Check for updates

Drought preconditioning improves freezing tolerance in drought-tolerant and -intolerant grape cultivars

Afsaneh Babajamali · Mahdiyeh Gholami · Bahram Baninasab

Abstract Plants preserve previous abiotic stress memory that may help achieving tolerance to subsequent stresses. We tested whether drought priming could alleviate freezing injury in two grape cultivars, namely 'Asgari' (drought-tolerant) and 'Yaghuti' (drought-sensitive). To study the factors involved in drought acclimation process and their role in inducing freezing tolerance, irrigation was withheld for 20 days and carbohydrate and proline concentrations were measured regularly during this time. The results indicated that withholding irrigation decreased the starch, fructose and total soluble carbohydrate (TSC) concentrations. Rewatering increased starch, fructose and TSC concentrations to values exhibited by the plants under water stress conditions. The results also indicated how irrigation regimes strengthened over time. At the end of the study, negative and positive correlation coefficient between starch and proline concentrations and shoot LT_{50} (lethal temperature at which 50% of the total ion leakage occurs) were found, respectively. Root LT_{50} also followed the same correlation response as shoot LT_{50}. 'Asgari' water-stressed plants performed better under drought by maintaining leaf water status and chlorophyll index, and less damage to photochemical efficiency as compared to 'Yaghuti'. Drought stress affected positively the cold hardiness, with higher freezing tolerance and less damage to shoot and root tissues.

Keywords Abiotic stress · Cold hardiness · Ion leakage · Stress memory

1 Introduction

Over the last century, the increase in the concentration of greenhouse gases due to the burning of fossil fuels has been the major reason for the global warming associated with changing climate patterns (Hassan et al. 2020). The reports issued by the intergovernmental panel on climate change (IPCC) indicate that because of continuously changing environmental conditions, global air temperature could increase up to 4–6 °C by 2100 (Rai et al. 2020), along with an increase in the rate and/or intensity of super-optimal temperatures (Hoffman et al. 2012). In addition to the observed warming during the last three decades, the warming trend has not been globally uniform and temperatures have increased more at northern high

A. Babajamali · M. Gholami (✉) · B. Baninasab
Department of Horticulture, College of Agriculture, Isfahan University of Technology, Isfahan 84156-83111, Iran
e-mail: mah.gholami@iut.ac.ir

A. Babajamali
e-mail: ababajamali@yahoo.com

B. Baninasab
e-mail: bbanin@iut.ac.ir

latitudes in winter months (Tomczyk et al. 2019). This will affect negatively the winter survival of perennial crops by inhibiting complete cold acclimation before freezing temperatures (Hoffman et al. 2012).

Overwintering temperate fruit trees cease growth and acclimatise during autumn in response to decreasing temperature alone or in combination with decreasing photoperiod (Hellman 2003). The process of acclimation consists of many synchronous activities including decrease in water content of some tissues, change in composition of membrane lipids and membrane permeability and synthesis of specific proteins and cryoprotectant molecules such as soluble sugars, sugar alcohols and low-molecular mass nitrogenous compounds that overall increase cold hardiness (Joudmand and Hajiboland 2019). When cold acclimated plants are exposed to temperatures below 0 °C, they increase their freezing tolerance further by preventing ice crystal formation. On the other hand, freezing damage in non-acclimated plants, is generally due to osmotic dehydration that may lead to water diffusion from the cells to the growing ice crystals (Hincha and Zuther 2020). Therefore, research is needed to recognize strategies that may help to promote cold acclimation and improve plants freezing tolerance.

One strategy to increase freezing tolerance might include subjecting plants to a transient limited environmental stimulus (priming) to respond effectively to a future freezing stress without advancing resources into continued maintenance of the acclimated state (Leuendorf et al. 2020; Wang et al. 2020). Throughout a range of natural ecosystems, plants often experience water stress and freezing concurrently, raising the potential of interactions between them. This interaction may be particularly important at temperate land-margins, where low winter temperatures are more likely to follow by low precipitation. Rajashekar and Panda (2014) addressed the question of whether the imposed water stress is as effective as typical cold acclimating conditions to induce freezing tolerance. Data obtained in such a study indicated that water stress is a main factor in acquired freezing tolerance in strawberry, resulting in 56% of freezing tolerance attained by normal cold acclimation (Medeiros and Pockman 2011).

Grape (*Vitis* spp.) is an important healthful temperate fruit with economic value and is generally cultivated in arid and semi-arid areas (Ju et al. 2020).

However, growth of the grapevine industry and its products has led to expansion of vineyards into many regions of the world (Londo and Kovaleski 2019). Although this may appear a considerable advantage, growing in cooler and more restrictive climates could frequently increase the injuries of environmental stress during grape production (Kaya 2020a). If given time to acclimate to low temperatures in the autumn, grapevine can tolerate temperatures as low as – 15 °C (Masocha et al. 2020). Nevertheless, the plant genetics based and its interaction with environmental signals play a significant role in freezing tolerance (Karimi 2019). According to Londo et al. (2018) during the process of cold acclimation in grapevine (covertion from a cold sensitive to a cold-hardy state), nearly 800 genes are upregulated and 2300 genes downregulated contributes to the enhancement of freezing tolerance. The severity of freezing damages in vineyards have been and will continue to be increasing as the incomplete cold acclimation are predicted to occur more frequently due to global warming (Wang et al. 2020). So, investigation of approaches or cultural practices for increasing freezing tolerance would be favorable to the security of grape production. In this study, we asked the following questions: are effects of cold acclimation on freezing tolerance influenced by water stress conditions? Is the effect of drought acclimation on freezing tolerance influenced by genotype differences? We hypothesized that genotypes with higher drought resistance would have better response under freezing conditions.

2 Material and methods

2.1 Experimental materials

1 year-old, own-rooted 'Asgari' and 'Yaghuti' grapevines (*V. vinifera* L.) were grown in 7 L pots, containing soil: sand: leaf mould (2:1:1, v/v/v), and a gravel layer at the bottom in the research greenhouse of the Isfahan University of Technology, Isfahan, Iran (32,72°N, 51,52°E). 'Asgari' is seedless, the most popular cultivar in Iran and semi-tolerant to drought stress and 'Yaghuti', is a drought sensitive and very precocious. In Iran, 'Asgari', 'Yaghuti' and other grape cultuvars are propagated by cutting and the majority of vines are self-rooted plants. The field capacity (FC) of the substrate soil used

for potting was determined according to the protocol described by Richards (1949). Potted grapevine trees were irrigated daily for 4 months to FC level (25.6 g H_2O 100 g^{-1} air-dried substrate), until the plants were established. Mean average day- and night-time temperature, relative humidity and photosynthetic photon flux density (PPDF) in the greenhouse were 25–29 °C, 11–13 °C, 61–65% and about 1200 mmol m^{-2} s^{-1} at midday, respectively.

