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Article

EFFECT OF DROUGHT ON AQUAPORIN EXPRESSION IN GRAFTED AND UNGRAFTED GRAPEVINE CULTIVARS

EFEITO DA SECA NA EXPRESSÃO DA AQUAPORINA EM CULTIVARES DE VIDEIRA ENXERTADAS E NÃO ENXERTADAS

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SUMMARY

Drought stress severely affects growth, development and productivity in most agricultural crops. Since ancient times, rootstocks have been used to enable crop cultivation in unsuitable soil conditions. In the present study, three factors were evaluated: 1) cultivar: *Vitis vinifera* L. cv. 'Horozkarası' (drought-tolerant) and cv. 'Kabarcık' (drought-sensitive) were used; 2) rootstock: each cultivar was self-rooted and grafted onto 'Rupestris du Lot' rootstock; 3) drought stress: half of each cultivar/rootstock combination underwent drought stress and the other half was irrigated at field capacity for seven days. In order to estimate the responses of the cultivars, relative water content, proline content and aquaporin isoform expression levels (VvPIP2;1, VvPIP2;2, VvTIP1;1, and VvTIP2;1) were quantified. The results revealed that drought stress caused more reduction in relative water content (RWC) in 'Kabarcık' cultivar (drought-sensitive) than in 'Horozkarası' cultivar (drought-tolerant). Proline content increased in both cultivars in response to drought stress but to a relatively greater extent in the grafted 'Kabarcık' cultivar. Considering expression levels of genes, VvPIP2;1, VvPIP2;2, and VvTIP2;1 were downregulated whilst VvTIP1;1 was upregulated in the leaf. Both 'Horozkarası' and 'Kabarcık' cultivars showed similar trends in terms of their responses to drought stress. Grafting significantly increased the proline content in both cultivars exposed to drought stress. The rootstock conferred better drought protection to 'Kabarcık' cultivar than to 'Horozkarası' cultivar.

RESUMO

O stress hídrico afeta consideravelmente o crescimento, o desenvolvimento e a produtividade na maioria das culturas agrícolas. Desde a antiguidade, os porta-enxertos têm sido utilizados para permitir as culturas em condições de solo inadequadas. No presente estudo, foram avaliados três fatores: 1) cultivar: foram utilizadas as cultivares 'Horozkarası' (tolerante à seca) e 'Kabarcık' (sensível à seca) da espécie *Vitis vinifera* L; 2) porta-enxertos: cada cultivar foi auto-enraizada e enxertada no porta-enxertos 'Rupestris du Lot'; 3) stress hídrico: metade de cada combinação cultivar/porta-enxertos foi sujeita a stress hídrico e a outra metade foi irrigada à capacidade de campo durante sete dias. Para estimar as respostas das cultivares, procedeu-se à quantificação do teor relativo de água, do teor de prolina e dos níveis de expressão da isoforma aquaporina (VvPIP2;1, VvPIP2;2, VvTIP1;1 e VvTIP2;1). Os resultados revelaram que o stress hídrico causou maior redução no conteúdo relativo de água (RWC na cultivar 'Kabarcık' (sensível à seca) do que na cultivar 'Horozkarası' (tolerante à seca). O teor de prolina aumentou em ambas as cultivares em resposta ao stress hídrico, mas em maior quantidade na cultivar enxertada 'Kabarcık'. Em relação aos níveis de expressão dos genes, foram regulados negativamente os genes VvPIP2;1, VvPIP2;2 e VvTIP2;1 enquanto o gene VvTIP1;1 foi regulado positivamente na folha. Tanto a cultivar 'Horozkarası' como a cultivar 'Kabarcık' mostraram tendências semelhantes no respeitante às suas respostas ao stress hídrico. A enxertia provocou um aumento significativo do teor de prolina em ambas as cultivares sujeitas ao déficit hídrico. O porta-enxertos conferiu melhor proteção contra a seca na cultivar 'Kabarcık' do que na cultivar 'Horozkarası'.

Keywords: Horozkarası, Kabarcık, drought-tolerant, rootstock, gene expression.
Palavras-chave: Horozkarası, Kabarcık, resistência à seca, porta-enxertos, expressão genética.

INTRODUCTION

The Mediterranean region, including Balkans, with a total of 4.2 million hectares meet over 50% of the world's wine grapes and one-third of all table and raisin grapes (Capone *et al.*, 2014). As for other annual or perennial plant species, grapevine cultivars are continuously under pressure of environmental fluctuations. The effects of the ever-changing environmental conditions are translated into the impaired physiological and biochemical attributes of the plants, which in turn cause critical reductions in productivity of grape (Gambetta *et al.*, 2020). The reports anticipate that the incidence of unexpected climatic scenarios such as excessive precipitation or dry seasons might increase in certain regions (IPCC, 2007). Being an iconic plant species such as the olive tree, the vine and related activities are intensely developed in the Mediterranean Basin. Researchers hypothesized that crop yield of grapes will critically decrease due

to environmental fluctuations (Iglesias *et al.,* 2007; Brás *et al*., 2021; Dinis *et al.,* 2022).

