways (Hochberg *et al.* 2015). Roots are severely affected by drought through their direct contact with drying soil and have an important role in drought tolerance mechanisms (Weidner *et al.* 2009). Moreover, roots serve as storage sites for stilbenes as protective compounds (Erb *et al.* 2009; Balmer *et al.* 2013). The critical role of roots in water stress adaptation, including stress perception, signalling, and defence response, has been reported (Alsina *et al.* 2011; Gambetta *et al.* 2012; Lovisolo *et al.* 2016). Other reports highlighted the importance of root ability to modulate metabolic pathways involved in oxidative stress regulation under water stress (Regier *et al.* 2009). Thus, genetic plasticity of the grapevine antioxidant system seems to be directly related to ability to tolerate water stress (Carvalho *et al.* 2014; Corso *et al.* 2015). Hence, the importance of studying various genetic backgrounds to better understand mechanisms of drought stress adaptation in grapevine.

Screening of an autochthonous *Vitis* accession set from the Mediterranean Basin revealed unique alleles not found in other *Vitis* cultivars (Zoghlami *et al.* 2009). Within this range of genetically diverse local grapevines investigated, the *V. vinifera* cultivar Razegui is considered, from an evolutionary point of view, as intermediate as it has both wild and cultivated grapevine characteristics (Trifa *et al.* 2015). This autochthonous grapevine variety exhibited enhanced osmotic tolerance and adaptive responses to various abiotic factors (Hanana *et al.* 2008, 2014; Jellouli *et al.* 2008; Daldoul *et al.* 2010, 2012). The European grape genotype Syrah is traditionally planted in French Mediterranean vineyards and is considered a drought-tolerant cultivar (Prieto *et al.* 2010). In addition, the Muscat d'Italie genotype is one of the most common cultivated table grape varieties in the Mediterranean area. We hypothesized that grapevine tolerance to drought stress may involve regulation of antioxidative potential in root tissues. Defence metabolites, such as stilbenes, have known antioxidant properties. We suggest that the ability of stressed grapevines to tolerate drought is linked to ability to maintain metabolic integrity, ensured by stilbene induction. Drought elicitation could provide an additional layer of protection against drought-induced damage by increasing production of stilbenes and thus enhancing antioxidant status. Our study provides valuable insights into important strategies for enhancing grapevine tolerance to water scarcity based on stilbene biosynthesis.

MATERIAL AND METHODS

Plant material, greenhouse growth conditions and water deficit treatment

The experiment was performed under greenhouse conditions, using three cultivated grape (*V. vinifera*) varieties: cv. Syrah (a conventional red wine grape), Muscat d'Italie (one of the most diffuse table grape varieties cultivated in the Mediterranean area), and Razegui (a Tunisian autochthonous table grape).

Lignified woody cuttings were grown in peat for 2 months. After which, the cuttings were transplanted into 10-l pots filled with inert sandy soil and covered with aluminium foil to reduce evaporation. The greenhouse conditions were 16-h light with photosynthetically active radiation (PAR) of 300 μmol m^2 s^{-1}, minimum and maximum temperatures between 20 and 33°C, respectively, and average humidity of 74.2%. Throughout the growth in thegreenhouse, plants were watered using Long Ashton nutrient solution (3.5 mM $(CaNO_3)_2$, 3 mM KNO_3, 2 mM NH_4NO_3, 0.6 mM K_2HPO_4, 1.5 mM $MgSO_4$, 1.6 mM KH_2PO_4, 90 μM Fe-EDTA, 9.1 μM $MnCl_2$, 0.76 μM $ZnSO_4$, 0.7 μM $CuSO_4$, 46.3 μM H_3BO_3, 0.21 μM $(NH_4)^{6-}MO_7O_{24}$, pH 6.0; Hewitt & Smith 1975). Plants were placed in a randomized complete block design and 0.5 m apart and 1.0 m between rows. Pots were irrigated with 700 ml Long Ashton nutrient solution, per plant three times a week. The soil water content was maintained above 75% pot capacity and drainage was approximately 100 mL. Six-month-old plants were then subjected to water deficit stress treatment. Stressed plants were not irrigated for 15 days (moderate stress) or 20 days (severe stress). Control plants were continuously watered. Roots and leaves were sampled for further analysis. The experiment consisted of two treatments: irrigated conditions, referred to as control or well-watered (WW), and stressed or water deficit (WD). Nine vines were used per treatment, with three replicates.

Shoot elongation rate

Shoot elongation rate (SER) was determined using the formula: SER = (T2–T1)/*t*, where T1 and T2 are main shoot length at the start and end of each water stress period (t = 15 days or t = 20 days), respectively. The length of the main shoot was measured from the base of the plant to its upper growth point.

Leaf water potential

Leaf water potential (Ψw) was measured with a Scholander pressure chamber (Model 1000; PMS Instruments, Corvallis, OR, USA). In each measurement set, three fully mature and expanded leaves (from the fourth node of the main shoot) of three grapevine plants were chosen. Measurements of leaf Ψw were taken at 10:00 h on days 15 and 20 of water stress treatment.

Leaf and root water relative content

To determine relative water content (RWC), fresh leaves and roots were harvest, immediately weighed (FW) before being submerged in distilled water for 24 h at room temperature in the dark, to allow them to reach maximum turgor, and tugid weight (TW) noted. Subsequently, these leaves and roots were dried at 60°C for 48 h to constant weight, and the resulting weight recorded as dry weight (DW). RWC was calculated following Guha *et al.* (2010) as: RWC (%) = (FW–DW)/(TW–DW) × 100.

Leaf and root membrane stability index

The leaf and root membrane stability indices were determined following Premachandra *et al.* (1990). Immediately after

harvest, leaf discs were carefully cut from the middle of leaves using a punch, while roots were cut in small parts using a scalpel. These samples were then washed thoroughly with running water, then with distilled water to eliminate surface contaminants. Subsequently, they were placed in stoppered vials containing 10 mL double-distilled water. The vials with the samples were incubated at room temperature (25°C) for 24 h. After which the initial electrical conductivity (EC1) of the water in the vials was measured. Then, samples were autoclaved at 120°C for 20 min, cooled to room temperature and electrical conductivity measured again (EC2). The membrane stability index (MSI) was calculated according as described by Sairam *et al.* (1997) as: $MSI = [1 - (EC1/EC2)] \times 100$.

Phenolics extraction from roots

Phenolics extraction from roots followed the method of Gouvinhas *et al.* (2018). For both control and stressed plants, 40 mg freeze-dried root tissue powder was mixed with 1.5 mL methanol/distilled water solution (70:30, v/v), vortexed to ensure mixing, agitated for 30 min at room temperature, then centrifuged for 15 min at 10,000 rpm and 4°C. The resulting supernatants were collected for further analysis using spectrophotometry.

Determination of total phenolic and total flavonoid content

Root total phenolic content (TPC) and total flavonoid content (TFC) were determined according to Machado & Domínguez-Perles (2017). The TPC in grape root extracts was determined using the Folin–Ciocalteu method with gallic acid as standard. In summary, 20 µL of each diluted extract was combined with 100 µL Folin–Ciocalteu reagent, then 80 µL Na_2CO_3 (7.5%) added. The reaction was conducted at 40°C for 30 min in the dark and absorbance at 760 nm recorded. Results are expressed as milligrams gallic acid equivalents per gram dry weight (mg GAE g^{-1} DW). For assessment of flavonoid content in grape root extracts, 24 µL diluted sample were mixed with 28 µL $NaNO_2$ (50 g L^{-1}) for 5 min, the 28 µL $AlCl_3$ (100 g L^{-1}) added, and the mixture allowed to react for 6 min. Subsequently, 120 µL NaOH (1 M) were added and absorbance immediately recorded at 510 nm. Flavonoid content was determined using catechin as standard and results were expressed as milligrams catechin per gram dry weight (mg CE g^{-1} DW).