2.2 Irrigation regimes

The experiment started in May 2018, when 60 plants of each cultivar were subjected to three irrigation regimes. One of them, termed well water (WW), maintained substrate water at FC. In the second regime, termed water deficit (WD), the plants were subjected to a water shortage period by withholding irrigation for 20 days until the plants showed a strong loss of turgescence and majority of leaves became withered. The third group (water deficit-recovery/ WDR) was subjected to water stress treatment by withholding irrigation for 13 days until the stressed plants began to show visual signs of water stress. In these plants, recovery of water stressed plants was carried out by re-watering of pots for 7 days. In both WD and WDR plants, in order to decrease the rate of development of water stress, the top of the pots was sealed with a plastic film.

To further confirm that the studied cultivars differ in drought tolerance, the proline, total soluble carbohydrates (TSC), sucrose, fructose and starch concentrations of WW, WD and WDS plants was determined in 5 days intervals. The relative water content (RWC), chlorophyll fluorescence (Fv/Fm) and chlorophyll index of plants was determined at the end of the water stress period.

2.3 Freezing procedure

To measure the freezing tolerance, shoot and root samples of both cultivars were tested in a programmable freezing chamber (Eyela, Tokyo, Japan) based on a stepwise lowering freezing program that started from the prevailing outdoor temperature. The shoots were cut at the soil base and roots cleaned carefully under tap water. For freezing the shoots and roots were cut into 10 cm stem and root pieces. The root and stem segments (containing at

least one node) were first washed in distilled/deionized water, covered in a moist paper towel and then with aluminium foil, and carried into the freezing chamber. The samples were subjected to low temperatures at 0, − 5, − 10, − 15, − 20 or – 25 °C for 12 h. The temperature was decreased stepwise down at 1.5 °C h^{-1} to 0 °C and then at 5 °C h^{-1} to − 25 °C (Cansev et al. 2009). Then, from each low temperature treatment, the samples were removed and recovery was done by increasing the temperature at the same rate until 5 °C for slow thawing (Hashempour et al., 2014). The electrolyte leakage of samples was quantified based on Saadati et al. (2019). Briefly, five pieces of 1 cm long shoot and root segments were placed in test tubes containing 10 mL of deionized water. These samples were then incubated at room temperature on a shaker (model: Gerber GmbH) set at 250 rpm for 1 h and kept for 24 h before the first electrical conductivity (EC1) measurement was carried out using an electrical conductivity meter (CC-501, Elmetron, Zabrze, Poland). The tubes were then transferred into a boiling water bath at 100 °C for about 20 min and then EC (EC2) was measured again. Finally, the electrolyte leakage was measured as EC1/EC2 and presented as a percentage.

2.4 Quantitative estimate of freezing tolerance

Freezing tolerance was calculated and expressed as LT_{50}. The LT_{50} in the case of our study is a lethal temperature at which 50% of the total ion leakage occurs. LT_{50} was calculated by fitting response curves using the following logistic sigmoid function:

Relative ion leakage percentage (based on LT_{50} estimation)

$$= \left(a/1 + e^{b(x-c)} \right) + d$$

In which: x represents treatment temperature; b shows the slope of the function at inflection point c, c denotes the temperature at the inflection point (LT_{50}) and a and d are the upper and lower asymptotes of the function, respectively (Saadati et al. 2020).

2.5 Determination of relative water content (RWC)

The RWC was measured according to the method of Farzi et al. (2017). Leaf segments

398

Theor. Exp. Plant Physiol. (2022) 34:395–407

of mature leaves were gathered, and the RWC was determined by the following equation: $(FW - DW) / (TW - DW) \times 100$, where FW is the fresh mass; DW is the dry mass after oven-drying the leaves in forced air circulation at 80 °C for 48 h, and TW is the turgid mass after re-hydrating the leaves for 24 h at 4 °C.

2.6 Chlorophyll *a* fluorescence

The maximum photochemical efficiency (Fv/Fm) of photosystem II was measured on young fully expanded leaves with a Plant Efficiency Analyzer (Hansatech Instruments Ltd.) after 30 min of dark-adaptation. The Fv/Fm was estimated by (Fm–F0)/ Fm where Fm is the maximal fluorescence, and F0 the minimal fluorescence from a dark-adapted leaves.

2.7 Chlorophyll index (SPAD)

SPAD chlorophyll meter reading is a tool for rapid evaluation of chlorophyll density in leaves. The chlorophyll index of the three youngest fully-expanded leaves on four plants was determined using a chlorophyll cocentration meter (CL-01 Chlorophyll Cocentration Meter- Hansatech Instrument Ltd, UK). The chlorophyll meter readings were used as relative values for chlorophyll contents (Moradi et al., 2017).

2.8 Assay of total carbohydrate, sucrose, fructose and starch

The carbohydrate concentrations were measured based on the anthrone colorimetric procedure proposed by Kang et al. (2009). Briefly, leaf samples (100 mg) were homogenized in 5 mL of 80% (v/v) ethanol, and the extract was centrifuged at 14,000 g for 10 min. This was repeated twice, and the supernatants were collected and pooled. To assay the total soluble carbohydrates (TSC), 0.1 mL of the extract was mixed with 4 mL of anthrone reagent (150 mg of anthrone in 100 mL of 98% (v/v) H_2SO_4) and shacked briefly. The mixture was placed into boiling water for 15 min and then was set on ice. The absorbance of the mixture was read at 620 nm using a spectrophotometer (UV-160A, Japan). The amount of TSC was measured using the standard curve prepared from glucose. The sucrose concentration was determined by reacting 250 µL of the ethanolic extract with 100 µL

of 2 M KOH and by placing the extract in a boiling water bath for 10 min. After cooling at room temperature, 3.0 mL of the anthrone reagent was added to the mixture and then incubated at 40 °C for 15 min. Likewise, the standard solutions of sucrose were tested to obtain the calibration curve. Fructose was analyzed by reacting 250 µL of the extract with 2.5 mL of the freshly-prepared anthrone reagent and placed in a water bath at 40 °C for 30 min. After cooling, the absorbance was read at 620 nm. Starch concentration was measured according to the method of Fukao et al. (2012). The ethanol-insoluble fraction was washed several times with water, resuspended in 2 mL of distilled water and incubated at 95 °C for 30 min. After cooling, the suspension was mixed with 2 mL of 52% (v/v) perchloric acid and then 6 mL of distilled water. After centrifugation at 4 °C for 20 min at 16,000 g the glucose concentration in the supernatant (100 µL) was quantified by the anthrone method described earlier.

2.9 Proline concentration

Proline was spectrophotometrically appraised based on the method developed by Bates et al. (1973). To put it in a nutshell, 0.5 g of the fresh leaf tissues was homogenized in 10 mL of 3% sulfosalicylic acid, and the homogenate was centrifuged at 15,000 g at 4 °C for 10 min. Then, 2 mL of the supernatant was mixed with 2 mL of acid ninhydrin and 2 mL of glacial acetic acid. This mixture was incubated at 100 °C for 1 h and then cooled by transferring the reacted mixture from water bath to ice bath. After this, 1 mL toluene was mixed with reaction mixture vigorously (for 30 s). The chromophore layer having toluene was taken from the upper aqueous phase and warmed to room temperature. The proline was spectrophotometrically quantified at 520 nm. The proline concentration was determined using a standard curve.