Among the stress factors, drought is one of the most devastating abiotic factors on grapevines. In order to cope with the relevant stress, grapevines, as other sessile plants, developed remarkable strategies at physiological, biochemical and molecular levels (Serra *et al*., 2014). For instance, grapevines effectively use hydraulic conductivity to mitigate the adverse impact of drought stress. Hydraulic conductivity is very critical in early stomatal closure to preserve foliar water, avoiding xylem vessel cavitation and embolism damage, and enhancing water uptake (Lovisolo and Schubert, 2006). Regarding to the regulation of hydraulic conductivity, aquaporins, called water channel proteins, play crucial roles in ensuring the continuous water transport from roots to leaves, controlling the permeability of membranes to water, and altering cellular hydraulic conductivity (Lovisolo and Schubert, 2006; Hayes *et al.*, 2007; Surbanovski and Grand, 2014). Aquaporins belong to the major intrinsic protein (MIP) family, having five sub-groups in grapevines based on their nucleotide sequence similarity and cellular location. Of the aquaporins available, plasma membrane intrinsic proteins (PIPs) and tonoplast intrinsic proteins (TIPs) are the most abundant in plant organs. Their locations in the cell suggest that they mainly regulate water transport (Chrispeels and Maurel, 1994; Johanson *et al.*, 2001). PIPs are mostly located in the plasma membrane whereas TIPs occur in the tonoplast (Kapilan *et al.*,2018). PIPs have higher nucleotide and amino acid similarity than TIPs. The PIP aquaporins are divided into PIP1 and PIP2 subgroups whilst the TIP aquaporins are divided into five different subfamilies (Shelden *et al*., 2009). Aquaporins control water movement and might be downregulated or upregulated depending on their location and the severity of abiotic stress on the plant (Jang *et al.*, 2013). In this regard, the former reports clearly showed that changes in the aquaporin isoform expression levels differ among grapevine varieties, plant organ types and locations, environmental stress duration and severity, and circadian rhythm (Kaldenhoff *et al.*, 2006; Heinen *et al.*, 2009; Leitão *et al.*, 2012; Pou *et al.*, 2013; Turgay, 2015; Shelden *et al.*, 2017; Abdelhakam *et al.*, 2021). Transpiration removes water from the leaf, resulting high tension. Xylem embolism is avoided by moving water from adjacent living cells to the xylem vessels via aquaporins (Daniela *et al*., 2021). PIP aquaporins are membrane proteins; hence, they play important roles in cell water exchange, regulating the intracellular traffic by modulating aquaporin gene transcription. PIP1 and PIP2 aquaporins are expressed independently of each other. However, PIP2 aquaporins play active roles in water flow whereas PIP1 aquaporins increase the efficiency of PIP2 aquaporins (Gautam and Pandey, 2021). TIPs regulate cell turgor by moving water to and from the vacuoles across the tonoplasts. TIP aquaporins are abundant along the tonoplast, control water flow between the vacuole and the cytoplasm, regulate the permeability of urea, hydrogen peroxide, and glycerol, and provide osmoregulation (Li *et al.*, 2014). Some TIP aquaporins may be organ-specific. TIP3;1 and TIP3;2 are expressed in seeds whereas TIP5;1 is found in pollen mitochondria and remobilises nitrogen (Mandlik *et al.*, 2022).

The scion × rootstock × environment interaction must be considered to elucidate the mechanisms by which grapevines contend with drought stress. American rootstocks have high tolerance to abiotic stress factors such as drought and biotic stress factors like phylloxera. Hence, the selection of a rootstock suitable for the local soil and climatic conditions and the scion variety is vital in sustainable viticulture development (Serra *et al.*, 2014). Rootstocks might play important roles in drought tolerance by controlling water uptake from the soil and regulating transpiration (Soar *et al.*, 2006; Tramontini *et al.*, 2013). Several studies have been conducted on drought stress in grapevine (Chaves *et al*., 2010). Nevertheless, few investigations have been performed on rootstock × scion interactions. Hence, under a given *terroir* condition, it is vital to select a suitable combination of variety and rootstock for a sustainable development of viticulture. For this reason, it was hypothesised that grafting would help to ensure a wide range of tolerance against drought stress. In order to test the hypothesis, a series of aquaporin associated genes (VvPIP2;1, VvPIP2;2, VvTIP1;1, and VvTIP2;1), RWC and proline content of two grapevine cultivars contrasting drought tolerance was estimated.

MATERIALS AND METHODS

Plant materials and experimental conditions

The experiment was conducted from February to August 2019 in a semi-controlled greenhouse at Kilis 7 Aralık University (Kilis, Turkey). Drought-tolerant ('Horozkarası') and drought-sensitive ('Kabarcık') *Vitis vinifera* L. cultivars and 'Rupestris du Lot' rootstock were used. The experiment was carried out with four replicates, and each replicate corresponded to five plants. Both ungrafted and grafted plants were cultivated in pots filled with 1:2:1 (w/w/w) peat:soil:perlite and well-watered at field capacity for six months. Their lateral branches were trained to create and maintain a homogeneous morphology. The plants were then exposed to drought stress by withholding water. The plastic pots containing the soil mixture were monitored with a soil tensiometer and the drought

stress phase was terminated by day 7. The control plants were irrigated at field capacity throughout the experiment. After a 7-day period, fully expanded leaves were collected. The experimental design (cultivar*rootstock*drought stress, 2^3=8) is presented in Table I.

