Physiological and Biochemical Changes of Some Grapevine Cultivars under Different Irrigation Regimes

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Summary

In this research, the responses of six grapevine cultivars to irrigation regimes were investigated using some physiological and biochemical traits in order to determine the water deficit tolerant cultivars and to select them as a rootstock. The potted plants were subjected to different irrigation treatments: well-watered (100% FC or Full Irrigation, FI), mild (75 % FI), moderate (50% FI) and severe water deficit (25% FI) for a period of 60 days. A factorial experiment (6 cultivars × 4 irrigation levels) was conducted in a completely randomized design (RCD) under greenhouse conditions. The results showed that 'Rotabi' cultivar had the highest quantum efficiency of photosystem II (Fv/Fm) and relative water content (RWC %) under severe water stress deficit and cultivar 'Thompson Seedless' had the lowest amount of these two above mentioned traits. The lowest total chlorophyll (TCh) occurred in 'Yaghouti' and 'Flame Seedless' and the highest one in 'Rotabi' and 'Thompson Seedless'. The highest amount of starch, proline and protein was observed in 'Rotabi' in response to different irrigation levels. 'Perlette' had the highest activity of POD, SOD and protein content as well as total chlorophyll content under 25% FI. 'Siah Samarghandi' and 'Thompson Seedless' cultivars indicated the same activity of superoxide dismutase (SOD) in different irrigation levels. In conclusion, it seems that 'Rotabi' and 'Perlette' are the most tolerant grapevine cultivars in response to progressive water deficit.

Key words

antioxidant enzymes activity, chlorophyll fluorescence, grapevines

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Introduction

Water restrictions and irregular rainfall distribution during the climate changes are the main crises for current and future agriculture (Chaves et al., 2007). Drought is one of the leading environmental stresses causing the decline of crop productivity in the world. Iran is one of the top 20 water-stressed countries in the world (Tianyi, 2018), where all crops and orchards (including vineyards) are highly affected by water resource limitation. Since breeding of grapevine is a slow and expensive process and a new crossing may take over 25 years before being released as a new cultivar (Bota et al., 2016), to explore the existing variability of genetic resource in search of more water efficient varieties seems a good alternative. It is well known that varieties of grapevine are characterized by high heterogeneity concerning ability of their adaptation to adverse environmental conditions (Beis and Patakas, 2012). In fact, genotype-related differences have been described in the responses to water stress, in terms of stomatal conductance, water use efficiency (WUE) and other properties. Even genotypes behave differently according to the growing conditions (e.g., greenhouse versus field or field plants versus potted plants) and the stress degree imposed to the vines (Bota et al., 2016). Various species or cultivars of grapevine use different mechanism to overcome water stress. Hence, it is necessary to classify the cultivars based on numerous biochemical and physiological parameters to select more precisely tolerant cultivar. Chlorophyll concentration, relative water content (RWC) and leaf water potential (WP) are generally important indices used to evaluate plant physiological responses to water deficit (Silva et al., 2010). Kranner et al. (2010) reported drought generates tensions such as osmotic and oxidative stresses, which have negative impact on the plant, causing changes in its normal growth. Due to stomatal closure resulted from water shortage leads to over-excitation of the photosystem II reaction centres and formation of reaction oxygen species (ROS) (Ahmed et al., 2009). These phenomena affect plant metabolism in different ways and bring about cellular damage, destabilization of nucleic acid structure and alteration of enzyme activity (Reddy et al., 2004; Lei et al., 2006; Dumont et al., 2011), chlorophyll degradation, membrane integrity disruption, reduction in metabolic efficiency and loss of carbon fixation and organelle function (De Campos et al., 2011). Cifre et al. (2005) explained the good relevance between stomatal conductance (gs) and leaf water potential and/or water content in some grapevine genotypes. Soluble sugars also maintain the leaf turgidity and prevent dehydration of membranes and proteins (Sawhney and Singh, 2002). Additionally, organic compound such as proline plays a crucial role in cell osmoregulation (Gholamhoseini et al., 2013). Today, commercial orchard production needs the use of drought tolerant cultivars. The aim of this research was to compare the six grapevines candidate rootstock cultivars in responses to irrigation regimes using physiological and biochemical changes and the selection of more tolerant cultivars.

