Effects of drought stress on total phenolics, phenolic acids, polyamines and some organic acids in two important Iranian grapevine cultivars

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Abstract

This study was conducted to assess the induced-changes in the content of total phenolics and individual phenolic acids, total and individual polyamines and some organic acids in the leaves of two important Iranian grapevine cultivars under different durations of drought stress, and also to determine their involvement in grapevine drought tolerance. For this purpose, 'Yaghuti' as a drought-tolerant and 'Bidanesefid' as a drought-sensitive vine were subjected to 6, 12 and 18 days of drought stress. Our results showed that as compared to the respective control vines, 18 days drought stressed plants of 'Yaghuti' and 'Bidanesefid' had the highest concentration of total phenolics (31.55 vs. 27.40 mg.g^{-1}Fw), total polyamines (98.40 vs. 70.11 ng.g^{-1}Fw) and total organic acids (3.30 vs. 3.08 mg.100g^{-1}Fw). Based on comparative analyses of individual phenolic acids, it seemed that in drought stress condition and especially in 'Yaghuti', Caffeic acid and Gallic acid could be used as biochemical markers of stress responses. In our study, only 18 days of drought stress resulted in a significant increase in spermidine concentration in 'Yaghuti' cultivar while putrescine and spermine concentration of this cultivar increased at the onset of stress, so it seemed that these two polyamines had a more important role in drought tolerance of 'Yaghuti'. In the current study, different durations of drought stress remarkably increased the amounts of individual organic acids (tartaric, malic, oxalic and ascorbic acid) in both cultivars. The contents of quantified organic acids were not often significantly different between the two cultivars in the same durations of drought stress. Totally it seemed phenolic acids and polyamines were more effective markers for drought resistance investigation of vines.

Introduction

Drought is one of the most significant abiotic stresses which have adverse effect on growth and development of plants (Xu *et al*., 2010). Being a multidimensional stress, it triggers a wide variety of plant responses ranging from physiological, biochemical to molecular levels. One of the inevitable consequences of drought stress is an increase in reactive oxygen species (ROSs) production in different cellular compartments, namely the chloroplasts and mitochondria which cause membrane injuries, protein degradation, enzyme inactivation and thus induce oxidative stress (Kaur and Asthir, 2017; Zlatev and Lidon, 2012). This enhanced ROSs production known as the oxidative burst is however, kept under tight control by a versatile and cooperative antioxidant system that modulates intracellular ROS content and sets the redox status of the cell (Kaur and Asthir, 2017).

Plants have evolved different defense systems to avoid the oxidative damage caused by drought stress including overproduction of antioxidant metabolites which inhibit the propagation of oxidative chain reactions (Caliskan *et al*., 2017). In recent years, the importance of antioxidant activities of phenolic compounds and their potential role in stress tolerance of plants as a natural antioxidant compounds has reached a new level. Phenolic compounds such as phenolic acids and flavonoids have been found to be the most widespread substantial groups of plant secondary metabolites produced from the shikimate-phenylpropanoid biosynthetic pathway (Quan *et al*., 2016). The drought tolerance mechanism controlled by endogenous phenolic compounds has been observed in many plants, but it differs among species, cultivars, plant tissues and drought intensity (Gharibi *et al*., 2015; Al Hassan *et al*., 2015; Akula and Ravishankar, 2011; Weidner *et al*., 2009).

Polyamines (PAs) are ubiquitous low molecular weight compounds, which are now known to be involved in plant stress tolerance. Modulation of PA

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metabolism with concomitant changes in their concentrations have been observed in a variety of crops under conditions of water-deficit stress and PAs have been considered as protective agents due to their ability to function either as antioxidants or as signaling molecules for initiation of other protective mechanisms through their catabolism (Loka *et al*., 2015). The anti-stress properties of polyamines in plants are illustrated by their roles in modulation of morphological growth parameters, elevation of photosynthetic pigments, as well as declined content of stress indices, antioxidative enzymes, and non-enzymatic antioxidants content (Khajuria *et al*., 2018).