Physiological and biochemical analyses

Relative water content (RWC)

To ensure homogeneous sample collection, the leaves were cut 1,5 cm diameter with a hole punch, and the fresh weight (FW) of the leaf discs was immediately determined. The leaf discs were then stored in double-distilled water for 4 h. Then excess surface water was blotted with paper towels, the turgid weights (TW) of the leaf discs were measured, the leaf discs were then dried at 60 °C for 24 h, and their dry weights (DW) were measured (Dhanda and Sethi, 1998). RWC was calculated according to Equation 1.

$$RWC\ (\%) = [(FW - DW) / (TW - DW)] \times 100 \quad \textbf{Eq. 1}$$

Table I

Experimental design of the study

Treatments	Acronym	Water regime
'Horozkarası' Ungrafted Control	HUC	Full-irrigation
'Horozkarası' Ungrafted Stress	HUS	Drought stress
'Kabarcık' Ungrafted Control	KUC	Full-irrigation
'Kabarcık' Ungrafted Stress	KUS	Drought stress
'Horozkarası' Grafted Control	HGC	Full-irrigation
'Horozkarası' Grafted Stress	HGS	Drought stress
'Kabarcık' Grafted Control	KGC	Full-irrigation
'Kabarcık' Grafted Stress	KGS	Drought stress

Proline content

Fifty milligrams (FW) leaf tissue was homogenised in 1 mL of 40:60 (v/v) ethanol:water with a TissueLyser (Qiagen, Hilden, Germany). The extract was mixed with 100 µL of a solution consisting of 1% (w/v) ninhydrin, 20% (v/v) absolute ethanol, 60% (v/v) glacial acetic acid, and 20% dd-H_2O in a Microwell plate. The plate was heated to 80 °C for 30 min and then cooled to 22 °C. OD_{520} was measured in a microplate reader (Thermo Scientific Multiskan GO; Thermo Fisher Scientific, Waltham, MA, USA). The proline content was determined using a standard curve dilution series of 1-0.4-0.2-0.1-0.04 mM (Carillo and Gibon, 2011).

Molecular analyses

RNA extraction and qRT-PCR

After a 7-day drought stress, leaves were excised from the plants, frozen in liquid nitrogen, and stored at -80 °C until the subsequent analyses. RNA was extracted with a Vivantis Total Plant RNA Extraction Kit (Vivantis Technologies, Selangor Darul Ehsan, Malaysia) according to the manufacturer's protocol. RNA concentration and purity were determined on a 1% agarose gel and with a Thermo Scientific Multiskan ™ GO spectrophotometer using the nanodrop reader.

The cDNA was synthesised with a Vivantis cDNA Synthesis Kit (Vivantis Technologies) following the manufacturer's protocol. The PCR was performed after 5 min incubation at 85 °C for 60 min treatment with reverse transcriptase enzyme at 42 °C.

Real-time PCR was performed with SYBR Green I Master Mix. Regiospecific primers for the targeted genes were used (Table II). Actin served as the reference gene. Each 20-µL reaction system comprised 0.2 µL of each primer, 5 µL of 1/10 diluted cDNA, and 10 µL of SYBR Green I Master Mix (Thermo Fisher). A melting curve analysis was performed to determine PCR efficiency and detect dimerization. The thermal cycling conditions were 10 min at 95 °C, 15 s at 94 °C, 20 s at 55–60 °C, and 20 s at 72 °C. The relative expression values were calculated by the $2^{-\Delta\Delta Ct}$ method (Schmittgen and Livak, 2008) using the Ct (cycle threshold) values obtained via a Light Cycler NanoReal-Time PCR (Roche Diagnostics, Basel, Switzerland).

Statistical analysis

All data were processed using the "agricolae" package in RStudio v. 1.3.1073 (de Mendiburu, 2016). ANOVA was carried out to identify significant differences, and Tukey HSD was used as post hoc test among treatments at each sampling time.