Materials and Methods

Plant Material and Different Irrigation Regimes

Cuttings of six grape cultivars were collected from Agricultural and Natural Resources Research Center in Fars province. One-year-old rooted cuttings of six grape cultivars including 'Thompson Seedless', 'Siah Samarghandi', 'Rotabi', 'Yagouti', 'Flame Seedless' and 'Perlette' were used in this research (16 rooted cutting for each cultivar). In total, 96 plants were transferred to the pots (12 liter) containing sandy loam soil (29% silt, 15% clay and 56% sand) without drainage. The field capacity of the soil used for potting was determined according to the protocol described by Richards (1949). Grapevine cultivars were grown in a greenhouse, under relative humidity 50 ± 5%, 25/18°C day/night cycle, located in the School of Agriculture (29° 36 N, 52° 32 E, 1810 m above mean sea level), Shiraz University, Shiraz, Iran. The plants were irrigated at field capacity (FC) for three months until growth was well established (with nearly 12 leaves). Then they were subjected to water deficit stress. Treatments were 4 levels of irrigation including field capacity or full irrigation (FI), 75% FI, 50% FI and 25% FI. These irrigation levels were applied by the help of a balance. The physiological and biochemical indices were measured after 2 months of irrigation treatments. For measuring proline and antioxidants enzymes, leaf samples were collected and immediately frozen in liquid nitrogen and stored at -80°C until further analysis.

Water Potential

At midday, leaf water potential (WP) was measured by a pressure bomb (PMS Instrument Co., Oregon, USA) using the youngest fully expanded leaves. The water potential rate (MPa) was recorded in the first drop exudation of cut petioles.

Relative Water Content

Fully developed leaves were used to measure relative water content (RWC). The fresh weight of leaf sample discs of 1 cm (10 discs) in diameter was recorded. The samples were submerged in distilled water for 24h at room temperature. Then the turgid weight (TW) was recorded. Finally, the dry weight (DW) was determined after putting the samples at 70°C for 24h. RWC was estimated using the following equation:

$$RWC\ (\%) = (FW - DW) / (TW - DW) \times 100$$

FW: fresh weight

DW: dry weight

TW: turgid weight.

Chlorophyll Fluorescence Analysis

Measurements were made using one fully developed leaf per plant. Chlorophyll fluorescence was measured after 30 min of leaf acclimation to dark under leaf clips, using a OS-30p hand held portable chlorophyll fluorometer (Opti-Sciences, Inc., Hudson, NH, USA) and a saturating light pulse of 3,500 μE m^{-2} s^{-1} was applied for 1 s, which closed all the reaction centers. The maximum quantum efficiency of photosystem II is calculated as

$$F_v / F_m = (F_m - F_o) / F_m$$

where F_m and F_o are maximal and minimal fluorescence of dark-adapted leaves, respectively and F_v is variable fluorescence.

Electrolyte Leakage (EL)

Membrane stability was measured using the procedure of Arora et al. (1998). Discs of 2 cm in diameter of fully expanded leaves were placed in test tubes containing 20 mL of distilled water. Samples were then shaken on a shaker at 250 rpm for 4 h at room temperature. Then, electrical conductivity of each solution was measured using a conductivity meter (Conductometer, Metrohm, Herisau, Switzerland). After measuring initial electrolyte leakage (E1), the samples were heat-killed (autoclaved at 121 °C, 124 KPa for 15 min) and final electrolyte leakage (E2) was measured at room temperature. Ion leakage was calculated using the equation of

$$EL\ (\%) = E1 / E2 \times 100.$$

Total Chlorophyll (TChl)