Organic acids (OAs) are important metabolites formed in plants mainly in the Krebs cycle and glyoxylate cycle, as well as in the processes of C4 and CAM photosynthesis. They can form both active cytosolic and storage vacuolar pools that can be used to maintain the ionic balance in plant cells. Organic acid machanisms are of fundamental importance at the cellular level for several biochemical pathways, including energy production, formation of precursors for amino-acid biosynthesis and, at the whole plant level, in modulating adaptation to the environment (Osmolovskaya *et al*., 2018).

Grapevine (*Vitis vinifera* L.), is among the most important fruit crop species worldwide. Water is critical for viticulture sustainability because grape production, quality, and economic viability largely depend on water availability (Medrano *et al*., 2015). Grapevine is considered a water stress avoidant species with a tight stomatal control. However, the most imminent challenges that grape and raisin industries must face, especially in arid and semi-arid regions, are increasing drought and salinity due to higher evaporation and declining water availability (Miras-Avalos *et al*., 2017). Adaptive changes in grapevine physio-biochemical processes lead to its acclimation to water deficit. Changes in concentration of proteins, proline and soluble sugars together with alteration in some secondary metabolites are some of the important adaptations in grapevine under drought stress condition (Li-Ping *et al*., 2006; Nazarli and Faraji, 2011; Akinci and Losel, 2009; Krol *et al*., 2014).

Iranian grapevine germplasm is estimated to include more than 800-1000 genotypes, among which, about 250 cultivars are grown and mostly used as table and dried fruit (Doulati-Baneh *et al*., 2013). Precise study of the compatibility mechanisms and the response of grapevine trees to water stress are indispensable for the development of tolerant cultivars and the prevention of future problems in vineyards. The effects of drought stress on morphological and physio-biochemical responses of some Iranian grapevine cultivars have been studied in varied investigations. Hadadinejad *et al*. (2013) assessed the photosynthetic traits and rubisco activase gene expression of three Iranian rootstocks. Soukhtesaraee *et al*. (2017) and Aran *et al*. (2017) investigated the effects of different levels of water

deficit on physio-biochemical characteristics and antioxidant enzyme activities in three grapevine cultivars. The antioxidative effects of secondary metabolites such as polyamines and phenolic compounds, and their positive role in plants drought tolerance have been demonstrated in several studies (Loka *et al*., 2015; Gharibi *et al*., 2015; Weidner *et al*., 2009; Al Hassan *et al*., 2015). However, the effects of drought stress on the organic acid, polyamine and phenolic acid content of vines have seldom been assessed. To the best of our knowledge, no study has been conducted concerning the changes of these mentioned metabolites in Iranian grapevine cultivars in response to drought stress.

In order to investigate the effect of drought stress on grapevine total phenolic and phenolic acids, polyamines and organic acid content, two cultivars differing in drought tolerance ('Yaghuti' as a tolerant, and 'Bidanesefid' as a sensitive cultivar) were used. The objectives of this study were to monitor the changes in the mentioned parameters in grapevine leaves as well as to determine their involvement in grapevine drought tolerance.