Table II

Aquaporin gene primers used in qPCR

Primer	Forward	Reverse	Reference
PIP2;1	5'-GCCTTGGAGCTGCAGTAATC-3'	5'-TGAATGAACCAAGGGCTTTC-3'	Sabir *et al.* (2014)
PIP2;2	5'-CCACGGTCATAGGCTACAAG-3'	5'-CGAAGGTCACAGCAGGGTTG-3'	Vandeleur *et al.* (2008)
TIP1;1	5'-CCAACGTGTCTGTGTGGAAC-3'	5'-GGGTTCATTGAAGCACCAGT-3'	Sabir *et al.* (2014)
TIP1;2	5'-GCCGATTTCGAGAATAGCTG-3'	5'-GCCGATTTCGAGAATAGCTG-3'	Leitão *et al.* (2012)
Actin	5'-GCCTCCGATTCTCTCTGCTC -3'	5'-TCACCATTCCAGTTCCATTGTCA -3'	Vandeleur *et al.* (2008)

RESULTS AND DISCUSSION

Changes in RWC and proline content in grafted and ungrafted plants under drought stress

RWC decreased in both cultivars in response to drought stress. However, the reduction in RWC was more severe in the sensitive cultivar 'Kabarcık' than the tolerant cultivar 'Horozkarası'. The lower RWC reduction was observed in KGS, whereas the higher RWC decline was recorded in KUS. However, the effect of drought stress (DS) was significant among treatments (Table III).

DS caused significant increase in proline content. Considering the experimental groups, the highest content of proline was observed in KUS, whilst the lowest content was recorded in HUC. Corresponding to DS, the wider range of changes in proline content was observed between KGC and KGS. The rootstock contributed to the proline content in both cultivars and also the changes in proline content in response to cultivar (C), grafting (G), drought stress (DS), and the G × DS were significant (Table III).

Table III

Effect of drought stress on RWC and proline content in leaves of grafted and ungrafted grapevine cultivars

			RWC (%)	Proline (nmol/mg FW)
Horozkarası	Ungrafted	Control	83.2 a	0.013 c
		DS	79.2 ab	0.026 bc
	Grafted	Control	82.4 a	0.015 c
		DS	80.1 ab	0.079 b
Kabarcık	Ungrafted	Control	82.2 a	0.023 bc
		DS	75.5 b	0.037 bc
	Grafted	Control	83.4 a	0.022 bc
		DS	77.7 b	0.155 a
Cultivar (C)			ns	*
Grafting (G)			ns	**
Drought Stress (DS)			***	***
C × G			ns	ns
C × DS			ns	ns
G × DS			ns	**
C × G × DS			ns	ns

For each parameter, values indicate the results of Tukey HSD test between control and drought stress for each cultivar (grafted and ungrafted); ns: not significant; ***p < 0.001; **p < 0.01; *p < 0.05.

Changes in aquaporin gene expression in grafted and ungrafted plants under drought stress

Corresponding to the expression levels of aquaporin genes, foliar PIP2;1, PIP2;2, and TIP2;1 aquaporins were down-regulated whilst foliar TIP1;1 aquaporin was up-regulated. Independent from tolerance levels, both cultivars exhibited similar responses with respect to the expression levels (Table IV).

PIP2;1 aquaporin exchange significantly decreased in response to drought stress. The highest relative gene expression of PIP2;1 was estimated in KGC, whereas the lowest expression level was quantified in KUS. Of the treatments, DS significantly affected expression level of PIP2;1. However, other factors were not statistically significant. Regarding PIP2;2, the highest and lowest relative expression levels were estimated in KUC and KGS, respectively. Variance analysis revealed that C × G × DS interaction was significant for PIP2;2. Concerning TIP1;1, the highest and lowest expression levels were recorded in KUS and KGC, respectively. The G × DS and C × G × DS interactions were not significant for TIP1;1 aquaporin. However, all other interactions were significant at various levels. The TIP2;1 aquaporin gene was downregulated in response to foliar drought stress. The highest expression level of TIP2;1 was noted in HUC and the lowest expression level was recorded in KUS. Variance analysis showed that the C × DS and C × G × DS interactions were not significant whereas the other interactions were significant (Table IV).

Table IV

Effect of drought stress on changes in expression of PIP2;1, PIP2;2, TIP1;1 and TIP2;1 aquaporin genes in leaves of grafted and ungrafted grapevine cultivars

			PIP2;1	PIP2;2	TIP1;1	TIP2;1
Horozkarası	Ungrafted	Control	3.32 ab	0.30 b	1.04 bc	0.81 a
		DS	0.86 bc	0.20 b	2.58 bc	0.46 bc
	Grafted	Control	2.52 abc	0.25 b	1.07 bc	0.55 abc
		DS	1.11 bc	0.16 b	2.12 bc	0.29 cd
Kabarcık	Ungrafted	Control	3.22 ab	1.51 a	1.88 bc	0.62 ab
		DS	0.34 c	0.18 b	6.09 a	0.15 d
	Grafted	Control	4.38 a	0.34 b	0.44 c	0.46 bc
		DS	0.59 bc	0.15 b	3.28 b	0.29 cd
Cultivar (C)			ns	***	**	**
Grafting (G)			ns	***	*	*
Drought Stress (DS)			***	***	***	***
C × G			ns	***	*	*
C × DS			ns	***	*	ns
G × DS			ns	***	ns	*
C × G × DS			ns	***	ns	ns

For each parameter, values indicate the results of Tukey HSD test between control and drought stress for each cultivar (grafted and ungrafted); ns: not significant; ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$.