Total antioxidant activity

Total antioxidant activity (TAA) was assessed using the DPPH (1,1-diphenyl-2-picrylhydrazyl) assay described by Yen & Chen (1995). A volume of 15 µL of each diluted extract was combined with 1 mL freshly prepared methanolic solution of DPPH (25 mg L^{-1}). After 10 min of incubation, absorbance was measured at 517 nm. A Trolox calibration curve was used, and results expressed as µmol Trolox equivalent per g dry weight (µmoL TE g^{-1} DW).

Resveratrol derivatives extraction and analysis

Resveratrol and its derivatives were extracted according to Rayne *et al.* (2008) with slight modifications. Freeze-dried grapevine root powder (100 mg) was subjected to a three-stage extraction process with continuous stirring at room temperature in the dark, using 800 µL ethanol/distilled water (80:20, v/v). In the first extraction stage, 20 µL *t*-hydroxystilbene (100 µg mL^{-1}) were added as internal standard. The extracts were then centrifuged at 13,000 rpm for 15 min at room temperature and the resulting supernatants combined. The pooled supernatants were then evaporated and the obtained residues reconstituted with 200 µL methanol/distilled water (50:50, v/v). Finally, quantification of resveratrol and its derivatives was carried out using an HPLC-DAD system.

HPLC-DAD identification and quantification of resveratrol derivatives

Analysis of stilbenes was performed as previously described (Vincenzi *et al.* 2013). Stilbenes were separated on a C18-Lichrospher column (4 mm x 250 mm, 5 µm; Agilent Technologies, Milan, Italy) at 40°C, using an HPLC system Nexera XR (Shimadzu Italia, Milan, Italy) equipped with a PDA detector SPD-M30A (Shimadzu Italia, Milan, Italy). The mobile phase gradient was 0.5% v/v formic acid in deionized water (solvent A) and 2% v/v formic acid in methanol (solvent B). The gradient program applied was 0 to 10% (solvent B) for 3 min, followed by 10% to 30% (solvent B) for 5 min, 30% to 44% (solvent B) for 35 min, 44% to 55% (solvent B) for 2 min, 55% to 75% (solvent B) for 15 min, and 75% to 100% (solvent B) for 1 min. Flow rate was 1.0 mL min^{-1} and injection volume was 20 µL. Detection was at 310 nm for *trans*-isomers including *t*-resveratrol, *t*-ε-viniferin, *t*-piceatannol and *t*-piceid. Concentration of individual stilbenes was determined based on peak areas of calibration curves of commercially available standards of *t*-resveratrol, *t*-ε-viniferin, *t*-piceatannol, and *t*-piceid. All stilbene standards were obtained from Extrasynthese (Genay Cedex, France). Data were analysed using the Shimadzu Lab Solution software.

Statistical analysis

Statistical analysis was conducted using IBM SPSS Statistics software for Windows (v 20.0; IBM, Armonk, NY, USA). To assess effects of individual main factors (genotype, drought treatment, harvesting time) and their interaction (drought treatment × genotype) and (drought treatment × harvesting time) on all measured parameters, two-way ANOVA was applied. ANOVA assumptions were checked by graphical diagnostic plot of residuals and with the Kolmogorov–Smirnov and Levene tests. Where the interaction between the two main factors was significant ($p \leq 0.05$), pairwise comparison of means was performed using Tukey's HSD test ($p \leq 0.05$). Where the interaction was not significant, the effect of the water deficit treatment was evaluated separately. Significant differences between well-watered and water deficit grapevines were determined using the Student *t*-test ($p \leq 0.05$).

Pearson correlation analysis was performed at the 1% significance level. To perform Principal Components Analysis (PCA), a correlation matrix was generated for Syrah, Razegui and Muscat d'Italie after 15 days of water deficit treatment, and for Syrah after 15 days and 20 days of treatment, and their corresponding response variables, including physiological, biochemical, and stilbene content in roots. Values for each evaluated parameter were plotted and the first two components were used to create a biplot using SPSS Statistics software.

All the experimental data are presented as mean of three independent biological replicates ± SD. Graphical illustrations of results with heat maps were generated using GraphPad Prism software (GraphPad Software v 8.0.0 for Windows; San Diego, CA, USA, www.graphpad.com).

RESULTS

Assessment of drought survival among grapevine genotypes

To identify the survival limit beyond which growth is impaired, resulting in mortality, two different water deficit treatments were applied to the three cultivars (Razegui, Muscat d'Italie and Syrah). After 15 days of water deficit, there was a marked reduction in growth of all tested genotypes. After a prolonged water deficit (20 days), obvious stress damage was observed, inducing mortality, especially for the Razegui and Muscat d'Italie genotypes. In cpntrast, Syrah survived these such harsh conditions, indicating a better drought tolerance capacity of this genotype (Fig. 1b).

Effect of moderate drought treatment on grapevine varieties

A two-way ANOVA found a significant impact of both genotype and treatment on morphological and physiological traits, including SER, leaf Ψ_W, leaf and root RWC, leaf and root MSI, TPC, TFC, TAA and stilbene content.

Shoot elongation rate variations

SER was adversely impacted by water deficit across all three cultivars. After a 15-day stress, reductions of 62.38%, 65.65%, and 74.59% were observed in Syrah, Razegui, and Muscat d'Italie, respectively, compared to the controls (Table 1). Nevertheless, the impact of water deficit was notably less pronounced in Syrah and more pronounced in Muscat d'Italie.

Water status parameters

All grapevine genotypes had similar water status under well-watered conditions. However, there was a notable decrease in leaf Ψ_W in response to water deficit, with stressed grapevines having more negative values than controls. Specifically, Syrah, Razegui, and Muscat d'Italie displayed average differences of −0.833, −0.769, and −0.4 MPa, respectively. Muscat d'Italie had the lowest reduction in leaf water potential under water stress compared to Syrah and Razegui (Table 1). RWC in both leaves and roots was affected by both water deficit treatment and the grapevine genotypes. Overall, arrest of vine irrigation resulted in a significant reduction in RWC in leaves and roots compared to controls. The percentage reductions were 11.45%, 10.21%, and 21.3% in leaves, and 17.15%, 28.04%, and 36.72% in roots for Syrah, Razegui, and Muscat d'Italie, respectively. Statistically, the decrease in RWC in leaves due to water deficit was similar for all stressed varieties (Syrah, Razegui, and Muscat d'Italie). However, in roots, the adverse impact of drought stress was more pronounced in Razegui and Muscat d'Italie than in Syrah (Table 1).

Membrane stability index

The MSI is one of the most important oxidative stress indicators. This parameter was used to estimate severity of drought-induced membrane damage in both leaves and roots of the studied varieties. Water stress resulted in a decrease in MSI in both root and leaf tissues. In response to water stress,