Material and methods
Plant material, growth conditions, and drought treatments: Two-year-old self-rooted cuttings of dormant *Vitis vinifera* cv. 'Bidanesefid' (as a drought sensitive cultivar) and cv. 'Yaghuti' (as a drought tolerant cultivar), pruned to two winter buds and planted in pots (25 cm high, 26 cm top and 18 cm bottom diameter) filled with a soil-sand-organic manure mixture (3:1:1 in volume). The soil characteristics were as follows: loam in texture, sand 43%; silt 39%; clay 18%; pH 7.79; and organic matter 1.23%. The seedlings were grown for the next four months in a greenhouse sited in Malayer (lat. 34°30′N, long. 48°85′E, alt. 1550 m), Iran with a 16 hrs. light and an eight hrs. dark photoperiod with an average day/night temperature of 22/16 °C. During the whole period, the plants were regularly watered to maintain the optimum soil moisture and fertilized every ten days, for a total of six times using 50 ml of a NPK fertilizer containing 9%N, 9%P (in the form of P_2O_5) and 9% K (in the form of k_2O). The experiment consisted of a completely randomized factorial design consisting of 4×2 (water stress period × cultivar) with six individually potted plants per water stress period and cultivar. Drought stress was performed by the method of cut off irrigation. The details of the water stress period factor were as follows: (i) 0-day water stress (0dws) as Control; (ii) 6-days water stress (6dws) as short-term; (iii) 12-days water stress (12dws) as medium-term; and (iv) 18-days water stress (18 dws) as long-term drought stress. The experiment began from 27 July to 13 August 2015. Watering of the 18 dws plants was completely withheld from the start of the test period. To synchronize the sampling from treatments, in 12 dws and 6 dws plants, water was withheld for 6 and 12 days after the start of the experiment, respectively.

Control plants were regularly watered to maintain the optimum soil moisture. The sampling of the plants was done at the end of the test period to study the response of two grapevine cultivars to varied days of drought.

Determination of total phenolic compound (TPC): TPC was determined by reaction with the Folin-Ciocalteu reagent, using the method of Velioglu et al. (1998). Gallic acid standard solutions were used as standard, and the amount of total phenolic concentrations in each sample was calculated as mg gallic acid g^{-1} of FW.

Extraction and quantitative estimation of phenolic acids: For extraction and quantitative estimation of phenolic acids, plant material was air-dried at room temperature and powdered afterward. The extraction was performed with constant shaking for 48 hrs., using 80% aqueous methanol. Plant material was removed by filtration; raw extracts were evaporated and re-dissolved in DMSO to the final concentration of 200 mg/ml. Extracts were diluted with mobile phase solvents A (0.05% aqueous formic acid) and B (methanol), premixed in 1:1 ratio, to obtain a final concentration 2 mg/ml (Orcic et al., 2014). Samples and standards were analyzed using Unicam-crystal-200, series high-performance liquid chromatograph. Ten microlitres were injected into the system, and compounds were separated on Zorbax Eclipse XDB-C18 (50 mm × 4.6 mm, 1.8 lm) rapid resolution column held at 50 °C. For detection, photo-diode array detector (Model 966) was used. The detection was monitored at 300 nm. Mobile phase was delivered at a flow rate of 1 ml/min in gradient mode (0 min 30% B, 6 min 70% B, 9 min 100% B, and 12 min 100% B, re-equilibration time 3 min).

Extraction and determination of polyamines: The extraction, separation, identification, and measurement of free PAs by direct dansylation and HPLC have been described elsewhere (Walter and Geuns, 1987). For this purpose, 250 mg of frozen leaves was homogenized in 2 ml of 4% perchloric acid ($HClO_4$) at 4 °C and kept in a cold chamber for 1 hour. After filtration through 0.45 μ filter, to 0.2 ml of the homogenate, 1 ml of carbonate buffer (pH 9) and 1 ml of dansyl chloride solution (10 mg ml^{-1} acetone) were added. After heating for one hour at 60 °C, the dansylated PAs were extracted with 3 ml of toluene. The extract was loaded on a 0.5 g silica gel column and washed with 5 ml of toluol and 5 ml of toluol-triethylamine (10/0.3, v/v). then, the dansylated PAs were eluted with 3 ml of ethyl acetate, and the volume reduced under N_2. Isocratic HPLC-analysis with acetonitrile/H_2O (72/28, v/v) on a 10-cm long 3 mm octadecyl silica column took 8 min. The solvent flow was 2 ml min^{-1}. Dansylated putrescine (PUT), spermidine (SPD), and spermine (SPM) were injected as references.