Drought is one of the damaging stressors that cause critical problems and deserves further investigation to reveal the mechanism of action on plants. After doing a basic search on SCOPUS with criteria inclusion “drought OR water stress” on January 11, 2023, about 264.397 documents were recorded. In spite of the high number of documents, the action mechanism of drought is not fully-elucidated due to the critical changes in severity of stress and frequency, being still very destructive on crop and non-crop species. Physiological and molecular basis responses of plants should be addressed and understood in order to cope with the stress and to propose solutions. In such analysis, different cultivars contrasting in tolerance are relatively significant to understand the response. For this reason, the present study was performed. Of the assayed parameters, RWC is closely related to leaf water potential, and simply and effectively determines the degree of drought stress tolerance in plants (Chaves, 1991). The cell structure collapses in response to a decrease in intracellular turgor, resulting in retarded physiological activity and loss of cell integrity. As the total cellular relative humidity declines to 75%, ATP and protein production are prevented (Lawlor and Cornic, 2002). As expected, significant decline in RWC of both cultivars was observed. The low rate of reduction in RWC was showed in drought-tolerant (Horozkarası) and both grafted cultivars. Buffering

the decline in water status in tissues of the plants are critical in coping with the stress conditions. Of the devoted strategies, plants highly accumulate osmolytes to maintain cell turgor in drought-suffering plants (Serraj and Sinclair, 2002). Among the stress indicators, proline is of the universal osmolytes estimated in stress-submitted plants, being a low molecular weight amino acid that is not cytotoxic (Ashraf and Fooland, 2007). Several studies clearly revealed that grapevine suffering from stress enhanced proline content (Şahin, 2009; Özden *et al*., 2009; Abdi *et al.*, 2016). In the present work, critical increases in proline content were observed in drought-sensitive cultivar 'Kabarcık' grafted on 'Rupestris rootstock'.

Effective control of the water transport system can increase drought tolerance in grapevines. Expression levels of VvPIP2;1 and VvPIP2;2 were down-regulated in stress-submitted plants. VvPIP2;2 aquaporin was more responsive in relation to VvPIP2;1 against stress and grafting. A study on 'Chardonnay' cultivar demonstrated a positive relationship between PIP2;1 and leaf hydraulic conductivity (Pou *et al.*, 2013). Decrease in leaf water potential in response to drought stress was reported for 'Touriga Nacional' cultivar (Zorrouk *et al.*, 2016). Concerning two cultivars ('Syrah' and 'Grenache') contrasting to drought stress, the expression levels of VvPIP2;1 did not change in 'Syrah' but decreased in isohydric 'Grenache' (Dayer *et al.*, 2020). Galmes *et al.* (2007) revealed an increase at the earlier developmental period of grapevine. The relevant increase was ascribed to the stomatal closure (Zorrouk *et al.,* 2016). The link between abscisic acid (ABA) level and PIP aquaporin in stomatal closure was well-known (Grondin *et al*., 2015). Significant alteration in pH corresponding to the stomatal closure might trigger critical changes in gene expression levels of PIP aquaporin (Shelden *et al.,* 2009). Orchestrated responses at physiological and physiological might leaf water integrity at the later stage of the stress.

TIPs play key roles in maintaining cell turgor pressure through osmotic adjustment. TIP1;1 maintains the osmotic balance of the cell in grapevine under arid conditions. In the present study, significant increases in TIP1;1 in both cultivars under drought stress were observed. VvTIP1;1 aquaporin increased in *Vitis* spp. leaves (Shelden *et al.*, 2009; Pou *et al.*, 2013; Zarrouk *et al.*, 2015). For this reason, VvTIP1;1 may be considered as a significant stress indicator for grapevine. Changes in another TIP2;1 aquaporin isoform was also investigated. VvTIP2;1 was strongly downregulated in response to drought stress in 'Horozkarası' and 'Kabarcık' cultivars. TIP 2;1 might be expressed at higher levels in the roots and root tips than the leaves (Nguyen *et al.*, 2013; Baiges *et al.,* 2001). VvTIP1;1 might increase the permeability of aquaporins to small-molecule solvents. VvTIP2;1 may regulate water exchange in the vacuole.

Changes in aquaporin gene expression may be important criteria in the selection of grapevine cultivars tolerant to drought stress (Joshi *et al.*, 2016). Regarding nucleotides and amino acids, VvTIP1;1 and VvTIP2;1 exhibited ~72% similarity. However, the similarity among PIP aquaporin genes may be as high as 99% (Shelden *et al.*, 2009). The efficiency of PIP aquaporins can be increased by upregulation of duplicate genes (Chaumont *et al.,* 2001). This approach may effectively identify different results in expression studies. Hence, the aquaporin family should be investigated from a more in-depth and broad perspective in platforms such as RNA sequencing.