2.10 Data analysis

A complete randomized design was used in this study with four replications and two pots per replication. The data were subjected to analysis of variance (ANOVA) based on a split-plot factorial experiment for characteristics measured drought stress period or based on factorial experiment (2×3 factorial scheme consisting of two grape cultivars and three irrigation

Theor. Exp. Plant Physiol. (2022) 34:395–407

399

regimes) for other characteristics. All statistical analysis was performed using SAS software Version 9.1 (SAS Inc., Cary, NC, USA) and the least significant difference (LSD) test was used to separate means at a probability value of $p \leq 0.05$.

3 Results

In the present study, the effect of irrigation regimes on the RWC, maximum photochemical efficiency (Fv/Fm), chlorophyll index and proline, TSC, starch, fructose and sucrose concentrations were significant. In addition, analyses of variance showed differences among cultivars for RWC, Fv/Fm, chlorophyll index and proline, TSC, starch, and sucrose concentrations. Cultivar × irrigation regime treatments were significant for proline, TSC, starch, and sucrose concentrations. The effect of time on the proline, TSC, starch, fructose and sucrose concentrations were significant as indicated by the F test and mean separation data in supplementary Tables S1 and S2.

To know whether the two cultivars 'Asgari' and 'Yaghuti' differed in the development of drought stress symptoms, irrigation was withheld for 20 days and the RWC was measured after this time. RWC was altered by water stress, decreased to reach 63.6% in 'Asgari' and 54.4% in 'Yaghuti'. There were statistically significant differences between RWC of WW, WD and WDR plants. A slow recovery of RWC values after rewatering was noted in the stressed plants. RWC in WDR plants was 19.9% greater than in the WD plants (Fig. 1). RWC was negatively correlated with LT_{50} in the shoots and roots ($p < 0.05$; Table 1). Regardless of the irrigation regimes, the Fv/Fm was significantly higher in 'Asgari'. The WD plants lost maximum photochemical efficiency of photosystem II after 20 days going from about 0.81 to 0.72. The values of Fv/Fm increased during rewatering, regardless of cultivar (Fig. 2). The chlorophyll index (SPAD) in WW plants was significantly higher than those in WD and WDR plants. 'Asgari' had 10.2% more SPAD value, as compared to 'Yaghuti' (Fig. 3). SPAD value was negatively correlated with LT_{50} in the shoots and roots ($p < 0.05$; Table 1).

F To know whether the two cultivars 'Asgari' and 'Yaghuti' differed in the accumulation of different osmolytes, irrigation was withheld for 20 days and the carbohydrate and proline concentrations were measured regularly during this time. After five days, TSC and fructose concentrations in both studied cultivars were similar in all treatments. Ten days after the last irrigation, the TSC of WD plants started to increase in both grape cultivars, but significantly stronger in 'Yaghuti' than in 'Asgari' (20.9 and 12.2%, respecively). Despite differences in TSC after 20 days of drought stress, both cultivars showed a decrease in TSC from about 44% in 'Asgari' to about 58% in 'Yaghuti'. In contrast to 'Yaghuti', TSC concentration of leaves in 'Asgari' tended to be higher in WDR plants than in WW plants (Table 2). In cultivar Asgari, the patterns of change in fructose concentration was similar in plants from both WW and WD treatments, while mean fructose concentration decreased over the same period in WD plants of 'Yaghuti' (Table 3). Different irrigation regimes

Fig. 1 Effect of drought and re-watering periods (right) on the cultivars Asgari and Yaghuti (left) on relative leaf water content. The values are presented as the mean ± standard deviation
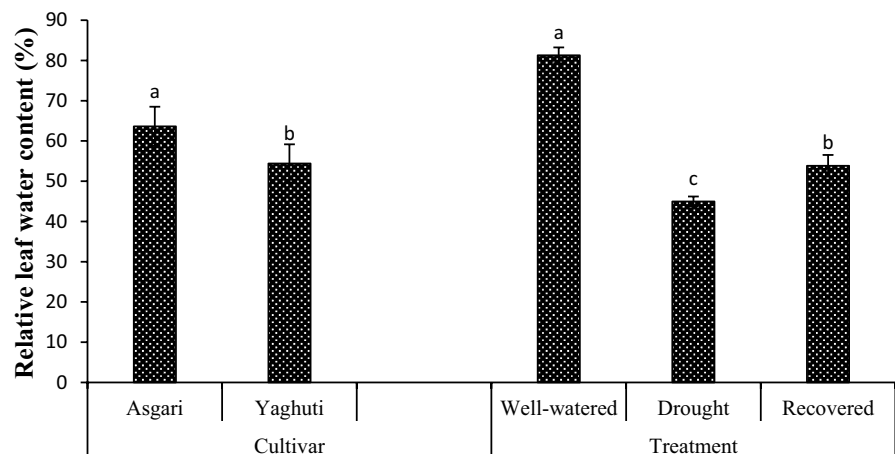
Table 1 Correlation coefficients of different characteristics with LT_{50} in different tissues of the grape cultivars Asgari and Yaghuti[a]

Time (day)	LT_{50} in shoots	LT_{50} in roots
5		
Proline	0.53**	0.47*
Starch	0.13[ns]	− 0.01[ns]
Sucrose	0.56**	0.50*
Fructose	− 0.20[ns]	− 0.18[ns]
Total soluble Carbohydrate	0.33[ns]	0.38[ns]
10		
Proline	0.72**	0.59**
Starch	− 0.56*	− 0.35[ns]
Sucrose	0.66**	0.57**
Fructose	0.35[ns]	0.28[ns]
Total soluble carbohydrate	0.72*	0.42*
15		
Proline	0.82**	0.67**
Starch	− 0.63**	− 0.45*
Sucrose	0.27[ns]	0.27[ns]
Fructose	− 0.03[ns]	0.12[ns]
Total soluble carbohydrate	0.18[ns]	0.24[ns]
20		
Proline	0.74**	0.44**
Starch	− 0.74**	− 0.46*
Sucrose	− 0.25[ns]	0.29[ns]
Fructose	− 0.48[ns]	− 0.40[ns]
Total soluble carbohydrate	− 0.32[ns]	− 0.25[ns]
Relative water content	− 0.71**	− 0.53**
Chlorophyll index	− 0.55**	− 0.39*
Fluorescence index	0.49*	− 0.15[ns]