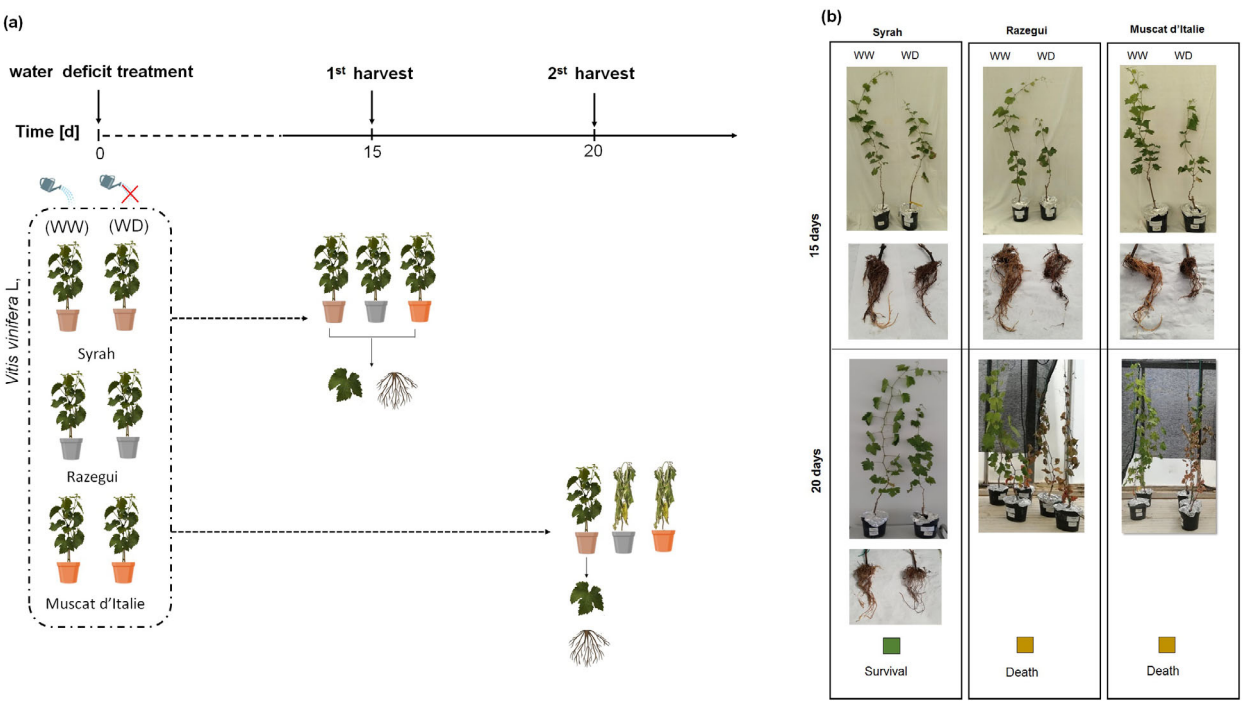

Fig. 1. (a) Overview of the experimental design, detailing application of water deficit treatment, grapevine genotypes, harvest time, and plant material. (b) Morphological aspects of whole plants, shoots and roots, of Syrah, Razegui and Muscat d'Italie under well-watered (WW, control) and water deficit (WD, stressed) conditions after 15 or 20 days.

Table 1. Changes in relative growth rate (SER), leaf water potential, relative water content (RWC) and membrane stability index (MSI) of leaves and roots of three grapevine genotypes under well-watered (WW) or water deficit (WD) conditions after 15 days.

genotype/water deficit treatment	SER (cm d^{-1})	$\Psi_{w\ Leaf}$ (MPa)	$RWC_{\ Leaf}$ (%)	$RWC_{\ root}$ (%)	$MSI_{\ Leaf}$ (%)	$MSI_{\ root}$ (%)
Syrah						
WW	4.442^{a}	-0.833^{a}	81.651^{b}	79.381^{a}	$65.763^{b,c}$	81.246^{a}
WD	1.671^{d}	-1.666^{c}	72.174^{c}	65.771^{b}	59.761^{c}	$71.555^{b,c}$
Razegui						
WW	3.643^{b}	-0.831^{a}	80.629^{b}	74.616^{a}	$71.122^{a,b}$	66.988^{c}
WD	$1.256^{d,e}$	-1.6^{c}	72.399^{c}	53.699^{c}	49.105^{d}	56.977^{d}
Muscat d'Italie						
WW	2.489^{c}	-0.9^{a}	90.662^{a}	78.549^{a}	75.741^{a}	74.501^{b}
WD	0.637^{e}	-1.366^{b}	71.352^{c}	49.705^{c}	43.335^{d}	54.125^{d}
F-values						
Water deficit treatment	340.83*	295.69*	443.84*	418.51*	284.30*	273.98*
Genotype	46.93*	3 ns	24.03*	29.65*	2.762 ns	122.62*
Interaction	4.42*	7.92*	35.79*	18.14*	41.33*	18.91*

Data are mean of three replicates. Data with different letters within a column are significantly different according to Tukey's HSD test at $p \leq 0.05$. Two-way ANOVA outcome given as *F*-values and significances.
ns, non-significant.
*$p \leq 0.05$.

MSI in leaves and roots decreased 1.1-fold, 1.4-fold, and 1.7-fold in leaves, and 1.1-fold, 1.2-fold, and 1.4-fold in roots for Syrah, Razegui, and Muscat d'Italie, respectively. Interestingly, despite the significant reductions in MSI, statistical analysis did not reveal significant differences between Razegui and Muscat d'Italie under water stress in both leaves and roots. However, in Syrah, the decrease was significantly different and less pronounced compared to that of Razegui and Muscat d'Italie under water deficit. These results indicate that Syrah could maintain more stable membrane integrity than Razegui and Muscat d'Italie in both leaves and roots after 15 days of water scarcity (Table 1).

Phenolic compounds and antioxidant activity

Under limited water supply, TPC significantly increased in Syrah and Razegui but decreased in Muscat d'Italie. TPC increased in drought-stressed samples by 43.82% and 17.48% in Syrah and Razegui, respectively, but decreased by 48.56% in Muscat d'Italie. Drought-induced accumulation of TPC was notably higher in Syrah than in Razegui (Table 2).

TFC followed a similar trend in both Syrah and Razegui. No significant differences in TFC were observed between these genotypes under control and drought conditions. In contrdast, Muscat d'Italie showed a significant decrease in TFC of 45.7% under water deficit stress compared to well-watered plants (Table 2).

There was a significant difference in total antioxidant activity (TAA) between Syrah and Muscat d'Italie. Under water deficit, TAA increased by 33.5% in Syrah but decreased by 50.07% in Muscat d'Italie, compared to controls. For Razegui, water stress did not affect TAA (Table 2). Overall, water deficit stress led to a significant decrease in TPF, TFC, and TAA in Muscat d'Italie. For Syrah, water deficit induced significant increases in TPC and TAA.

Stilbene accumulation

We compared the amounts of stilbenic compounds, including *t*-resveratrol, *t*-piceatannol, *t*-ε-viniferin, and *t*-piceid, in roots of Syrah, Razegui, and Muscat d'Italie genotypes in response to a moderate water deficit of 15 days. Two-way ANOVA revealed significant effects of 'grapevine genotype' and 'water deficit treatment' factors on root stilbene accumulation (Table 2).

There were marked differences in initial aptitude for stilbene biosynthesis among the three grapevine genotypes. Under well-watered conditions, total stilbene content (TSC) was highest in Syrah, followed by Muscat d'Italie then Razegui. Syrah had a TSC 4.1 times higher than Razegui and 2.06 times higher than Muscat d'Italie. Under moderate water stress, TSC significantly increased in Syrah by 22.8% compared to control plants, while TSC decreased in Muscat d'Italie (by 34.57%) and remained unchanged in Razegui (Fig. 2a).

Stilbene fractions maintained similar distribution patterns in all three grapevine genotypes, despite the significant differences in TSC. The stilbene biosynthetic pathway showed a preference for resveratrol oligomerization by oxidation, leading to *t*-ε-viniferin synthesis, followed by resveratrol glycosylation, leading to *t*-piceid synthesis, and then resveratrol hydroxylation, leading to *t*-piceatannol synthesis, in Syrah, Razegui, and Muscat d'Italie (Fig. 2b–d). When comparing the three genotypes under control conditions, Syrah contained the highest amount of *t*-ε-viniferin, a resveratrol dimer, and *t*-piceid, a glycosylated resveratrol monomer, which may explain the TSC highest level in roots of this genotype. Muscat d'Italie had the highest level of the resveratrol-hydroxylated derivative, *t*-piceatannol (Fig. 2a–d).

However, there were significant changes in the content of resveratrol derivatives within 15 days of water deficit. In Syrah roots, the contents of *t*-resveratrol, *t*-piceatannol, *t*-ε-viniferin, and *t*-piceid were elevated by 158.12%, 47.63%, 19.42%, and 22.56%, respectively, in water-stressed plants compared to the controls. Conversely, *t*-resveratrol, *t*-piceatannol, *t*-ε-viniferin and *t*-piceid levels decreased by 52.38%, 69.59%, 19.07% and 41.09%, respectively, in Muscat d'Italie roots compared to control plants.