Extraction and determination of organic acids: For the extraction and quantitative estimation of OAs, fresh sample was homogenized in mobile phase solution (10 ml) with a manual blender, and extracted twice. The

filtrate was collected and volumed to 25 ml with mobile phase solution. Then, it was centrifuged at 12000×g for 10 min at 4 °C and then filtered through a 0.22-μm filter for HPLC. The HPLC used in this study consisted of a Unicam, Model; Crystal-200, England. Separations were performed on a reversed phase column (Waters Atlantis dC18 column (4.6×250 mm length and 5 μm particle diameter), and column was placed in a column oven (CTO-15C) set at 25 °C. We used a UV double wavelength detector (SPD-15C). Detection of L-ascorbic acid was carried out at 243 nm, and the rest of organic acids at 210 nm. Mobile phase was prepared fresh daily and was used with a constant flow rate of 0.7 ml/min and consisted of 0.01 mol/L KH_2PO_4-H_3PO_4 in water (pH 2.80). Individual compounds were identified by co-injections of reference compounds with the samples and also by comparison of their retention times of the standards. External standard calibration was done for quantification (Zheng et al., 2009).

Experimental design: The current research was performed as a factorial experiment (4×2) based on completely randomized design. Data were analyzed by SAS software package (version 9.0, SAS Institute Inc., Cary, NC, USA) using the general linear model procedure. Statistical differences between treatments were determined using Tukey's test. Mean differences were considered significant to be P<0.05.

Results

TPC and phenolic acid contents: Drought periods and cultivars had a significant interaction effect on TPC (P≤0.005) and individual phenolic acid content (Table 1). The total phenolics enhanced gradually in the drought-stressed vines. In 6 dws plants of both cultivars, no significant change was observed in the content of total phenolics compared to the respective control vines, but two other stress levels caused a significant increase in the content of phenolic compounds of both cultivars. No significant difference was detected between the TPC in 12 dws plants of 'Yaghuti' and 18 dws plants of 'Bidanesefid' cultivar. The highest value of TPC in all treatments was observed in 18 days water stressed plants of Yaghuti cultivar (Table 1). Quantitative estimation of phenolic acids on grapevine leave extracts demonstrated that they contained ferulic acid (FA), p-coumaric acid (PCA), Gallic acid (GA), Chlorogenic acid (COA), Caffeic acid (CAA) and resveratrol in both 'Yaghuti' and 'Bidanesefid' (Table 1). Similar to total phenolics, those vines which experienced 6 dws treatment, exhibited no significant difference in individual phenolic acid content (except PCA in 'Yaghuti') compared to the controls. Drought-stressed vines of 'Yaghuti' significantly showed higher amounts of CAA and PCA, than those of 'Bidanesefid'. In 'Bidanesefid', the content of CAA in drought-stressed plants (12 and 18 dws) dropped significantly compared to the control plants, while the amount of other phenolic acids extensively increased under water stress conditions. Among these compounds, GA had the

Table 1- Total phenolics and phenolic acids in leaves of 'Yaghuti' and 'Bidanesefid' grapevines under different periods of drought stress