[a]Pearson correlation coefficient

[ns] not significant

*Significant at $p < 0.05$; **Significant at $p < 0.01$

Fig. 2 Effect of drought and re-watering periods (right) on maximum photochemical efficiency (Fv/Fm) for the grape cultivars Asgari and Yaghuti (left). The values are presented as the mean ± standard deviation
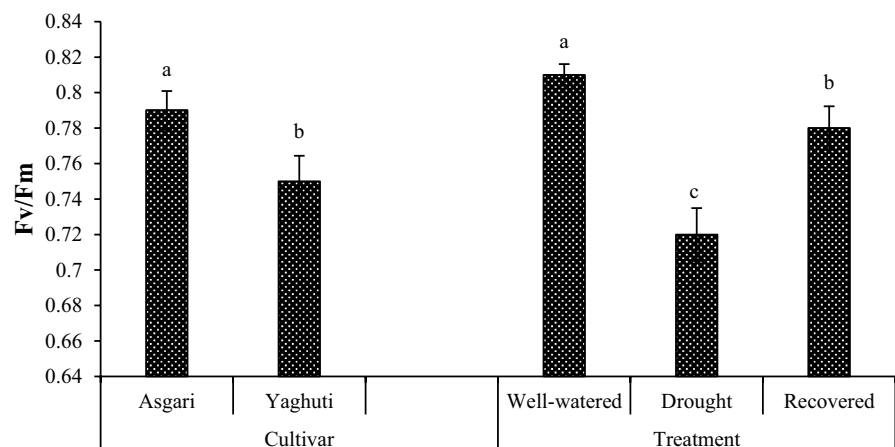
did not affect sucrose concentration of 'Yaghuti' leaves during the experiment. The results show that WDR plants in 'Asgari' presented the highest values of sucrose concentration. Among the two tested cultivars, 'Asgari' had higher sucrose concentration than 'Yaghuti' under water deficit conditions (Fig. 4). Regardless of the cultivar, sucrose in WW plants was unchanged by the water stress period, but began to significantly decline in WD plants by the 20 days (Fig. 5). This decline led to decrease in TSC concentration over the same time period (Table 2). Under water stress conditions, leaf starch concentration dropped in 'Asgari' by 45.0% and in 'Yaghuti' by 39.7% below control concentration. No statistically significant differences in the starch concentration was found among the WD and WDR plants for either of the cultivars (Fig. 6). Water stress duration, on the other hand, was found to significantly alter the starch concentration of grape cultivars. Although there was a significant increase in starch concentration during the whole stress course from WW treatment, however, there was a sharp decrease in starch concentration from WD and WDR treatments after 10 days of drought stress (Fig. 7). Control plants of both studied cultivars, which had not been exposed to water stress, had a low proline concentration in the leaves (Table 4). After 10 days of drought treatment, the proline concentration of the WD plants increased by 90.1 and 11.1% in 'Asgari' and 'Yaghuti', respectively in comparison with that of the control plants. Afterwards, the amount of proline in WD plants continuously increased, whereas that in the control plants remained constantly. At the end of the drought stress (20 days), the highest proline concentration was recorded in WD plants of both cultivars (Table 4).

Fig. 3 Effect of drought and re-watering periods (right) on cultivars Asgari and Yaghuti (left) on chlorophyll index. The values are presented as the mean ± standard deviation
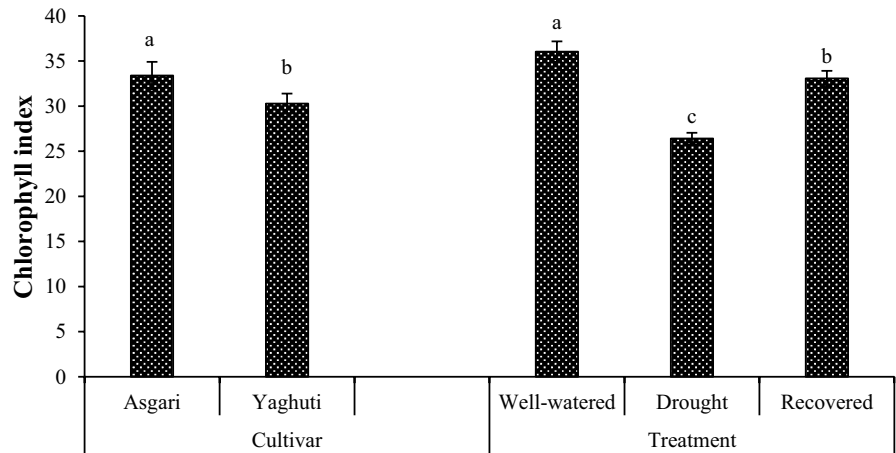
Table 2 Change in total soluble carbohydrates over time for grape cultivars Asgari and Yaghuti under control and drought treatments

Cultivar	Drought treatment	Days			
		5	10	15	20
		Total soluble carbohydrates concentration (mg g^{-1} dm)			
Asgari	Well-watered	126.61 ± 10.56 def	142.57 ± 8.78 b–e	150.99 ± 12.62 bcd	148.68 ± 5.39 bcd
	Drought	143.92 ± 2.88 b–f	172.42 ± 5.21 ab	165.16 ± 5.12 b	83.46 ± 8.93 ij
	Recovered	127.57 ± 6.58 c–f	150.48 ± 4.18 bcd	161.75 ± 15.06 bc	180.45 ± 8.10 a
Yaghuti	Well-watered	99.60 ± 8.79 ghi	117.18 ± 9.16 fgh	123.69 ± 6.90 efg	122.68 ± 7.48 efg
	Drought	82.44 ± 5.54 ghi	131.49 ± 16.41 b-e	69.48 ± 9.12 ij	51.09 ± 12.55 j
	Recovered	84.22 ± 11.11 hi	143.52 ± 11.12 bcd	69.78 ± 10.50 ij	80.07 ± 16.51 hij

Mean values ± standard deviation (n = 4) for each variable with the same letters are not significantly different at p ≤ 0.05 by the LSD test

Table 3 Change in leaf fructose concentration over time for grape cultivars Asgari and Yaghuti under control and drought treatments

Mean values ± standard deviation (n = 4) for each variable with the same letters are not significantly different at p ≤ 0.05 by the LSD test

| Cultivar	Drought treatment	Days			
		5	10	15	20
		Fructose concentration (mg g^{-1} dm)			
Asgari	Well-watered	7.60 ± 0.77 bcd	6.86 ± 1.66 b–e	7.31 ± 1.09 bcd	5.99 ± 0.18 d–g
	Drought	6.37 ± 0.38 def	8.39 ± 0.24 a–d	8.49 ± 0.14 a–d	2.43 ± 0.81 gh
	Recovered	6.25 ± 1.20 de	8.79 ± 1.03 a	8.67 ± 0.76 ab	8.71 ± 1.95 a
Yaghuti	Well-watered	8.31 ± 0.61 a–d	6.62 ± 1.13 def	7.77 ± 0.21 bcd	8.52 ± 1.22 a–d
	Drought	5.93 ± 0.88 cde	6.68 ± 0.80 de	3.63 ± 0.54 fgh	3.49 ± 0.82 h
	Recovered	6.12 ± 0.75 de	7.26 ± 0.25 b–e	6.17 ± 0.67 de	4.81 ± 1.66 efg