Next to Syrah and Muscat d'Italie, water deficit also modulated synthesis of TSC in Razegui, as shown by the increase in

Table 2. Changes in total phenolic content (TPC), total flavonoid content (TFC), total antioxidant activity (TAA), and stilbene compounds in roots of three grapevine genotypes under well-watered (WW) or water deficit (WD) conditions after 15 days.

genotype/water deficit treatment	TPC	TFC	TAA	TSC	*t*-resveratrol	*t*-piceatannol	*t*-ε-viniferin	*t*-piceid
Syrah								
WW	46.747^{b,c}	29.547^{a}	373.529^{b}	10.17^{b}	0.066^{c}	0.46^{b}	6.33^{b}	3.32^{b}
WD	67.232^{a}	30.500^{a}	498.654^{a}	12.50^{a}	0.172^{a}	0.68^{a}	7.56^{a}	4.06^{a}
Razegui								
WW	40.984^{c,d}	9.285^{c}	273.895^{c}	2.47^{d}	0.042^{d}	0.33^{b,c}	1.29^{e}	0.80^{d}
WD	48.570^{b}	12.000^{b,c}	338.848^{b,c}	2.75^{d}	0.088^{b}	0.15^{d}	1.65^{d,e}	0.85^{d}
Muscat d'Italie								
WW	67.152^{a}	26.571^{a}	327.293^{b,c}	4.93^{c}	0.084^{b}	0.74^{a}	2.37^{c}	1.76^{c}
WD	34.542^{d}	14.428^{b}	163.362^{d}	3.22^{d}	0.040^{d}	0.22^{c,d}	1.92^{d}	1.03^{d}
F-values								
Water deficit treatment	1.61 ns	15.62*	341.83 ns	4.18 ns	24.38*	22.03*	34.74*	0.08 ns
Genotype	35.12*	245.12*	56,944.41*	1397.88*	27.12*	45.94*	2882.6*	419.01*
Interaction	180.59*	42.99*	34,890.73*	64.94*	42.02*	53.41*	57.38*	24.72*

Data are mean of three replicates. Data with different letters within a column are significantly different according to Tukey's HSD test at $p \leq 0.05$. Two-way ANOVA outcome as *F*-values and significances. TPC, TFC, TAA and stilbene content expressed in mg GAE.g^{-1} DW, mg CE. g^{-1} DW, µmoL TE. g^{-1} DW and mg. g^{-1} DW, respectively.
ns, non-significant.
*$p \leq 0.05$.

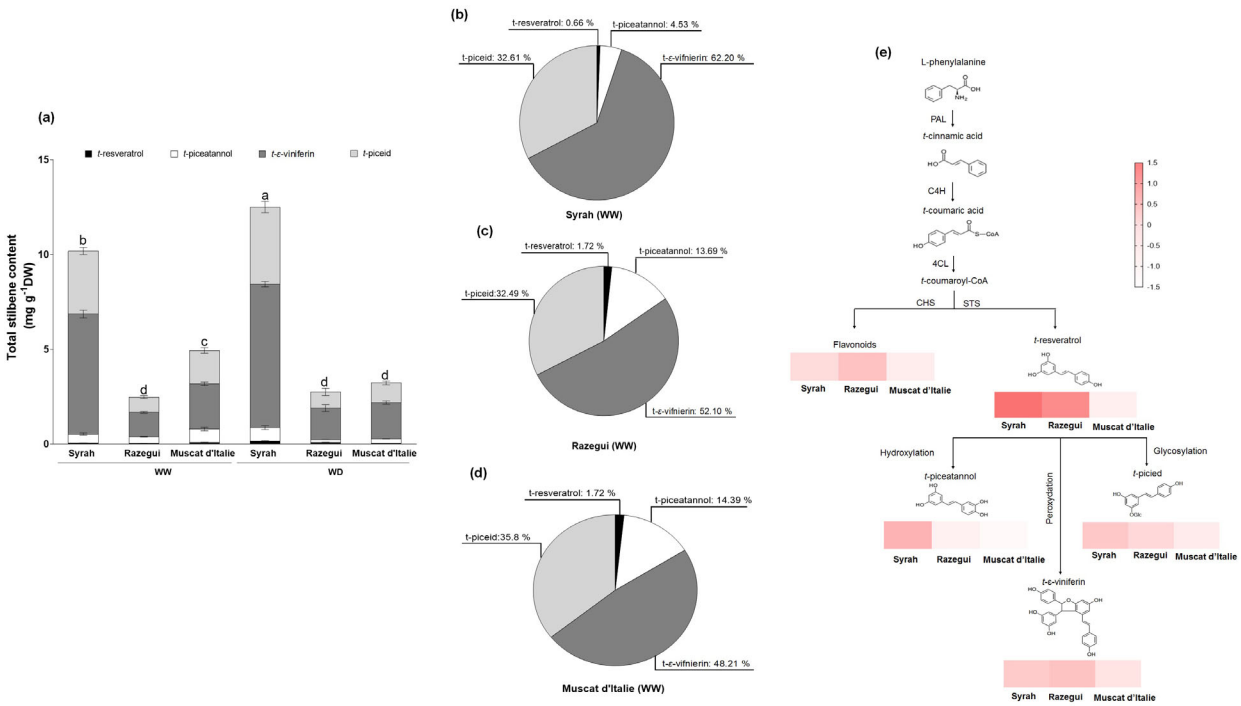

Fig. 2. (a) Changes in total stilbene content (TSC) (sum of *t*-resveratrol, *t*-piceatannol, *t*-ε-viniferin and *t*-piceid) in roots of Syrah, Razegui and Muscat d'Italie under well-watered (WW) and water deficit (WD) conditions after 15 days. There were 3 biological replicates and 9 plants per treatment. Different letters denote significant differences between groups according to Tukey's HSD test at $p \leq 0.05$. Identical letters mean no significant differences. (b–d) Proportion of individual stilbenic compounds (*t*-resveratrol, *t*-piceatannol, *t*-ε-viniferin, and *t*-piceid) compared to TSC in Syrah, Razegui and Muscat d'Italie, respectively, after 15 days under well-watered condition (WW). (e) Heat map summarizing the effect of 15 days of water deficit treatment on stilbene biosynthesis. Colour intensity for each heat map rectangle corresponds to log 2-fold change (WD/WW) of the content of each stilbenic compound for each grapevine genotype on Day 15 of water deficit treatment.

t-resveratrol by 106.23% together with a considerable decrease in *t*-piceatannol of 53.33%, compared to control plants. There were no significant differences between stressed and unstressed Razegui roots for *t*-ε-viniferin and *t*-piceid (Table 2). These changes indicate efficient induction of the stilbene biosynthesis pathway under water stress (Fig. 2e).

Response of Syrah under moderate and severe drought stress

As Syrah has the highest tolerance to water deficit stress for an extended period of up to 20 days, subsequent analyses were limited for this variety for physiological, biochemical, and stilbene changes to two time intervals (15 and 20 days after stress treatment). Two-way ANOVA revealed significant combined effects of 'harvesting time' and 'water deficit treatment' on leaf water potential, leaf RWC, leaf MSI, TPC, TFC, total detected stilbene, *t*-resveratrol and *t*-piceid (Tables 3 and 4). However, there was no significant interaction between 'time x water deficit treatment' for SER, root RWC, root MSI, TAA, *t*-ε-viniferin, and *t*-piceatannol.

Shoot elongation rate variations

Shoot growth rate in Syrah was notably reduced after prolonged water deficit. Extending the water deficit treatment to 20 days significantly delayed shoot growth in this genotype. A similar trend was observed on day 15 after the onset of water deficit treatment (following the initial harvest). Notably, Syrah subjected to water stress for 20 days exhibited a more pronounced decline in SER compared to 15-day water-stressed plants. Relative to well-watered plants, SER decreased by 65.49% on day 20 of water stress treatment, compared to 62.38% on day 15 (Table 3).