TChl was measured using dimethyl sulfoxide (DMSO) described by Hiscox and Israelstam (1979). In this method, 7 mL of DMSO was added to 100 mg leaf pieces (free vein) and then they were placed in an incubator (60°C for 30 minutes). After filtering, by adding the DMSO extract volume was reached to/at 10 mL. Finally, absorbance was read at 470, 645 and 663 nm using spectrophotometer (Spectrophotometer Epoch, company Bio Tek, USA). Total chlorophyll

$$(mg\ g^{-1}\ F.W.) = [20.2\ (A645) + 8.02\ (A663)] * V / 1000 * W$$

where V = extract volume in ml and W = fresh weight of sample gram.

Proline Concentration

According to the method described by Bates et al. (1973), the leaf samples (500 mg) were crushed in 10 mL of sulphosalycylic acid (3% aqueous) and filtered. Two mL of the extract were added into the test tube containing 2 mL of glacial acetic acid and 2 mL of ninhydrin reagent. The reaction mixture was heated in a boiling water bath at 100°C for one hour. After cooling the mixture by ice, toluene (4 mL) was added and thoroughly mixed. Finally, the absorbance was measured at 520 nm using a spectrophotometer (Spectronic 200^{+}) against toluene blank.

Antioxidant Enzymes

First, the fresh leaves (1 g) were homogenized into 4 mL potassium phosphate (50 m M) buffer (pH = 7), 3 mM 2-mercaptoethanol, 2 mM Na-EDTA and 1 % (w/v) polyvinyl polypyrrolidone (PVPP) in a chilled mortar. Then homogenate was centrifuged at 16000 g for 10 min at 4°C (Ozden et al., 2009) and subsequently supernatant was used for enzymes assay. Superoxide dismutase (SOD, EC 1.15.1.1) activity was measured using a spectrophotometer at 560 nm as described by Giannopolitis and Ries, (1977). The reaction mixture (3 mL) was mixed to 50 mL of potassium phosphate 50 mM (pH = 7.8), 0.1 mM EDTA, 13 mM L-Methionine, 75 µM nitroblue tetrazolium (NBT), 4 µM riboflavin, and 250 µM enzyme extract. The reaction was done under light over 15 min.

Catalase (CAT) (EC 1.11.1.6) activity was assayed by following the H_2O_2 consumption (extinction coefficient, 39.4 mM^{-1} cm^{-1}) at 240 nm over a 1 min interval (Dhindsa et al., 1981). Reaction mixture (3 mL) containing potassium phosphate 50 mM (pH 7), 10 mM H_2O_2 and 50 µl enzyme extract was used.

Peroxidase (POD) (EC 1.11.1.7) activity assay, the guaiacol oxidation was measured by the increase in absorbance at 470 nm. The assay mixture included 2.9 ml of K-phosphate buffer (10 mM, pH 7.0), 0.05 mL of guaiacol (20 mM) and 50 µl enzyme extract. The reaction was initiated by adding 20 mL of H_2O_2 (40 mM) (Ozden et al., 2009).

Total Soluble Carbohydrate (TSC) and Starch Concentration

According to Dubois et al (1956), the dry leaf samples were extracted by adding 10 mL of ethanol 80% and they were centrifuged (5000 rpm) for 10 minutes. After separating of supernatants, the extraction and centrifugation were repeated on residue. Then, 25 µl of phenol solution (5 %) was added to target solution. Immediately, 25 µl of concentrated sulphuric acid was also added. Then the absorbance was recorded at 490 nm using Epoch set (Spectrophotometer Epoch, company Bio Tek, USA). The soluble sugar concentration was calculated using the standard curve of glucose and the results were expressed as mg g^{-1} dry weight. The concentration of starch was determined using a modified protocol of McCready et al. (1950). After reading the soluble free sugars, the sample residue was washed with 200 µl of ice cold distilled water and 260 µl of perchloric acid (52%) and then the samples were shaken. Again, 400 µl of distilled water was added to falcons and then they were centrifuged at 5000 rpm for 10 min. The supernatant was taken and added to residue of 200 µl ice-cold distilled water and 130 µl perchloric acid (52%). The starch concentration was determined using the 100 µl of sample solution plus 200 µl of anthrone reagent (2g L^{-1} in the cooled sulphuric acid method). After holding the plates in the oven at 60°C (20 min), the final solution swirled when immersed in ice-cold water. Epoch device (Spectrophotometer Epoch, company Bio Tek, USA) was used in order to measure the starch at the wavelength of 630 nm.