Table 5 expresses the osmoregulatory substances of two grape cultivars under controlled and water stress conditions. As shown in Table, the 'Asgari' proline, TSC, sucrose and starch concentrations were significantly higher than those of 'Yaghuti'. When grown under water stress conditions, the starch,
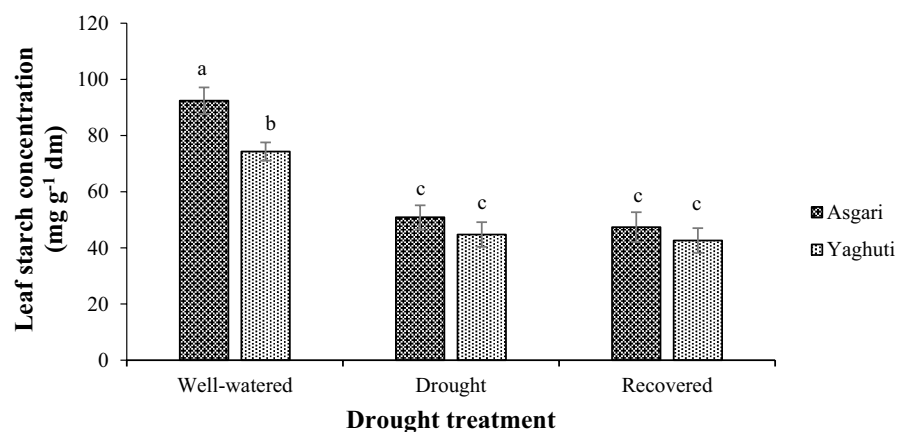
Fig. 4 Effect of drought and re-watering periods on sucrose concentration in the leaves of the grape cultivars Asgari and Yaghuti. The values are presented as the mean ± standard deviation
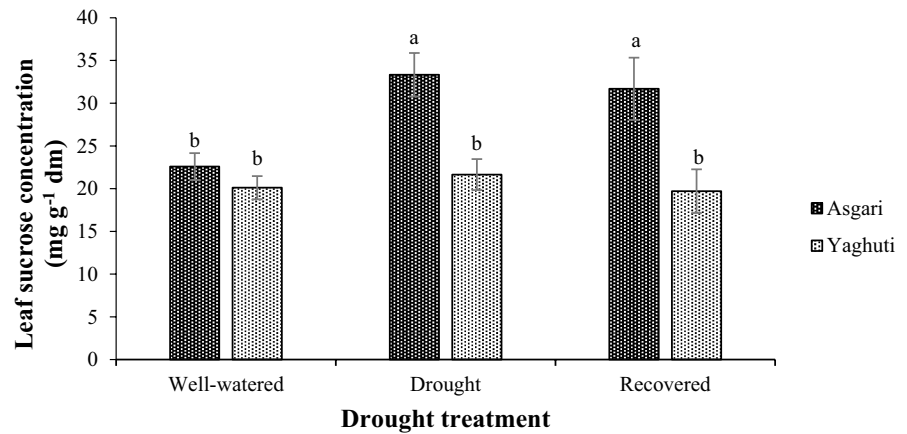
Fig. 5 Changes in sucrose concentration over time for the grape cultivars Asgari and Yaghuti under drought and re-watering treatments. The values are presented as the mean ± standard deviation
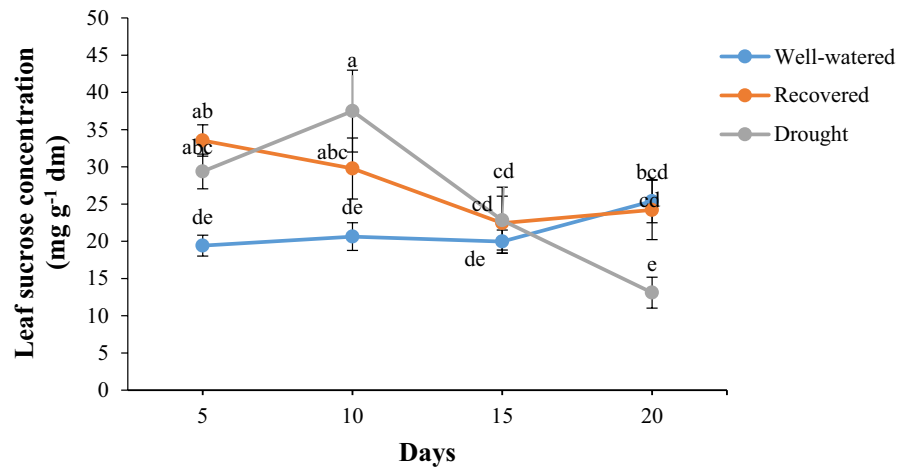
Fig. 6 Effect of drought and re-watering periods on starch concentration in the leaves of the grape cultivars Asgari and Yaghuti. The values are presented as the mean ± standard deviation


fructose and TSC concentrations were reduced. Interruption of water stress by rewatering increased starch, fructose and TSC concentrations to the concentration achieved by the plants under WW conditions. The results also indicated how the effect of irrigation regimes strengthened with the passage of time. At the

Fig. 7 Changes in starch concentration over time for the grape cultivars Asgari and Yaghuti under drought and re-watering treatments. The values are presented as the mean ± standard deviation
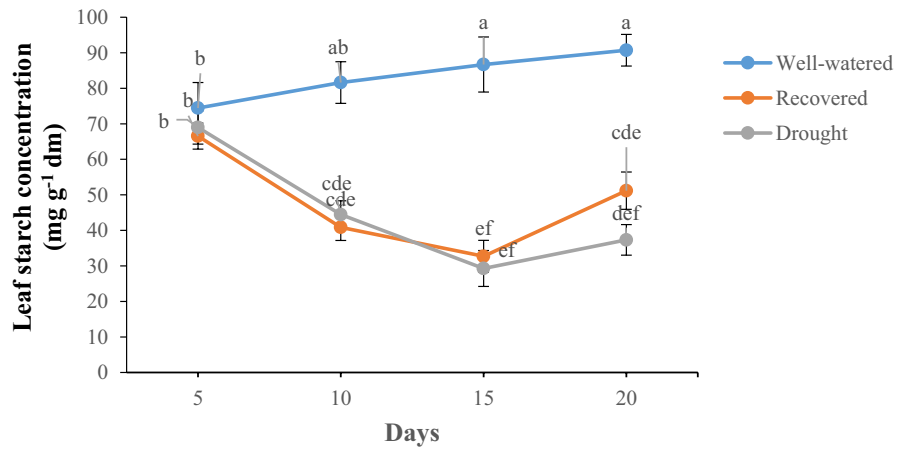
Table 4 Change in leaf proline concentration over time for the grape cultivars Asgari and Yaghuti under control and drought treatments

Mean values ± standard deviation (n = 4) for each variable with the same letters are not significantly different at p ≤ 0.05 by the LSD test

Cultivar	Drought treatment	Days			
		5	10	15	20
		Proline concentration (µmol g⁻¹ dm)			
Asgari	Well-watered	0.077 ± 0.004 e	0.077 ± 0.004 de	0.072 ± 0.004 e	0.075 ± 0.002 e
	Drought	0.085 ± 0.002 de	0.147 ± 0.005 b	0.166 ± 0.007 a	0.176 ± 0.020 a
	Recovered	0.073 ± 0.003 e	0.136 ± 0.004 b	0.127 ± 0.002 b	0.075 ± 0.002 e
Yaghuti	Well-watered	0.079 ± 0.002 de	0.081 ± 0.003 de	0.077 ± 0.002 e	0.080 ± 0.001 e
	Drought	0.072 ± 0.008 e	0.090 ± 0.014 c	0.121 ± 0.015 cd	0.124 ± 0.012 c
	Recovered	0.074 ± 0.004 e	0.102 ± 0.005 c	0.087 ± 0.002 d	0.086 ± 0.003 e

Table 5 Effect of drought and re-watering periods, Asgari and Yaghuti cultivars and time on leaf proline and carbohydare concentrations