Water status parameters

Leaf water potential (Ψ_W) was significantly reduced at both time points of water deficit treatment. Under control conditions, Syrah had a similar water status at both 15 and 20 days of stress treatment. However, after 20 days of water stress, mean Ψ_W decreased by 148% in Syrah leaves compared with controls. This indicates that leaf Ψ_W decreases with increasing

Table 3. Changes in relative growth rate (SER), leaf water potential (Ψ_w), relative water content (RWC), and membrane stability index (MSI) of leaves and roots of Syrah under well-watered (WW) or water deficit (WD) conditions after 15 days or 20 days.

harvest time/water deficit treatment	SER (cm d^{-1})	$\Psi_{w\ Leaf}$ (MPa)	$RWC_{\ Leaf}$ (%)	$RWC_{\ root}$ (%)	$MSI_{\ Leaf}$ (%)	$MSI_{\ root}$ (%)
Syrah						
15 days						
WW	4.442^{a}	−0.833^{a}	81.510^{a}	79.381^{a}	65.763^{b}	81.246^{a}
WD	1.671^{b}	−1.666^{b}	72.174^{c}	65.771^{b}	59.761^{b}	71.555^{b}
20 days						
WW	3.495^{a}	−0.766^{a}	80.691^{a}	75.307^{a}	77.827^{a}	72.743^{a}
WD	1.206^{b}	−1.900^{c}	75.950^{b}	59.951^{b}	62.090^{b}	64.662^{b}
F-values						
Harvest time	20.32*	2.77 ns	3.12*	24.18*	20.91*	115.97*
Water deficit treatment	261.11*	368.77*	79.81*	207.34*	47.71*	154.52*
Interaction	2.37 ns	9*	8.85*	0.75 ns	9.56*	1.26 ns

Data represent mean of three replicates. Data with different letters within column are significantly different according to Tukey's HSD test at $p \leq 0.05$. Data with different italic letters represent significant differences between well-watered and water deficit Syrah at each harvest time, according to Student's *t*-test at $p \leq 0.05$. Two-way ANOVA given as *F*-values and significances.
ns, non-significant.
*$p \leq 0.05$.

Table 4. Changes in total phenolic content (TPC), total flavonoid content (TFC), total antioxidant activity (TAA), and stilbene compound content of roots of Syrah under well-watered (WW) or water deficit (WD) conditions after 15 days or 20 days.

harvest time/water deficit treatment	TPC	TFC	TAA	TSC	*t*-resveratrol	*t*-piceatannol	*t*-ε-viniferin	*t*-piceid
Syrah								
15 days								
WW	46.747^{c}	29.574^{b,c}	373.529^{b}	10,179.9^{c}	0.066 c	0.46^{b}	6.33^{b}	3.32^{b}
WD	67.232^{a}	30.500^{b}	498.654^{a}	12,501.0^{b}	0.172 a	0.68^{a}	7.56^{a}	4.06^{b}
20 days								
WW	52.031^{b}	25.619^{c}	348.000^{b}	13,562.2^{a}	0.068 c	0.71^{a}	5.34^{b}	7.44^{a}
WD	63.638^{a}	74.261^{a}	451.998^{a}	12,037.1^{b}	0.143 b	0.79^{a}	6.59^{a}	4.50^{b}
F-values								
Harvest time	0.61 ns	51.04*	5.93*	50.43*	21.78*	31.48*	59.3*	210.83*
Water deficit treatment	221.94*	158.25*	59.8*	3.51 ns	1210.99*	21.71*	94.88*	48.48*
Interaction	16.98*	132.69*	0.5 ns	85.51*	27.69*	4.33 ns	0.006 ns	137.74*

Data represent mean of three replicates. Data labelled with different letters within a column are significantly different according to Tukey's HSD test at $p \leq 0.05$. Data labelled with italic different letters are significant differences between well-watered and water deficit-treated Syrah at each harvest time, according to Student's *t*-test at $p \leq 0.05$. Two-way ANOVA outcome given by *F*-values and significances. TPC, TFC, TAA and stilbene contents expressed in mg GAE.g^{-1} DW, mg CE. g^{-1} DW, µmoL TE. g^{-1} DW and mg. g^{-1} DW, respectively.
ns, non-significant.
*$p \leq 0.05$.

duration of water stress, reaching its lowest value on day 20 (Table 3). The variation in leaf RWC was influenced by both water deficit treatment and stress duration. Control leaves of Syrah maintained similar a water content at both time points (15 and 20 days), whereas water stress significantly reduced leaf RWC at both time points. The negative impact of water stress was more pronounced at day 15 than on day 20. The estimated percentage reduction was 11.45% to 5.87% after 15 and 20 days, respectively. Regarding roots, water deficit induced significant reductions in RWC at both time points. The decrease in RWC was 17.14% at day 15 and 20.39% on day 20 in stressed roots compared to non-stressed samples (Table 3).

Membrane stability index

Interestingly, prolonging water deficit did not significantly alter membrane integrity of Syrah leaves. After 15 days of water stress, there were no significant differences between control and stressed leaves in MSI. Despite a significant decrease after 20 days of stress compared to the control group, stressed leaves had statistically similar MSI to those in leaves subjected to control and 15-day water deficit treatment. In contrast, water stress was more pronounced in roots, which seem more sensitive to water stress than leaves in Syrah. Throughout the stress period, MSI decreased in stressed roots compared with control roots, with percentage reductions of 11.92% and 11.1% (Table 3).

Phenolic compounds and antioxidant activity

Under limited water supply, TPC significantly increased in Syrah roots at both time points (15 and 20 days). Statistical analysis indicated that average TPC values on days 15 and 20 increased compared to control plants, with a smaller increase in 15 days (43.82%) and 22.3% after 20 days of water deficit (Table 4).

On the other hand, water deficit stress did not affect TFC within 15 days, but 20 days significantly increased TFC content in Syrah roots by 2.8-fold as compared to the controls (Table 4). A similar trend was observed for the total antioxidant activity (TAA) in Syrah roots in response to water deficit treatment. Indeed, water deficit-stressed roots showed an increased TAA by 1.33- and 1.29-fold changes within 15 and 20 days, respectively, compared to well-watered roots (Table 4).

Stilbene accumulation

TSC was affected by water deficit as well as duration. Within 15 days of water deficit, there was a significant increase in TSC in stressed roots of 1.22-fold compared to the controls (Fig. 3a). However, within 20 days of drought treatment, Syrah roots exhibited a significant decrease in TSC of 1.13-fold compared to the controls. The modulation of the stilbene biosynthesis pathway in Syrah depended on the physiological age of plant roots, regardless of stress treatment. Indeed, within 15 days, the most abundant stilbene in Syrah roots under normal conditions was *t*-ε-viniferin, followed by *t*-piceid. However, 5 days later, *t*-piceid became the major stilbenic compound in Syrah roots (Fig. 3b,c).

On the other hand, water deficit significantly increased *t*-resveratrol and *t*-ε-viniferin content at both time points (15 and 20 days of drought) in Syrah roots, compared to the controls. The *t*-resveratrol content in water-deficit roots increased by 133.4% and 110.86% within 15 and 20 days, respectively, and *t*-ε-viniferin content increased by 19.43% and 23.4% within 15 and 20 days, respectively, compared to the controls. Also, significant induction of *t*-piceatannol was observed within 15 days of water deficit. Regarding to *t*-piceid, there was no significant difference between well-watered and water-deprived roots after 15 days of drought stress, but this decreased within 20 days in stressed roots compared to the controls (Fig. 3d).