Several theories have been postulated to explain the relationships between the scion and the rootstock of grapevine (Tsegay *et al.*, 2014). Though there have been numerous studies on drought stress in grapevine, very few have been conducted on the responses of rootstocks and scions to drought stress. The present study showed the rootstock effects on the PIP2;2, TIP1;1, TIP2;1 aquaporin levels in the scion, and this effect varied with scion vigour. Rootstocks can affect the scion through phytohormones and signalling pathways and meet the water demand of the scion (Zhang *et al.*, 2016). A previous study demonstrated that the genes regulating ABA biosynthesis in the roots of various graft combinations of 'Cabernet Savignon' cultivar were expressed at higher levels in the roots than in the leaves. Nevertheless, more ABA accumulated in the leaves than the roots (Prinsi *et al.*, 2021). Rootstocks do not modulate the impact of drought stress resistance on physiological parameters such as fruit yield but transcriptionally modify secondary metabolism and reduce the severity of drought stress in the grape (Zombardo *et al.*, 2020). Rootstocks might cause partial modulations in physiological and biochemical attributes of the scions. The phytohormones and other substances produced by the roots are translocated to the scion and protect it against drought and other abiotic stress.

The negative effects of drought on grape production are increasing, particularly in the Mediterranean region, in which viticulture is intense. For this reason, the selection of the appropriate cultivars/rootstocks is vital. 'Horozkarası' and 'Kabarcık' grape cultivars with different drought tolerances showed similar trends in the expression levels of their various foliar aquaporin isoforms, albeit at different levels of significance. Although rootstocks are effective at critical modulations of the scion, biochemical modulations are used more intensively depending on the vigour of the scion. Hence, it is crucial to consider the rootstock × scion × environment interaction in future endeavours to elucidate the

drought stress response mechanism. In this way, more reasonable variety-rootstock combinations while maintain the viticultural sustainability in response to ongoing climate warming should be explored.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

REFERENCES

Abdelhakam, S., Rabei, S.H., Nada, R.M., Abogadallah, G. M., 2021. The complementary role of root and leaf PIP1 and PIP2 aquaporins drives the anisohydric behavior in *Helianthus annuus* L. *Environ. Exp. Bot*., **182**, 104314.

Abdi, S., Abbaspur, N., Avestan, S., Barker, A., 2016. Sana physiological responses of two grapevine (*Vitis vinifera* L.) cultivars to cycoceltm treatment during drought. *J. Hort. Sci. Biotechnol.,* **91**, 211-219.

Ashraf, M.F.M.R., Foolad, M.R., 2007. Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environ. Exp. Bot.,* **59**, 206-216.

Brás, T. A., Seixas, J., Carvalhais, N., & Jägermeyr, J., 2021. Severity of drought and heatwave crop losses tripled over the last five decades in Europe. *Environ. Res. Lett.,* **16** (6), 065012.

Capone, R., El-Moujabber, M., Cardone, G., Adinolfi, F., Di Pasquale, J., El Chami, D., 2014. Trade and logistics: The case of the wine industry. *In: MediTERRA,* 245-262.

Carillo, P. Gibon, Y., 2011. Protocol: Extraction and determination of proline. Prometheus Wiki. Available at: https://prometheusprotocols.net/function/tissue-chemistry/primary-metabolites/extraction-and-determination-of-proline/ (accessed on 18.04.2019).

Chaves, M.M., 1991. Effects of water deficits on carbon assimilation. *J. Exp. Bot.,* **42**, 1–16.

Chaves, M. M., Zarrouk, O., Francisco, R., Costa, J. M., Santos, T., Regalado, A. P., Rodrigues, M.L., Lopes, C. M., 2010. Grapevine under deficit irrigation: hints from physiological and molecular data. *Annals of botany*, **105**, 661-676.

Dayer, S., Scharwies, J.D., Ramesh, S.A., Sullivan, W., Doerflinger, F.C., Pagay, V., Tyerman, S.D., 2020. Comparing hydraulics between two grapevine cultivars reveals differences in stomatal regulation under water stress and exogenous ABA applications. *Front. Plant Sci*., **11**, 705.

De Mendiburu, F., 2016. Agricolae: Statistical procedures for agricultural research. R v. 1.2-4. R Core Team, Vienna, Austria.

Dhanda, S.S., Sethi, G.S., 1998. Inheritance of excised-leaf water loss and relative water content in bread wheat (*Triticum aestivum*). *Euphytica,* **104**, 39-47.

Dinis, L. T., Bernardo, S., Yang, C., Fraga, H., Malheiro, A. C., Moutinho-Pereira, J., Santos, J. A., 2022. Mediterranean viticulture in the context of climate change. *Ciência Téc. Vitiv.*, **37**, 139-158.

Galmés, J., Pou, A., Alsina, M.M., Tomàs, M., Medrano, H., Flexas, J., 2000. Aquaporin expression in response to different water stress intensities and recovery in Richter110 (*Vitis* spp.): Relationship with ecophysiological status. *Planta,* **226**, 671–681.

Gambetta, G. A., Herrera, J. C., Dayer, S., Feng, Q., Hochberg, U., Castellarin, S. D., 2020. The physiology of drought stress in grapevine: towards an integrative definition of drought tolerance. *Journal of experimental botany*, **71**, 4658-4676.