Treatment	Proline (µmol g⁻¹ dm)	Soluble carbohydrate (mg g⁻¹ dm.)	Starch (mg g⁻¹ dm.)	Fructose (mg g⁻¹ dm.)	Sucrose (mg g⁻¹ dm.)
Cultivar					
Asgari	0.11 ± 0.01 a	146.17 ± 4.25 a	63.56 ± 4.02 a	7.16 ± 0.36 a	29.20 ± 1.68 a
Yaghuti	0.09 ± 0.00 b	97.94 ± 4.93 b	53.90 ± 3.11 b	6.28 ± 0.32 a	20.49 ± 1.12 b
Drought treatment					
Well-watered	0.08 ± 0.00 b	129.00 ± 4.24 a	83.36 ± 3.25 a	7.38 ± 0.34 a	21.35 ± 1.1 b
Drought	0.12 ± 0.01 a	112.43 ± 8.31 b	45.01 ± 3.42 b	5.68 ± 0.43 b	25.70 ± 2.44 a
Recovered	0.09 ± 0.00 b	124.73 ± 7.80 a	47.82 ± 3.06 b	7.10 ± 0.44 a	27.49 ± 1.86 a
Time (day)					
5	0.08 ± 0.00 b	110.73 ± 5.92 c	69.98 ± 3.05 a	6.77 ± 0.34 a	27.44 ± 1.65 a
10	0.11 ± 0.01 a	142.94 ± 5.05 a	55.64 ± 4.60 bc	7.44 ± 0.40 a	29.30 ± 2.68 a
15	0.11 ± 0.01 a	123.48 ± 9.22 b	49.56 ± 6.39 c	7.01 ± 0.43 a	21.74 ± 1.91 b
20	0.11 ± 0.01 a	111.07 ± 9.95 c	59.74 ± 5.38 b	5.66 ± 0.67 b	20.90 ± 2.06 b

Mean values ± standard deviation (n = 4) for each variable with the same letters are not significantly different at p ≤ 0.05 by the LSD test

end of the experiment, there was negative and positive correlation coefficient between starch and proline concentrations and shoot LT_{50}, respectively. Root LT_{50} also followed the same correlation trend as shoot LT_{50}. There also was negative correlation coefficient between starch concentration and shoot LT_{50} after 10 and 15 days of the drought treatment. Overall, there were positive correlation between the root and shoot LT_{50} and proline concentration (Table 1).

The results demonstrate that freezing tolerance expressed as the LT_{50} was significantly affected by cultivars and irrigation regimes, but was not affected by the interaction of cultivars and irrigation regimes. According to EL of shoot tissue values, between two tested cultivars, 'Asgari' presented higher freezing tolerance (by lowering LT_{50}) in comparison to 'Yaghuti'. Withholding irrigation significantly affected LT_{50} for grape shoots and roots, indicating that summer drought can lead to a significant decrease in LT_{50} (i.e., more negative values). Cold hardiness in grape shoots and roots (LT_{50} in controlled freezing) in the plants subjected to WD treatment, increased by 29.1 and 27.1%, respectively. Compared with the WW plants, the recorded levels of decreased in LT_{50} in the shoots and roots were 16.1 and 13.9% for WDR plants, respectively (Table 6). The effect of irrigation on LT_{50} was almost consistent between two tested cultivars. In grape shoots, both WD and WDR plants always exhibited the highest freezing tolerance (lower LT_{50} values) (Table 7). In cultivar 'Asgari', although WD decreased the root LT_{50} value by 30.7%, there was no measurable improvement in freezing tolerance of 'Yaghuti' roots, when compared with control

Table 6 Mean lethal temperatures for 50% (LT_{50}) of shoot or root damage in the grape cultivars Asgari and Yaghuti under control and drought treatments

	LT_{50} in shoot (°C)	LT_{50} in root (°C)
Cultivar		
Asgari	− 12.36 ± 1.27 a	− 10.54 ± 2.00 a
Yaghuti	− 11.29 ± 1.54 b	− 9.44 ± 1.14 a
Well-watered	− 10.28 ± 0.90 c	− 8.79 ± 0.90 b
Drought treatment		
Drought	− 13.27 ± 0.82 a	− 11.17 ± 2.18 a
Recovered	− 11.93 ± 0.83 b	− 10.01 ± 0.73 ab

Mean values ± standard deviation (n = 4) for each variable with the same letters are not significantly different at p ≤ 0.05 by the LSD test

Table 7 Mean lethal temperatures for 50% (LT_{50}) of shoot or root damage for the grape cultivars Asgari and Yaghuti under control and drought treatments

Cultivar	Drought treatment	LT_{50} in shoot (°C)	LT_{50} in root (°C)
Asgari	Well-watered	− 11.05 ± 0.37 d	− 9.29 ± 0.64 b
	Drought	− 13.82 ± 0.59 a	− 12.14 ± 1.83 a
	Recovered	− 12.21 ± 0.56 bc	− 10.20 ± 0.78 ab
Yaghuti	Well-watered	− 9.51 ± 0.43 e	− 8.28 ± 0.91 b
	Drought	− 12.72 ± 0.66 b	− 10.21 ± 0.80 b
	Recovered	− 11.64 ± 1.03 cd	− 9.82 ± 0.74 ab

Mean values ± standard deviation (n = 4) for each variable with the same letters are not significantly different at p ≤ 0.05 by the LSD test

group. In both cultivars, differences between the LT_{50} values in irrigated and non-irrigated plants reduced with re-irrigation. Regardless of the sample type (shoot or root), the cultivar 'Asgari' exhibited lower LT_{50} values in all WW, WD or WDR treatments than 'Yaghuti' did (Table 7).

4 Discussion

Abiotic stresses are perceived by different signaling mechanisms, among which few are specific, and some may have cross-talk at different stages and are also expected to interact in a similar manner. Moreover, numerous abiotic stress pathways share common elements that are likely 'nodes' for cross-talk (Divya et al. 2019). When exposed to low intensity of a stressor (priming), plants exhibit several physiological and morphological changes (Thomas and Puthur 2017). According to the results, drought-primed plants developed a stress imprint that improved tolerance to subsequent freezing stress.

In this study, drought stress decreased leaf RWC. Nonetheless, 'Asgari' exhibited higher RWC than 'Yaghuti'. The capacity of plants to absorb water is widely reduced under drought conditions, which can lead to reduction in RWC (Bagheri et al. 2021). It was observed that RWC was affected by cultivar. Grapes were considered to withstand drought stress and as a result of the higher accumulation of osmolytes, the reduction in RWC in the studied cultivars alleviated when re-irrigation was applied. These findings were in line with the results of Erdal et al. (2021). Although

grapes are considered drought-tolerant species that can sustain long periods of water stress conditions, we observed variations in tolerance between the two studied cultivars in response to water stress. 'Asgari' plants were able to maintain higher rates of Fv/Fm with higher values of chlorophyll index and could thus better mitigate the damages of drought stress. Our results indicate that the plants of the drought susceptible 'Yaghuti' exhibited a lower photochemical efficiency than 'Asgari'. However, no decrease in growth due to drought stress was observed, suggesting that plants in our experiment were able to regulate to the low water content conditions (Mendanha et al. 2020). The lower values of Fv/Fm found for drought susceptible cultivar is in accordance with literature (Sousaraei et al. 2021).