Pearson correlation coefficients between root TPC, TFC, individual stilbenes, and TAA in grapevine genotypes under water deficit stress

To explore relationships between different stilbene compounds and antioxidant capacity, correlation analyses between TPC, TFC, individual stilbenic compounds, and TAA were carried out using Pearson correlations (r). Significant positive correlations were observed between TAA and TPC, TFC, *t*-resveratrol content, *t*-piceatannol content, *t*-ε-viniferin content, and *t*-piceid content, with $r = 0.752$, $r = 0.644$, $r = 0.862$, $r = 0.770$, and $r = 0.777$, respectively, at $p = 0.01$ (Fig. 4a). These results indicate that these metabolites are involved in the antioxidant potential of roots under moderate water deficit. Moreover, as indicated in Fig. 4b, there were significant positive correlations between TAA and TPC, *t*-resveratrol and *t*-ε-viniferin in Syrah under both moderate (15 days) and severe (20 days) water deficit, with $r = 0.83$, $r = 0.935$ and $r = 0.855$, respectively, ($p = 0.01$). However, TFC, *t*-piceatannol, and *t*-piceid content did not show significant correlations with TAA. These correlations suggest that antioxidant activity of root extracts of Syrah genotype is largely attributable to total polyphenols, as well as stilbene compounds.

Principal components analysis

A PCA of the studied parameters within 15 days of water deficit for the different grapevine genotypes (Fig. 4c) found that the first two principal components accounted for 82.984% of the total variation. PC1 accounted for 54.971% and was positively correlated with root resveratrol derivatives (*t*-piceatannol, *t*-piceid, *t*-ε-viniferin, and *t*-resveratrol), TPC, TFC, and TAA, while PC2 explained 28.013% of total variance, and was associated with physiological traits, such as SER, leaf RWC, leaf MSI, and root MSI. Moreover, water-stressed Syrah was distinct from the other two tested cultivars in PC1, mainly attributable to root resveratrol derivatives (*t*-resveratrol, *t*-piceatannol, *t*-ε-viniferin, *t*-piceid), TAA, TPC, and TFC traits.

A second PCA was performed for Syrah using data from both moderate and severe water deficit (Fig. 4d). This revealed a clear distinction between control and stressed individuals of Syrah. PC1 and PC2 explained 89.453% of variance. PC1 explained 68.913% of the variance and was positively correlated with TPC, TFC, *t*- resveratrol, TAA, *t*-ε-viniferin, and leaf water potential, but negatively correlated with some physiological traits (leaf and root RWC, SER and leaf and root MSI). However, *t*-piceid and *t*-piceatannol content were positively correlated with PC2.

DISCUSSION

Coping with severe water deficit: Distinct physiological strategies of the drought-tolerant Syrah

In our present study, the prolonged water stress experiment revealed that Syrah was the most drought-tolerant genotype,

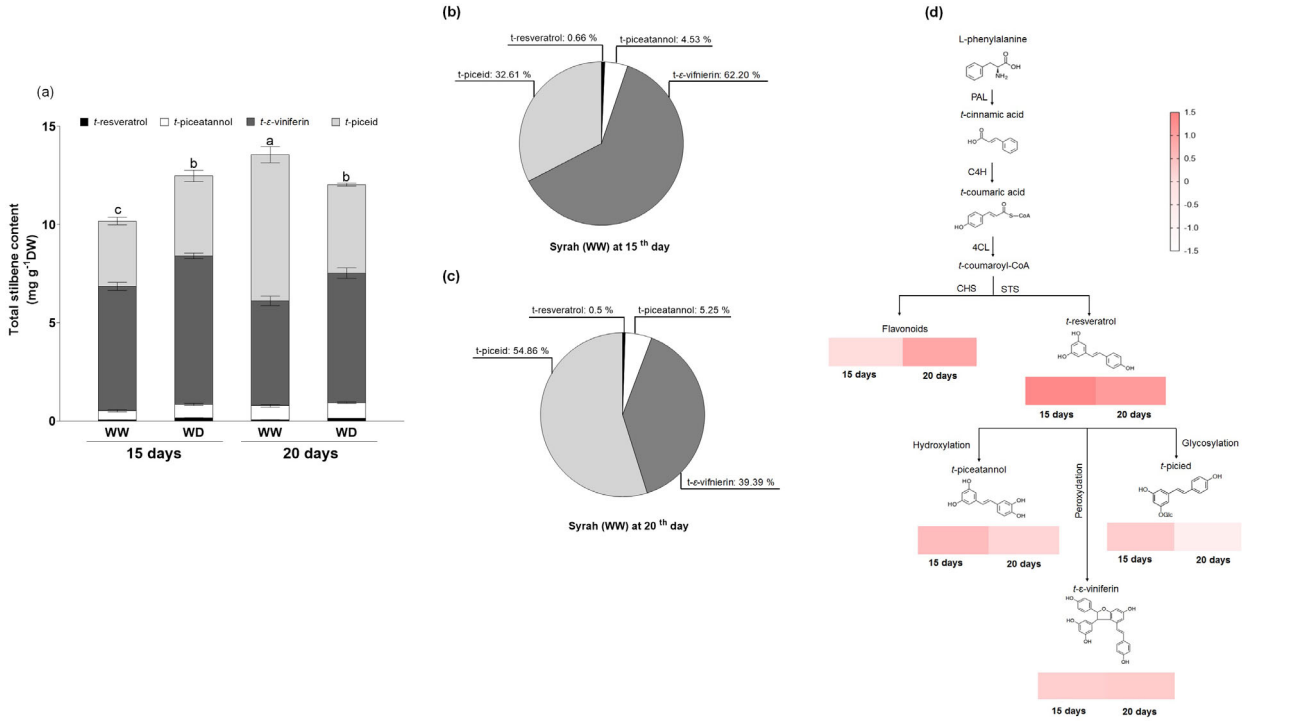

Fig. 3. (a) Changes in total stilbene content (TSC) (sum of *t*-resveratrol, *t*-piceatannol, *t*-ε-viniferin and *t*-piceid) in roots of Syrah under well-watered (WW) and water deficit (WD) conditions after 15 days or 20 days. There were 3 biological replicates and 9 plants were per treatment. Different letters denote significant differences between groups according to Tukey's HSD at $p \leq 0.05$. (b and c) Proportion of individual stilbenic compounds (*t*-resveratrol, *t*-piceatannol, *t*-ε-viniferin and *t*-piceid) compared to TSC in Syrah after 15 days and 20 days, respectively, under well-watered conditions (WW). (d) Heat map summarizing the effect of 15 days and 20 days of water deficit on stilbene biosynthesis. The colour intensity for each heat map rectangle corresponds to log2-fold change (WD/WW) in content of each stilbenic compound for Syrah at days 15 and 20 of water deficit treatment.

compared to Razegui and Muscat d'Italie (Fig. 1). This was confirmed for several processes associated with water deficit tolerance described above. In our study, Syrah maintained a higher RWC compared to Razegui and Muscat d'Italie. The higher RWC suggested greater ability of Syrah to maintain cell turgor at low water potentials. Water status is an important physiological parameter and the RWC of plant leaves can reflect the level of drought resistance and water absorption efficiency (Anjum *et al.* 2011; Kadioglu *et al.* 2011). A positive correlation between the high RWC and drought tolerance ability has previously been proposed for many plant species (Ahmed *et al.* 2015; Amoah *et al.* 2019; Du *et al.* 2020).