cultivar	Drought period	Total phenolic (mg.g^{-1}Fw) and phenolic acid (µg.g^{-1}Fw)						
	(days)	Ferulic acid	p-coumaric acid	Gallic acid	Chlorogenic acid	Caffeic acid	Resveratrol	Total phenolic
Yaghuti	0	$4.82 \pm 0.11^{e*}$	2.72 ± 0.08^{e}	8.25 ± 0.23^{cd}	0.72 ± 0.03^{d}	3.46 ± 0.12^{cd}	1.39 ± 0.06^{e}	23.03 ± 0.31^{de}
	6	5.53 ± 0.14^{de}	3.40 ± 0.14^{d}	7.71 ± 0.18^{de}	0.88 ± 0.05^{cd}	3.63 ± 0.10^{c}	$1.89 \pm 0.1d^{e}$	24.21 ± 0.51^{cd}
	12	5.91 ± 0.15^{bc}	4.16 ± 0.16^{bc}	9.17 ± 0.25^{bc}	1.10 ± 0.04^{bc}	4.19 ± 0.08^{b}	2.93 ± 0.12^{b}	27.59 ± 0.38^{b}
	18	6.44 ± 0.11^{ab}	5.10 ± 0.17^{a}	10.69 ± 0.18^{a}	1.46 ± 0.07^{a}	4.79 ± 0.10^{a}	3.61 ± 0.15^{a}	31.55 ± 0.18^{a}
Bidanesefid	0	5.62 ± 0.19^{cd}	2.16 ± 0.08^{e}	7.12 ± 0.16^{e}	0.66 ± 0.03^{d}	3.07 ± 0.11^{de}	1.88 ± 0.08^{de}	21.84 ± 0.40^{e}
	6	5.10 ± 0.17^{cde}	2.57 ± 0.13^{e}	7.51 ± 0.20^{de}	0.81 ± 0.03^{d}	2.80 ± 0.12^{e}	2.29 ± 0.11^{cd}	21.71 ± 0.07^{e}
	12	6.58 ± 0.18^{bc}	3.60 ± 0.09^{cd}	8.76 ± 0.20^{c}	0.90 ± 0.04^{cd}	2.21 ± 0.10^{f}	2.71 ± 0.09^{bc}	25.12 ± 0.22^{c}
	18	7.12 ± 0.17^{ab}	4.20 ± 0.09^{b}	9.86 ± 0.19^{ab}	1.22 ± 0.10^{ab}	1.93 ± 0.07^{f}	3.03 ± 0.12^{b}	27.40 ± 0.32^{b}

*Mean values marked with the different letters are significantly different (P≤0.05) according to Tukey test. Values are means of four replicates ± SE

highest concentration in leaves of the control and stressed samples. The next most abundant compound was FA. COA had the lowest concentration in leaves of the control, and stress samples in both cultivars.

PAs: Drought periods and cultivars had a significant interaction effect on total PA and individual PA content (P≤0.001). In both cultivars, total PA contents increased in drought-stressed vines to a higher level, by increasing the length of the drought period and maximum values were recorded in 18 days drought-stressed plants (Table 2). At the same level of drought period, the concentration of total PAs in 'Yaghuti' was significantly higher than 'Bidanesefid'. Depending on the PA kind, grapevine cultivar and drought period, different responses were seen among cultivars. The putrescine content in 12 dws plants of 'Yaghuti' reached the peak, then decreased slightly in 18 dws plants (Table 2). Spermidine had a similar response in Bidanesefid. In other cases, a gradual increase in individual PAs content was observed with increasing the drought period to 18 days in both cultivars. In 18 dws treatment, the highest values of all three individual PAs were detected in Yaghuti cultivar

Organic acid contents: A total of four OAs with significant change including tartaric acid, malic acid, oxalic acid, and ascorbic acid were detected in the leaves of both vine cultivars (Table 3). Drought periods and cultivars had a significant interaction effect on the OA contents. As compared to the respective control vines, the content of total OA and four mentioned individual OAs exhibited significant increases under different durations of drought stress, in both cultivars. Total amounts of organic acids in the leaves of 6dws and 12 dws Yaghuti were significantly higher than those in Bidanesefid. The concentrations of malic acid in 6 dws and 12 dws vines as well as tartaric acid concentration in 18 dws vines were found to be significantly higher in 'Yaghuti' than 'Bidanesefid'. In other treatments, at similar stress periods, there were no significant differences between organic acid concentrations of two grapevine cultivars. The ascorbic acid content in 12 dws plants of 'Yaghuti' reached the peak, then decreased slightly in 18 dws plants while in 'Bidanesefid', its highest content was observed in

18dws plants (Table 3)

Discussion

Drought creates an imbalance between light capture and its utilization, finally leading to generation of reactive oxygen species (ROSs). All plants have developed several antioxidant systems, both enzymatic and non-enzymatic, to scavenge these toxic compounds (Lisar *et al.*, 2012). Besides proper activation of the primary metabolic responses that lead to restoration of chemical and energetic imbalances created by stress, secondary metabolites such as phenolic acids and polyamines, are attracting increasing interest due to their potential role in mitigating the effects of stress (Fraire-Velazquez and Balderas-Hernandez, 2013; Rivas-Ubach *et al.*, 2012).