Protein

Total concentration of soluble protein was determined as method described by Bradford (1976) by applying bovine serum albumin (protein standard). This method is based on the connection of acidic reagent colour to protein. Frozen leaf samples (1g) were homogenized with buffer of Na-Phosphate (pH=7.2). For preparation of the reagent, 0.01 coomassie Brilliant Blue G-250 dissolved in 5 mL of ethanol (96%) in the dark. Then, 10 mL of phosphoric acid (85%) was gradually added to the mixture. The final volume was adjusted to 100 mL by adding distilled water. Then 20 µl of the extracts were diluted in 80 µl extraction buffer plus 5 mL of coomassie Brilliant Blue G-250. Finally, this solution was stirred for 2 minutes. After 5 minutes, its absorbance was read at 595 nm.

Statistical Analysis

The factorial experiment of 4 levels of irrigations and 6 cultivars was conducted in a completely randomized design (CRD) with 4 replications. Data were analysed using SAS software version 9.4. The means were compared using Duncan's multiple range tests at probability of 5%.

Results and Discussion

Water Potential (WP)

Due to non-significant interactions between different grapevine cultivars and irrigation levels on the stem water potential, the main effects are presented (Fig 1 and Fig 2). The six studied cultivars clearly indicate a difference in their response to various water supply levels. The highest and the lowest amount of WP in the vines were observed in the 'Perlette' and 'Yaghouti' cultivars, respectively. Regardless of cultivar types, with increasing water deficit levels, leaf WP decreased and the maximum and minimum amount of WP was related to FI and 25% FI treatments, respectively (Fig. 2).

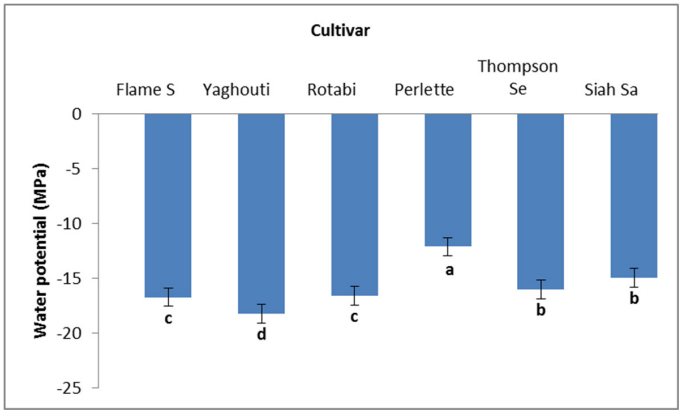

Figure 1. Leaf water potential of different grapevine cultivars. Means with the same letter are not significantly different using Duncan's multiple range test at 5% of probability. Each mean = ± SD, n = 3. Flame S, 'Flame Seedless'; Thompson Se, 'Thompson Seedless'; Siah Sa, 'Siah Samarkandi'