Drought reduced the growth rate of different grapevine genotypes, as shown by the decline in SER. Syrah plants were least affected, followed by Razegui and Muscat d'Italie, whose growth was almost completely arrested within 20 days of water deficit (severe drought). Shoot elongation was proved to be a very sensitive measure of plant growth and a good indicator of soil water availability changes and drought tolerance (Cramer *et al.* 2007). Survival of plants under stress is mediated by integrity of their cell membrane, which is considered as the initial site of injury from ongoing stress. MSI was used as a reliable oxidative damage indicator to assess the rate of injury to cell membranes under abiotic stress (Hniličková *et al.* 2019). Therefore, assessment of cell membrane integrity (evidenced by MSI) is pivotal for evaluating plant drought tolerance level. In this study, the integrity of the cell membrane in Syrah grown under drought stress was examined by measuring MSI of both leaf and root tissues. We found that MSI was less affected in Syrah compared to the other two genotypes, indicating that this genotype is better protected against oxidative stress. A decrease in MSI is an indicator of membrane damage due to lipid peroxidation by ROS (Dhindsa *et al.* 1981; Upadhyaya *et al.* 1990; Dhindsa 1991). According to Aran *et al.* (2017), the drought-tolerant Iranian grapevine genotype 'Yaghooti' had the lowest reduction in MSI under water deficit stress. Under drought, cell membranes may undergo changes, such as increased permeability and a decreased selectivity, which can be evidenced by increases in electrolyte leakage (Blokhina *et al.* 2003). Drought-resistant grapevine genotypes had higher ROS scavenging ability (Li *et al.* 2021). In this study, the TAA increased most under drought stress essentially in roots of Syrah, followed by Razegui, but decreased in roots of Muscat d'Italie. Since the antioxidant defence response plays an important role in drought tolerance (Laxa *et al.* 2019), we deduce that the observed drought tolerance in the Syrah genotype may rely on its ability to reduce ROS to non-toxic levels.

Phenolic defence strategies in grapevine genotypes under drought stress: Implications for antioxidant systems and membrane stability

Plants have evolved different defence processes to mitigate drought-induced oxidative damage, including over-production

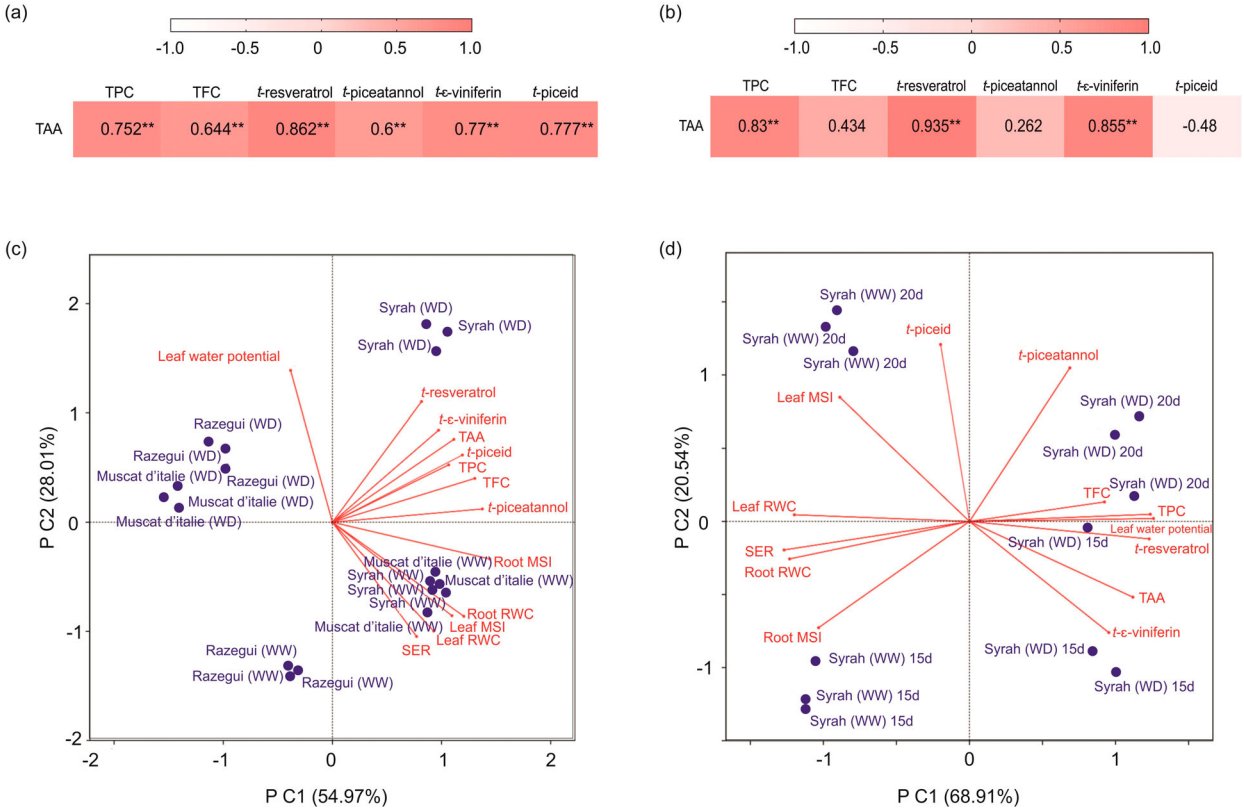

Fig. 4. (a and b) Heat map showing Pearson correlation between total antioxidant activity (TAA) and phenolic compound content (TPC, TFC, and stilbenes (*t*-resveratrol, *t*-piceatannol, *t*-ε-viniferin and *t*-piceid)) for Syrah, Razegui and Muscat d'Italie after 15 days of water deficit and for Syrah after 15 days and 20 days of water deficit, respectively. Pink indicates a correlation close to 1; white indicates a correlation close to −1. Asterisks (**) show significant differences at $p = 0.01$. (c and d) PCA biplot based on variance in physiological traits, biochemical parameters, and stilbene content of Syrah, Razegui and Muscat d'Italie under well-watered (WW) and water deficit (WD) after 15 days, and for Syrah after 15 days and 20 days of water deficit.

of antioxidant metabolites that can limit propagation of oxidative chain reactions (Caliskan *et al.* 2017). The involvement of endogenous phenolic compounds in drought tolerance mechanisms has been described in many plants. This is because phenolic compounds have high antioxidant properties in plants. The magnitude of some phenolic compounds in mitigating drought depends on species, cultivar, tissue and drought intensity (Weidner *et al.* 2007; Akula & Ravishankar 2011; Gharibi *et al.* 2015). The impact of environmental stresses on plants is multifaceted, as evidenced by studies showing that these stresses can either decrease (Weidner *et al.* 2007; Król *et al.* 2014) or increase the TPC in plants (Weidner *et al.* 2009; Gharibi *et al.* 2015). Here, the TPC in Syrah and Razegui genotypes was increased by drought, but decreased in Muscat d'Italie. Our results agree with those of Weidner *et al.* (2009), who described an increase in TPC in grapevine roots under drought.

On the other hand, drought can damage to membrane fluidity and stability, which is commonly used as a physiological index for evaluation of plant drought tolerance (Premachandra *et al.* 1990). Our results showed that Syrah had the highest TPC and the lowest cell membrane damage index, hence, higher ability to counteract drought compared to Razegui and Muscat d'Italie. Phenolics are secondary metabolites that accumulate under water deficit conditions (Hessini *et al.* 2022). Total phenolic compounds contribute to plant drought mitigation through scavenging ROS generated during stress, thereby preventing cellular oxidative damage (Bettaieb *et al.* 2011). Generally, TPC in plants positively correlate with TAA (Bettaieb *et al.* 2011; Gharibi *et al.* 2015). This is in line with our findings, where Syrah had increased levels of both TPC and TAA compared to the other two genotypes. Hence, the Syrah genotype may have a more effective non-enzymatic antioxidant system due to phenolics accumulation. Additionally, phenolics can be used to form covalent bonds with carbohydrates in the cell wall, which helps to maintain cell turgor under osmotic stress (Hura *et al.* 2012).