The phenolic compounds contain several secondary metabolites in plants that have antioxidant properties and are involved in prevention of stress-induced oxidative damages. Environmental stresses can cause a decrease (Weidner *et al.*, 2007; Krol *et al.*, 2014) or an increase (Gharibi *et al.*, 2015; Weidner *et al.*, 2009; Al Hassan *et al.*, 2015) in the TPC of plants. In this research, drought stress significantly increased the TPC in both cultivars, and longer drought stress duration caused a higher increase in TPC especially in 'Yaghuti' cultivar. This result firstly shows that vine phenolic compounds are affected by drought stress and secondly confirms that 'Yaghuti' cultivar has a more effective non-enzymatic antioxidant system than the less tolerant 'Bidanesefid'. Therefore, it seems that the enhanced phenolic content in vine leaf tissue under drought stress may be a good indicator for its drought tolerance. Our results were in agreement with the findings of Weidner *et al.* (2009) who reported increased TPC of grapevine roots under drought stress. Increased TPC was also reported in Achillea Species (Gharibi *et al.*, 2015) and Cherry tomato (Al Hassan *et al.*, 2015). On the otherhand, opposite results have been reported in research by other researchers. Krol *et al.* (2014) have found out that drought stress caused the reduction in TPC in grapevine leaves and roots. Such large discrepancies in experimental results can be attributed to differences in stress violence and its length, the stages of plant development and the biological substances,

Table 2- Total and individual polyamines in leaves of 'Yaghuti' and 'Bidanesefid' grapevines under different periods of drought stress

cultivar	Drought period (days)	Putrescine (ng.g^{-1}Fw)	Spermidine (ng.g^{-1}Fw)	Spermine (ng.g^{-1}Fw)	Total PAs (ng.g^{-1}Fw)
Yaghuti	0	11.69 ± 0.46^{d*}	13.8 ± 0.41^{cd}	22.13 ± 0.61^{e}	47.62 ± 0.29^{e}
	6	15.72 ± 0.65^{c}	14.75 ± 0.40^{c}	30.46 ± 1.19^{cd}	60.93 ± 1.28^{d}
	12	23.36 ± 0.63^{a}	14.75± 0.40^{c}	48.71 ± 1.17^{b}	86.82 ± 1.91^{b}
	18	20.12 ± 0.66^{b}	24.10± 0.70^{a}	54.18 ± 1.46^{a}	98.40 ± 2.01^{a}
Bidanesefid	0	9.60 ± 0.36^{d}	11.66 ± 0.63^{d}	17.74 ± 0.46^{e}	39.00 ± 1.03^{f}
	6	11.21 ± 0.63^{d}	15.39 ± 0.49^{c}	21.30 ± 0.73^{e}	47.90 ± 0.51^{e}
	12	14.96 ± 0.83^{c}	21.37 ±0.91^{ab}	27.81 ± 0.85^{d}	64.14 ± 0.92^{cd}
	18	16.42 ± 0.35^{c}	19.17 ± 0.58^{b}	34.52 ± 1.20^{c}	70.11 ± 1.68^{c}

*Mean values marked with the different letters are significantly different (P≤0.05) according to Tukey test. Values are means of four replicates ± SE