The cold hardiness of the grape cultivars is influenced by a combination of genetic factors, cultural practices, environmental conditions such as photoperiod and temperatures fluctuations and physiological and biochemical features including carbohydrate concentration, and bud water content (Kaya 2020a). Drought stress caused modifications in source-sink relationship of grapes and the resulting carbohydrates accumulation in leaves improves the freezing response in shoots and roots. In this study, the TSC, fructose and starch concentrations differed significantly between the WW and WD treatments and were higher in WW and WDR plants compared to WD plants (Table 5). The higher concentrations of TSC and sucrose found in the drought-tolerant cultivar 'Asgari' compared to 'Yaghuti' (Table 5), which confirms the involvement of carbohydrates in water stress protection in grapevine. Although TSC and fructose concentrations increased up to 10 days after drought stress in both cultivars, we observed decrease in TSC and fructose concentrations after 15 and 20 days of exposing to drought stresses in 'Yaghuti' and 'Asgari', respectively (Tables 2 and 3). A positive correlation coefficient between TSC and shoot and root LT_{50} ($p \leq 0.05$) at day 10 was also found (Table 1). At the end of the experiment however, the correlation coefficients between TSC and shoot and root LT_{50} were not significant. Nonetheless, upon exposure to drought stresses for 20 days, starch and soluble sugars distinctly followed an increasing and reducing trend, respectively. Although some researchers believe that changes in the TSC concentrations could be used as an indicator to evaluate

freezing damage (Kaya 2020b), this non-significant effect might be due to this fact that some soluble sugars may have persisted trapped in the insoluble starch and would, hence, be unavailable to contribute to the osmotic potential and consequently cold hardiness of the organs (Xu et al. 2020). On the other hand, carbohydrate metabolism plays a central role in the induction of plant tolerance to environmental stress, by acting as the primary source of energy and carbon (Li et al. 2021). Plants allocate carbohydrates produced at the early phases of stress to produce carbon based secondary metabolites such as antioxidants (Hernandez et al. 2021). According to Jørgensen et al. (2020), the increase in TSC in timothy (*Phleum pratense*) when grown under water stress conditions, was a reallocation of storage carbohydrates from roots to leaves. This could partly explain the higher LT_{50} values we found in roots after freezing compared to shoots.

Here, drought primed plants clearly increased the accumulation of proline as compared with non-primed plants. Proline accumulation was involved in the protection of plants from the detrimental effects of water stress. High endogenous proline level proceeded not only by mediating osmotic adjustment, but also by meliorating deleterious effects of drought-induced oxidative stress (Gohari et al. 2021). In this study, the higher proline concentration by WD and WDR was accompanied with a more effective cold hardiness compared with WW treatment. According to Xu et al. (2020), glycerophosphoserines -the dominant component of biological membranes in plants- and some unsaturated fatty acids such as eicosanoid and linoleic acid, are upregulated under water-deficit conditions. The higher proportion of these fatty acids in the membrane lipids increases the stability and cellular functions of membranes and improves membrane cryotolerance and functional integrity during freezing stress. In the present study WD and WDR treatments increased the proline and soluble sugar concentrations, especially of sucrose. Therefore, it seems both increased osmolyte accumulation and membrane stabilization as a result of changes in lipid composition might play significant roles in improving the freezing tolerance of grapes under drought conditions.

Drought primed plants showed less LT_{50} values under controlled freezing stress as compared with non-primed plants, indicating that drought priming effectively alleviated the adverses effects of freezing

406

Theor. Exp. Plant Physiol. (2022) 34:395–407

stress (Table 6). The finding is in agreement with those, which reported stress imprint developed by the stress priming to encourage resistance against subsequent stress in plants (Tankari et al. 2021). The improvement value of shoot LT_{50} in the plants under WD and WDR conditions occurred in both monitored cultivars. However, the highest concentrations of carbohydrate and proline coincided with the lowest LT_{50} in 'Asgari'. Oraee et al. (2020) also reported that drought stress-induced accumulation of carbohydrate and proline caused higher cold tolerance of viola (*Viola×wittrockiana*). In contrast, the lowest carbohydrate and proline accumulation in 'Yaghuti' was related to the susceptibility to low temperatures, especially of roots. It appears that in a drought-susceptible cultivar, responses to freezing could be more severe than the effects found in drought-hardy cultivar.

Winter survival of vineyards have been and will continue to decrease as the incomplete cold acclimation are predicted to occur more frequently due to global warming. As grapevines are grown in more restrictive climates, genotypes and practices, which present adaptive traits and help to bring about positive changes with regard to cold stress, increases the necessity for a sustainable viticulture. In this study we have documented that drought pretreatment induces an adaptation to freezing temperature in grapes by improving the osmoregulatory capability. We found higher concentrations of proline, TSC and sucrose and lower LT_{50} values in WD and WDR treatments in drought-tolerant cultivar 'Asgari' compared to 'Yaghuti', which confirm our hypothesis. This study brings purposes for the viticulture, specially in regions were the two drought and freezing factors takes place with recurrence.

Declarations

References

Bagheri M, Gholami M, Baninasab B (2021) Role of hydrogen peroxide pre-treatment on the acclimation of pistachio seedlings to salt stress. Acta Physiol Plant 43:51

Bates LS, Waldren RP, Teare ID (1973) Rapid determination of free proline for water-stress studies. Plant Soil 39:205–207

Cansev A, Gulen H, Eris A (2009) Cold-hardiness of olive (*Olea europaea* L) cultivars in cold-acclimated and non-acclimated stages: seasonal alteration of antioxidative enzymes and dehydrin-like proteins. J Agric Sci 147:51–61

Divya K, Bhatnagar-Mathur P, Sharma KK, Reddy PS (2019) Heat shock proteins (Hsps) mediated signalling pathways during abiotic stress conditions. In: Khan MI, Reddy PS, Ferrante A, Khan N (eds) Plant signaling molecules, 1st edn. Woodhead Publishing, pp 499–516

Erdal ŞÇ, Eyidoğan F, Ekmekçi Y (2021) Comparative physiological and proteomic analysis of cultivated and wild safflower response to drought stress and re-watering. Physiol Mol Biol Plants 27:281–295

Farzi R, Gholami M, Baninasab B (2017) Water-retention additives' effects on plant water status and some physiological parameters of two olive cultivars under reduced irrigation regimes. Acta Physiol Plant 39:126

Fukao T, Yeung E, Bailey-Serres J (2012) The submergence tolerance gene SUB1A delays leaf senescence under prolonged darkness through hormonal regulation in rice. Plant Physiol 160:1795–1807

Gohari G, Panahirad S, Sepehri N, Akbari A, Zahedi SM, Jafari H, Dadpour MR, Fotopoulos V (2021) Enhanced tolerance to salinity stress in grapevine plants through application of carbon quantum dots functionalized by proline. Environ Sci Pollut Res. https://doi.org/10.1007/s11356-021-13794-w

Hashempour A, Ghasemnezhad M, Fotouhi Ghazvini R, Sohani MM (2014) Olive (*Olea europaea* L) freezing tolerance related to antioxidant enzymes activity during cold acclimation and non acclimation. Acta Physiol Plant 36:3231–3241