Stilbene accumulation as part of the antioxidant defence strategy against drought

The baseline content of stilbenic compounds in roots of plants grown under control conditions showed significant genotypic variability, suggesting intrinsic differences in stilbene synthesis capacity (Corso *et al.* 2015). This aligns with existing literature highlighting the genetic diversity of stilbene metabolism across *Vitis* species (Duan *et al.* 2015). In our present work, among the stilbene derivatives, the oxidized resveratrol oligomer, viniferin, was the most abundant compound in Syrah, and was further enhanced within 15 days of drought stress exposure, probably due to elevated Peroxidases Class III activity (Morales *et al.* 1997; Barceló *et al.* 2003), thereby contributing to the

drought tolerance. Such oxidation is mediated by the resveratrol-oxidizing basic peroxidase isoenzyme (Calderón *et al.* 1992; Pedreno *et al.* 1996) known for its role as a free radical scavenger (Mikulski & Molski 2010). The oxidative dimerization of resveratrol into viniferin has been linked to resistance of grapevine cultivars to downy mildew (Pezet *et al.* 2004). Recently, Khattab *et al.* (2021) reported that wild grapevine genotypes with a high viniferin conent had strong resistance to pathogens. Additionally, drought-tolerant grapevine plants exhibited had enhanced phytoalexin (resveratrol and viniferin) accumulation compared with sensitive plants (Hatmi *et al.* 2015). The enhancement of *t*-resveratrol accumulation under severe drought treatment for 20 days can be linked to drought resilience. Previous studies have proposed that the constitutive accumulation of stilbenes in lignified organs may serve as a defence against pathogens, indicating their potential allelopathic role (Chong *et al.* 2009; Pugajeva *et al.* 2018; Goufo *et al.* 2020). Resveratrol has efficient free radical scavenging activity (Rodríguez-Bonilla *et al.* 2017; Šamec *et al.* 2021). The antioxidant activity of stilbene derivatives is generally attributed to phenolic structure and the presence of hydroxyl (–OH) groups (Charlton *et al.* 2023). Studies indicated that dimers of resveratrol, known as viniferins, contain four phenyl and five OH groups and exhibits increased antioxidant activity, underscoring the significance of hydroxyl group number (Privat *et al.* 2002).

The significant positive correlations found between TAA and TPC, TFC and the four-stilbene compounds, supports their involvement in antioxidant response of the drought-tolerant grapevine genotype Syrah after 15 days of water stress. These findings underscore the potential positive correlation between stilbene levels and grapevine drought tolerance, suggesting that stilbenes could be key players in plant defence mechanisms in response to moderate drought stress. The abundance of different stilbenic forms was reported to vary under drought stress. Post veraison stage, water deficit stress increased piceid (glycosylated form of resveratrol) and δ-viniferin content but reduced resveratrol content (Herrera *et al.* 2017). In contrast, Sun *et al.* (2023) reported an increase in both *cis*- and *trans*-resveratrol levels and a decrease in both *cis*- and *trans*-piceid content under drought. In our study, Syrah activated the stilbene biosynthesis pathway leading to increases in *t*-resveratrol, *t*-ε-viniferin, *t*-piceatannol, and *t*-piceid (Fig. 3d), all of which were positively correlated with TAA (Fig. 4a). After 20 days of drought, *t*-resveratrol and *t*-ε-viniferin were the only stilbene compounds showing significant positive correlations with TAA (Fig. 4b). The glucoside form of *t*-resveratrol, *t*-piceid, was mainly produced under moderate water deficit stress (15 days). The increased accumulation of *t*-resveratrol and *t*-ε-viniferin might partly explain the Syrah genotype's better survival under severe drought stress. In addition, our findings showed that *t*-ε-viniferin biosynthesis was induced to the same extent in Syrah roots under water deficit stress after both 15 days and 20 days, highlighting the pivotal role of *t*-ε-viniferin in the drought tolerance mechanisms of Syrah (Fig. 3d).

Evidence suggests that plant antioxidant status regulated by stilbene biosynthesis (Jeandet *et al.* 2021) is critical for overcoming severe plant water deficit stress (Laxa *et al.* 2019). Faurie *et al.* (2009) and Belchí-Navarro *et al.* (2013) demonstrated that biosynthesis of stilbenes in grapevine cell suspensions relied on the production of O_2^- and H_2O_2 under methyl jasmonate and cyclodextrin treatments. More recently, Bai *et al.* (2019) proposed a model where stilbene accumulation in cv. *Vitis labrusca* 'Concord' following Al^{3+} and UV-C treatment involves ROS production as an early signalling event.

The accumulation of stilbenes as defence molecules in roots under water stress in tolerant grape genotypes supports the hypothesis that stilbene stimulation contributes to grapevine adaptation to stress (Erb *et al.* 2009; Balmer *et al.* 2013). Furthermore, the fact that stilbenes were found in forms covalently bound to cell wall components suggests their involvement in cell wall reinforcement during infection response, consolidating their importance in plant defence (Morales *et al.* 2000). Monitoring these stress biomarkers is of great interest in assessing the effectiveness of priming agents in inducing natural defence responses in grapevine.

CONCLUSION

Analysing changes in phenolic content, antioxidant activity, and stilbene levels in cultivated grape genotypes can help to reveal the metabolic mechanisms contributing to drought stress tolerance in *Vitis* species. Our research providees insights into the implications of different stilbene derivatives in drought-tolerance mechanisms of *V. vinifera* Syrah, potentially through the regulation of cell redox homeostasis. Our results further support the hypothesis that stilbene metabolism contributes to drought tolerance mechanisms in grapevine. Moreover, roots offer an available and cost-effective source of bioactive molecules, like stilbenes. Further investigations based on molecular analysis of key genes involved in stilbene biosynthetic pathways would expand our knowledge of their agricultural and commercial value. Stilbene metabolic targets might be introduced into grapevine varieties to improve drought-tolerance, leading to the development of more resilient genotypes capable of thriving in the face of challenging climate changes. These findings potentially open new avenues for metabolic engineering aimed at enhancing biosynthesis of secondary metabolites in *Vitis* species.

AUTHOR CONTRIBUTIONS

SD and AM conceived and designed the study. FH and SD conducted research including greenhouse culture and physiology. FH performed biochemical and stilbene analysis. SV supervised HPLC analysis. SD and FH wrote the original draft of the manuscript with contribution from MG and HB. All authors read and contributed to review and editing of the final manuscript.

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CONFLICT OF INTEREST STATEMENT

On behalf of all authors, the corresponding author states that there is no conflict of interest.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Fig. S1. A representative HPLC-UV (310 nm) profile of root ethanolic extracts from Syrah Razegui, and Muscat d'Italie under well-watered conditions (a), and (c) and (e) and under water deficit stress for 15 days (b), (d) and (f), respectively, comparing to standard stilbene solution. Each arrow with corresponding number at peaks refers to (1) *t*-piceid, (2) *t*-piceatannol, (3) *t*-resveratrol, (4) *t*-*ε*-viniferin, and (5) *t*- hydroxystilbene. Peak (6) refers to *t*-pterostilbene not detected in root samples.

Fig. S2. A representative HPLC-UV (310 nm) profile of root ethanolic extracts from Syrah under well-watered conditions (a) and water deficit (b) for 20 days compared to standard stilbene solution. Each arrow with the corresponding number at peaks refers to (1) *t*-piceid, (2) *t*-piceatannol, (3) *t*-resveratrol, (4) *t*-*ε*-viniferin, (5) *t*- hydroxystilbene. Peak (6) refers to *t*-pterostilbene, not detected in root samples.

Table S1. Retention time (min) of stilbene compounds ((1) *t*-piceid, (2) *t*-piceatannol, (3) *t*-resveratrol, (4) *t*-*ε*-viniferin and (5) *t*-hydroxystilbene) in standard solution and grapevine root ethanolic extracts from grapevines under well-watered and water deficit stress for 15 days analysed by HPLC-UV. Stilbene identification is based on comparison of retention times and UV spectra of each stilbene compound in standard solution and in grapevine sample extract.

Table S2. Retention time (min) of stilbene compounds ((1) t-piceid, (2) *t*-piceatannol, (3) *t*-resveratrol, (4) *t*-*ε*-viniferin, and (5) *t*-hydroxystilbene) in standard solution and in grapevine root methanolic extracts from Syrah under well-watered conditions and water deficit stress from Syrah drought stressed for 20 days, analysed by HPLC-UV. Stilbene identification is based on comparison of retention time and UV-spectra of each stilbene compound in standard solution and in grapevine sample extract.

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