Table 3- Total and individual organic acids in leaves of 'Yaghuti' and 'Bidanesefid' grapevines under different periods of drought stress

cultivar	Drought period (days)	Organic acid (mg.100g^{-1}Fw)				
		Tartaric acid	Malic acid	Oxalic acid	Ascorbic acid	Total organic acid
Yaghuti	0	0.71 ± 0.03^{d*}	0.49 ± 0.03^{ef}	0.08 ± 0.007^{e}	0.12 ± 0.006^{e}	1.41 ± 0.04^{e}
	6	0.96 ± 0.05^{c}	0.78 ± 0.02^{c}	0.14 ± 0.009^{cd}	0.14 ± 0.011^{de}	2.02 ± 0.02^{c}
	12	1.53 ± 0.06^{b}	1.24 ± 0.05^{a}	0.20 ± 0.011^{ab}	0.20 ± 0.008^{bc}	3.16 ± 0.03^{a}
	18	1.85 ± 0.04^{a}	1.07 ± 0.03^{b}	0.23 ± 0.010^{a}	0.15 ± 0.008^{cde}	3.30 ± 0.09^{a}
Bidanesefid	0	0.62 ± 0.02^{d}	0.41 ± 0.02^{f}	0.11 ± 0.007^{de}	0.14 ± 0.012^{de}	1.28 ± 0.03^{e}
	6	0.81 ± 0.04^{cd}	0.59 ± 0.03^{de}	0.12 ± 0.012^{cde}	0.18 ± 0.010^{bcd}	1.70 ± 0.06^{d}
	12	1.35 ± 0.04^{b}	0.71 ± 0.03^{cd}	0.17 ± 0.015^{bc}	0.21 ± 0.013^{b}	2.44 ± 0.009^{b}
	18	1.52 ± 0.05^{b}	1.12 ± 0.04^{ab}	0.19 ± 0.009^{ab}	0.26 ± 0.014^{a}	3.08 ± 0.07^{a}

*Mean values marked with the different letters are significantly different (P≤0.05) according to Tukey test. Values are means of four replicates ± SE

such as roots or leaves, which are characterized by a great diversity of secondary metabolites (Weidner et al., 2009).

Phenolic acids which vary in the number and location of hydroxyl groups on the aromatic ring are one of the main classes of phenolic compounds within the plant kingdom (Pereira et al., 2009). In the present study, drought stress significantly affected caffeic, p-coumaric, ferulic, Gallic, chlorogenic acid and resveratrol contents of both cultivars. These results indicate that these detected phenolic acids have the key role as an advantageous adaptive mechanism for protection of grapevine cultivars against drought stress. Under different duration of drought stress, the levels of most identified phenolic acids in the leaves of both cultivars were increased, but the most abundant was gallic acid, and the least abundant was chlorogenic acid. Most phenolic acids in the two cultivars showed a similar response under drought stress conditions and drought stress resulted in an increase in their concentration. Results of this study suggest that in drought stress condition and especially in 'Yaghuti', CAA and GA might play a more pronounced role in the protective mechanisms in grapevine than other phenolic acids and can be used as biochemical markers of stress responses. However, to determine the real role of these compounds in grapevine and for a more precise and comprehensive conclusion, it is necessary to examine their presence in other cultivars and stress conditions. Increased accumulation of phenolic compounds and phenolic acids due to environmental stresses has been

reported in numerous studies (Quan et al., 2016; Weidner et al., 2009; Corso et al., 2015).

Several studies have demonstrated the involvement of the metabolic pathways associated with polyamines metabolism in plant responses to various stresses (Loka et al., 2015; Hatmi et al., 2014; Moschou et al., 2008). By acting as signaling molecules or compatible solutes under water-limiting conditions, PAs serve in the adaptation to osmotic stress (Hussain et al., 2011). The results of various experiments showed that compared to the non-resistant plants, stress-resistant plants have a higher potential to increase the concentration of PAs (Yang et al., 2007). The results of our study showed that grapevine PA concentrations vary depending on the types of cultivar and the duration of drought stress. As compared with corresponding control plants, total PA levels increased in different durations of drought stress in both cultivars and 'Yaghuti' had significantly higher concentrations of total PAs than 'Bidanesefid'. This result confirms higher drought tolerance of 'Yaghuti'. In our study, the concentrations of all three individual polyamines in both cultivars, were significantly increased compared to the control. Evidence is indicates that that polyamine interactions with phosphoric acid residues in DNA, uronic acid residues in cell wall matrix, and negative groups on membrane surfaces contribute to the maintaining of their functional and structural integrity (Edreva et al., 2008). In our study, only 18 days of drought stress resulted in a significant increase in SPD concentration in 'Yaghuti' cultivar while PUT and SPM concentration of this cultivar