Hassan MU, Chattha MU, Khan I, Chattha MB, Barbanti L, Aamer M, Iqbal MM, Nawaz M, Mahmood A, Ali A, Aslam MT (2020) Heat stress in cultivated plants: nature, impact, mechanisms, and mitigation strategies-a review. Plant Biosyst 155:211–234

Hellman EW (2003) Grapevine structure and function. Oregon State University Press, Corvallis

Hernandez JA, Díaz-Vivancos P, Martínez-Sánchez G, Alburquerque N, Martínez D, Barba-Espín G, Acosta-Motos JR, Carrera E, García-Bruntón J (2021) Physiological and biochemical characterization of bud dormancy: evolution of carbohydrate and antioxidant metabolisms and hormonal profile in a low chill peach variety. Sci Hortic 281:109957

Hincha DK, Zuther E (2020) Plant cold acclimation and Freezing Tolerance. In: Hincha DK, Zuther E (eds) Plant cold acclimation. Humana, New York, pp 1–7

Hoffman L, DaCosta M, Ebdon JS, Zhao J (2012) Effects of drought preconditioning on freezing tolerance of perennial ryegrass. Environ Exp Bot 79:11–20

Jørgensen M, Torp T, Mølmann JAB (2020) Impact of waterlogging and temperature on autumn growth, hardening and freezing tolerance of timothy (*Phleum pratense*). J Agron Crop Sci 206:242–251

Joudmand A, Hajiboland R (2019) Silicon mitigates cold stress in barley plants via modifying the activity of apoplasmic enzymes and concentration of metabolites. Acta Physiol Plant 41:29

Ju YL, Yue X, Min Z, Wang XH, Fang YL, Zhang JX (2020) VvNAC17, a novel stress-responsive grapevine (*Vitis vinifera* L.) NAC transcription factor, increases sensitivity to abscisic acid and enhances salinity, freezing, and drought tolerance in transgenic *Arabidopsis*. Plant Physiol Biochem 146:98–111

Kang YY, Guo SR, Li J, Duan JJ (2009) Effect of root applied 24-epibrassinolide on carbohydrate status and fermentative enzyme activities in cucumber (*Cucumis sativus* L.) seedlings under hypoxia. Plant Growth Regul 57:259–269

Karimi R (2019) Cold hardiness evaluation of 20 commercial table grape (*Vitis vinifera* L.) cultivars. Int J Fruit Sci 20:433–450

Kaya O (2020a) Bud death and its relationship with lateral shoot, water content and soluble carbohydrates in four grapevine cultivars following winter cold. Erwerbs-Obstbau 62:43–50

Kaya O (2020b) Defoliation alleviates cold-induced oxidative damage in dormant buds of grapevine by up-regulating soluble carbohydrates and decreasing ROS. Acta Physiol Plant 42:106

Leuendorf JE, Frank M, Schmülling T (2020) Acclimation, priming and memory in the response of *Arabidopsis thaliana* seedlings to cold stress. Sci Rep 10:689

Li S, Wang T, Guo J, Dong Y, Wang Z, Gong L, Li X (2021) Polystyrene microplastics disturb the redox homeostasis, carbohydrate metabolism and phytohormone regulatory network in barley. J Hazard Mater 415:125614

Londo JP, Kovaleski AP (2019) Deconstructing cold hardiness: variation in supercooling ability and chilling requirements in the wild grapevine *Vitis riparia*. Aust J Grape Wine Res 25:276–285

Londo JP, Kovaleski AP, Lillis JA (2018) Divergence in the transcriptional landscape between low temperature and freeze shock in cultivated grapevine (*Vitis vinifera*). Hortic Res 5:10

Masocha VF, Li Q, Zhu Z, Chai F, Sun X, Wang Z, Yang L, Wang Q, Xin H (2020) Proteomic variation in *Vitis amurensis* and *V. vinifera* buds during cold acclimation. Sci Hortic 263:109143

Medeiros JS, Pockman WT (2011) Drought increases freezing tolerance of both leaves and xylem of *Larrea tridentata*. Plant Cell Environ 34:43–51

Mendanha T, Rosenqvist E, Nordentoft Hyldgaard B, Doonan JH, Ottosen CO (2020) Drought priming effects on alleviating the photosynthetic limitations of wheat cultivars (*Triticum aestivum* L.) with contrasting tolerance to abiotic stresses. J Agron Crop Sci 206:651–664

Moradi S, Baninasab B, Gholami M, Ghobadi C (2017) Paclobutrazol application enhances antioxidant enzyme activities in pomegranate plants affected by cold stress. J Hort Sci Biotechnol 92:65–71

Oraee A, Tehranifar A, Nezami A, Shoor M (2020) The effects of three levels of irrigation water on the improvement of cold tolerance of acclimated viola. Acta Physiol Plant 42:111

Rai KK, Pandey N, Rai SP (2020) Salicylic acid and nitric oxide signaling in plant heat stress. Physiol Plantarum 168:241–255

Rajashekar CB, Panda M (2014) Water stress is a component of cold acclimation process essential for inducing full freezing tolerance in strawberry. Sci Hortic 174:54–59

Richards LA (1949) Methods of measuring soil moisture tension. Soil Sci 68:95

Saadati S, Baninasab B, Mobli M, Gholami M (2019) Measurements of freezing tolerance and their relationship with some biochemical and physiological parameters in seven olive cultivars. Acta Physiol Plant 41:51

Saadati S, Baninasab B, Mobli M, Gholami M (2020) Cold tolerance in olive leaves of three cultivars related to some physiological parameters during cold acclimation and de-acclimation stages. J Agric Sci Technol 22:1313–1326

Sousaraei N, Mashayekhi K, Mousavizadeh SJ, Akbarpour V, Medina J, Aliniaeifard S (2021) Screening of tomato landraces for drought tolerance based on growth and chlorophyll fluorescence analyses. Hortic Environ Biotechnol. https://doi.org/10.1007/s13580-020-00328-5

Tankari M, Wang C, Ma H, Li X, Li L, Soothar RK, Cui N, Zaman-Allah M, Hao W, Liu F, Wang Y (2021) Drought priming improved water status, photosynthesis and water productivity of cowpea during post-anthesis drought stress. Agric Water Manage 245:106565

Thomas TT, Dhanya Puthur Jos T (2017) UV radiation priming: a means of amplifying the inherent potential for abiotic stress tolerance in crop plants. Environ Exp Bot 138:57–66

Tomczyk AM, Sulikowska A, Bednorz E, Półrolniczak M (2019) Atmospheric circulation conditions during winter warm spells in Central Europe. Nat Hazards 96:1413–1428

Wang W, Wang X, Zhang J, Huang M, Cai J, Zhou Q, Dai T, Jiang D (2020) Salicylic acid and cold priming induce late-spring freezing tolerance by maintaining cellular redox homeostasis and protecting photosynthetic apparatus in wheat. Plant Growth Regul 90:109–121

Xu H, Li Z, Tong Z, He F, Li X (2020) Metabolomic analyses reveal substances that contribute to the increased freezing tolerance of alfalfa (*Medicago sativa* L.) after continuous water deficit. BMC Plant Biol 20:15