Gautam, A., Pandey, A.K., 2021. Aquaporins responses under challenging environmental conditions and abiotic stress tolerance in plants. *Bot. Rev.,* **87**, 467-495.

Hayes, M.A., Davies, C., Dry, I.B., 2007. Isolation, functional characterization and expression analysis of grapevine (*Vitis vinifera* L.) hexose transporters: differential roles in sink and source tissues. *J. Exp. Bot.,* **58**, 1985-1997.

Heinen, R.B., Ye, Q., Chaumont, F., 2009. Role of aquaporins in leaf physiology. *J. Exp. Bot.,* **60**, 2971-2985.

Joshi, R., Wani, S.H., Singh, B., Bohra, A., Dar, Z.A., Lone, A.A., Pareek, A., Singla-Pareek, S.L., 2016. Transcription factors and plants response to drought stress: current understanding and future directions. *Front. Plant Sci.,* **7**, 1029.

Kaldenhoff, R., Fischer, M., 2006. Functional aquaporin diversity in plants. *Biochim. Biophys. Act. (BBA)-Biomembr.,* **1758**, 1134-1141.

Kaldenhoff, R., Rıbas-Carbo, Mıquel., Sans, J.F., Lovisolo, C., Heckwolf, M., Uehlein, N., 2008. Aquaporins and plant water balance. *Plant Cell Environ*., **31**, 658-666.

Kamangar, A., Haddad, R., 2016. Effect of water stress and sodium silicate on antioxidative response in different grapevine (*Vitis vinifera* L.) cultivars. *J. Agric. Sci. Technol.,* **18**, 1859-1870.

Kapilan, R., Vaziri, M., Zwiazek, J.J., 2018. Regulation of aquaporins in plants under stress. *Biol. Res.* **51**, 1-11.

Keller, M., Deyermond, L.S., Bondada, B.R., 2015. Plant hydraulic conductance adapts to shoot number but limits shoot vigour in grapevines. *Funct. Plant Biol.,* **42**, 366-375.

Król, A., Weidner, S., 2017. Changes in the proteome of grapevine leaves (*Vitis vinifera* L.) during long-term drought stress. *J. Plant Physiol.,* **211**, 114-126.

Leitão, L., Prista, C., Moura, T.F., Loureiro-Dias, M.C., Soveral, G., 2012. Grapevine aquaporins: Gating of a tonoplast intrinsic protein (TIP2; 1) by cytosolic pH. *PLoS One*, **7**, e33219.

Li, G., Santoni, V., Maurel, C., 2014. Plant aquaporins: Roles in plant physiology. *Biochim. Biophys. Act. (BBA)-Gen. Subj*., **1840**. 1574-1582.

Lovisolo, C., Schubert, A., 2006. Mercury hinders recovery of shoot hydraulic conductivity during grapevine rehydration: Evidence from a whole-plant approach. *New Phytol.,* **172**, 469-478.

Mandlik, R., Singla, P., Kumawat, S., Khatri, P., Ansari, W., Singh, A., Sharma, Y., Singh, A., Solanke, A., Nadaf, A., Sonah, H., Deshmukh, R., 2022. Understanding aquaporin regulation defining silicon uptake and role in arsenic, antimony and germanium stress in pigeonpea (*Cajanus cajan*). *Environ. Pollut.,* **294**, 118606.

Merlaen, B., De Keyser, E., Ding, L., Leroux, O., Chaumont, F., Van Labeke, M.C., 2019. Physiological responses and aquaporin expression upon drought and osmotic stress in a conservative vs prodigal *Fragaria* x *ananassa* cultivar. *Plant Physiol. Biochem.,* **145**, 95-106.

Nguyen, M.X., Moon, S., Jung, K.H., 2013. Genome-wide expression analysis of rice aquaporin genes and development of a functional gene network mediated by aquaporin expression in roots. *Planta*, **238**, 669-681.

Ozden, M., Demirel, U., Kahraman, A., 2009. Effects of proline on antioxidant system in leaves of grapevine (*Vitis vinifera* L.)

exposed to oxidative stress by H_2O_2. *Scient Horticult*., **119**, 163-168.

Pou, A., Medrano, H., Flexas, J., Tyerman, S.D., 2013. A putative role for TIP and PIP aquaporins in dynamics of leaf hydraulic and stomatal conductances in grapevine under water stress and re-watering. *Plant Cell Environ*., **36**, 828-843.

Prinsi, B., Simeoni, F., Galbiati, M., Meggio, F., Tonelli, C., Scienza, A., Espen, L., 2021. Grapevine rootstocks differently affect physiological and molecular responses of the scion under water deficit condition. *Agronomy*, **11**, 289.

Richards, D., 1983. The grape root system. *Hort. Res*., **5**, 127-168.

Sabir, F., Leandro, M.J., Martins, A.P., Loureiro-Dias, M.C., Moura, T.F., Soveral, G., Prista, C., 2014. Exploring three Pips and Three Tips of grapevine for transport of water and typical substrates through heterologous expression in Aqy-null yeast. *PLoS One,* **9**, e102087.