increased at the onset of stress, so it seems that these two PAs have a more important role in drought tolerance of 'Yaghuti', probably due to their effective roles in maintaining and stabilizing of cell structures.

Organic acids represent intermediates of major carbon metabolism in plant cells and are involved in various biochemical pathways, such as glycolysis, the tricarboxylic acid (TCA) cycle, photorespiration, the glyoxylate cycle, or the photosynthetic C4 cycle (Drincovich *et al*., 2016). Organic acids as well as other compatible solutes were found among the solutes accumulated during osmotic adjustment in drought-stressed plants. In current research, total leaf and individual OA content were shown to increase under drought stress in both cultivars. These results are consistent with those obtained by Du *et al*. 2012 who reported 65.99% increase in total content of OAs in hybrid bermudagrass under 18 days of drought stress. Total OA accumulation in cultivars was in accordance to their drought tolerance levels and 'Yaghuti' accumulated higher amounts of organic acids than 'Bidanesefid' under 6 and 12 days of drought stress. Organic acid metabolism was shown to be involved in abiotic stress responses like drought tolerance (Du *et al*., 2012) and cold acclimation (Dyson *et al*., 2016).

In the current study, different durations of drought stress remarkably increased the amounts of individual organic acids (tartaric, malic, oxalic and ascorbic acid) in both cultivars. Among four evaluated organic acids, tartaric and malic acids constructed 80 to 88% of total organic acids concentration. In the present study, the contents of quantified OAs were not often significantly different between the two cultivars in the same durations of drought stress. Except malic acid that its contents were higher in 'Yaghuti' than 'Bidanesefid', there were no significant differences between the contents of other OAs in 6dws and 12dws vines of both cultivars. In concordance with the results of our study, in a research carried out by Guo *et al*. 2018, malic acid and oxalic acid were among the metabolites that showed significant increases under drought stress and the extents of their changes were more apparent in drought-tolerant wheat genotype compared to the drought-sensitive one. As an important intermediate of the TCA cycle in all plant species, malate is an essential storage carbon molecule that has, been defined as a pH regulator and exhibits partial control over the efficacy of nutrient uptake and over stomatal function (Fernie and Martinoia, 2009). In the present study, increased content of malic acid in the drought-treated vines, indicates that energy production in the TCA cycle was enhanced under drought stress in both cultivars and more strongly in 'Yaghuti'. In plants, Ascorbic acid plays a protective role against reactive oxygen species that are formed from photosynthetic and respiratory processes. Ascorbic acid is linked to cell growth, being involved in the cell cycle and other mechanisms of plant cell growth and division, as well as acting as a co-factor for many enzymes (Barata-Soares, 2004). Compared to the well-watered control plants, increased content of ascorbic acid in drought-treated plants of both cultivars showed that this substance may act as an effective antioxidant in grapes.

Conclusions

Totally, the present results indicated that drought stress had a marked influence on the content of total phenolic and phenolic acids, total and individual polyamines and organic acids in grapevine cultivars. It seemed that increased contents of the investigated parameters, specially total phenolics, phenolic acids, and polyamines was probably a response to the generation of ROS, so these compounds played significant physiological roles in grapevine drought tolerance. However, to determine the real role of these compounds in grapevine and for a more precise and comprehensive conclusion, it is necessary to examine their presence in other cultivars and stress conditions.

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