Şahin, Ö., 2009. *Evaluation of salt and boron tolerance of sultana seedless grapevines (Vitis vinifera L.) grafted on different grapevine rootstocks with physiological parameters and antioxidant enzymes symptomatic for oxidative stress.* 152 p. MSc Thesis, University of Ankara.

Sakr, S., Alves, G., Morillon, R., Maurel, K., Decourteix, M., Guilliot, A., Fleurat-Lessard, P., Julien, J.L., Chrispeels, M.J., 2003. Plasma membrane aquaporins are involved in winter embolism recovery in walnut trees. *Plant Physiol.*, **133**, 630-641.

Schley, T.R., Franco, D.M., Junior, J.P.A., de Godoy Maia, I., Habermann, G., de Almeida, L.F.R., 2022. TIP1; 1 expression could modulate the recovery of stomatal opening during rehydration in *Sorghum bicolor*. *Environ. Exp. Bot.*, **200**, 104908.

Secchi, F., Lovisolo, C., Schubert, A., 2007. Expression of Oepip2.1 aquaporin gene and water relations of *Olea europaea* twigs during drought stress and recovery. *Ann. Appl. Biol.* **150**, 163-167.

Serra, I., Strever, A., Myburgh, P.A., Deloire, A., 2014. Review: the interaction between rootstocks and cultivars (*Vitis vinifera* L.) to enhance drought tolerance in grapevine. *Aust. J. Grape Wine Res.*, **20**, 1-14.

Serraj, R., Sinclair, T.R., 2002. Osmolyte accumulation: Can it really help increase crop yield under drought conditions? *Plant Cell Environ*., **25**, 333-341.

Shao, H.B., Song, W.Y., Chu, L.Y., 2008. Advances of calcium signals involved in plant anti-drought. *Compt. Rend. Biol.*, **331**, 587-596.

Shelden, M.C., Vandeleur, R., Kaiser, B.N., Tyerman, S.D., 2017. A comparison of petiole hydraulics and aquaporin expression in an anisohydric and isohydric cultivar of grapevine in response to water-stress induced cavitation. *Front. Plant Sci.*, **8**, 1893.

Soar, C.J., Dry, P.R., Loveys, B.R., 2006. Scion photosynthesis and leaf gas exchange in *Vitis vinifera* L. cv. Shiraz: Mediation of rootstock effects via xylem sap ABA. *Aust. J. Grape Wine Res.*, **12**, 82-96.

Šurbanovski, N. Grant, O.M., 2014. The emerging role of aquaporins in pant tolerance of abiotic stress. *In: Emerging technologies and management of crop stress tolerance*. 431-447. Academic Press, San Diego.

Tramontini, S., Vitali, M., Centioni, L., Schubert, A., Lovisolo, C., 2013. Rootstock control of scion response to water stress in grapevine. *Environ. Exp. Bot.*, **93**, 20-26.

Tsegay, D., Amsalem, D., Almeida, M., Crandles, M., 2014. Responses of grapevine rootstocks to drought stress. *Int. J. Plant Physiol. Biochem.*, **6**, 1-6.

Turgay, G., 2015. *Asma' da (Vitis vinifera L.) aquaporin genlerinin biyoinformatik analizi ve farklı dokularda ifade profillerinin belirlenmesi*. 142 p. PhD Thesis, University of Ege.

Vandeleur, R.K., Mayo, G., Shelden, M.C., Gilliham, M., Kaiser, B.N., Tyerman, S.D., 2008. The role of plasma membrane intrinsic protein aquaporins in water transport through roots: diurnal and drought stress responses reveal different strategies between isohydric and anisohydric cultivars of grapevine. *Plant Physiol.,* **149**, 445-460.

Zarrouk, O., Francisco, R., Pinto-Marijuan, M., Brossa, R., Santos, R.R., Pinheiro, C., ... Chaves, M.M., 2012. Impact of irrigation regime on berry development and flavonoids composition in Aragonez (Syn. Tempranillo) grapevine. *Agr. Water. Manage.*, **114**, 18-29.

Zarrouk, O., Garcia-Tejero, I., Pinto, C., Genebra, T., Sabir, F., Prista, C., David, T.S., Dias, M.C., Chave, M. M. (2016). Aquaporins isoforms in cv. Touriga Nacional grapevine under water stress and recovery—regulation of expression in leaves and roots. *Agr. Water. Manage.*, **164**, 167-175.

Zhang, L., Marguerit, E., Rossdeutsch, L., Ollat, N., Gambetta, G.A., 2016. The influence of grapevine rootstocks on scion growth and drought resistance. *Theor. Exp. Plant Physiol.,* **28**, 143-157.

Zombardo, A., Mica, E., Puccioni, S., Perria, R., Valentini, P., Mattii, G.B., Cattivelli, L., Storchi, P., 2020. Berry quality of grapevine under water stress as affected by rootstock–Scion interactions through gene expression regulation. *Agronomy*, **10**, 680.