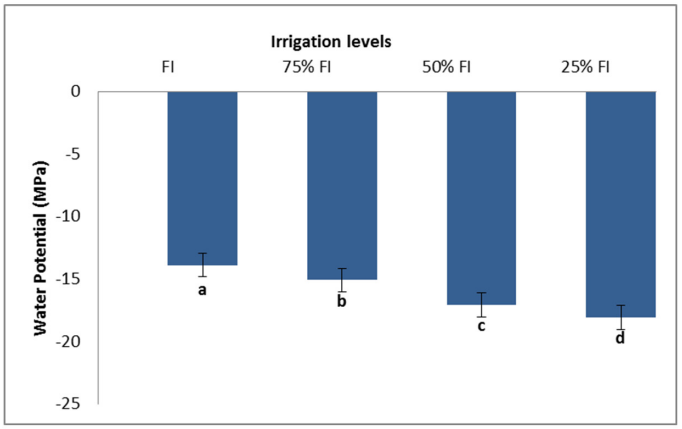

Figure 2. The effect of water stress on leaf water potential. Means with the same letter are not significantly different using Duncan's multiple range test at 5% of probability. Each mean = ± SD; n = 3; FI= full irrigation

Insufficient water uptake by the roots from dry soil results in decreasing water potential and leaf turgor simultaneously (Pereria and Chaves, 1995). Typically, the stress of drought activates water removal from the cytoplasm to the extracellular space causing a decrease in the vacuolar and cytosolic size, consequently the wilted appearance of the vine shoot. Water potential of leaf is extensively used as an index to reveal the plant water stress level. More negative potentials of predawn leaf water reflect restricted water availability to roots or an ineffectual conducting system of water (Zwack and Graves, 1998). In Oleander (*Nerium oleander*) clones, Niu et al. (2008) reported the similar phenomenon of rapid decrease in potential of leaf water around the critical content of soil moisture.

Electrolyte Leakage

Regardless of irrigation levels, the highest EL was observed in 'Yaghouti' (20.48 %) and the lowest in 'Thompson Seedless' (10.41 %). In all cultivars, with increasing water stress, EL significantly increased (Table 1). However, this increment was not significant in 'Flame Seedless' when compared with FI. The highest leakage increment (52%) occurred in 'Rotabi' compared with its control (FI). The high electrolyte leakage (EL) reflects low cell membrane stability (CMS).

It has been reported that drought stress causes membrane peroxidation of lipid and produce certain compounds such as ethylene and malondialdehyde (MDA) resulting in instability of cell membrane (Sairam et al., 2001). These results, consistent with the results obtained by Ghaderi et al. (2011), reported that drought stress decreased the index of cell membrane stability in some grapevine cultivars. In addition, the ability of different cultivars to protect the integrity of the cell membrane was different.

Relative Water Content

The RWC decreased significantly when drought stress levels were increased. In each cultivar, a significant difference was observed in the RWC (%) between control and highly stressed plants (Table 1). Water stress had the lowest impact on RWC in the cultivars 'Rotabi' and 'Perlette' (reduction of 9.7 and 9.53% respectively), whereas the highest amount of RWC decrease was observed in the cultivar 'Yaghouti' and 'Siah Samarghandi' (16.4 %).

Measurement of RWC is a general method used to determine leaf water balance in plants during drought stress periods (Uzildaya et al., 2012) and evaluates the percentage of water existent in the leaves as a total volumetric water fraction that the leaves can maintain at full turgor (Blum, 1998). Water accessibility limitation is one of the important factors, which affects the leaf RWC. In well-irrigated condition, cultivars 'Flame Seedless', 'Perlette' and 'Yaghouti' showed significantly higher RWC. However, with mild water stress (75% FI), a significant reduction was observed in these cultivars compared with their controls, whereas in 'Rotabi', 'Siah Samarghandi' and 'Thompson Seedless' cultivars, 50% FI reduced RWC significantly. In agreement with our results, Karami et al. (2017) reported that drought significantly decreased RWC in Bedaneh Sefid cultivar. Contrary to our findings, it has been reported that there is no significant difference in RWC under different water irrigation regimes, where the isohydric conduct would be expected to restrict the response of the leaf water potential range to the differences in soil water content (Ghaderi et al., 2011).

Table 1. Interaction between different grape cultivars and irrigation levels on the electrolyte leakage (EL), Relative water content (RWC) and F_v/F_m

Mean	F_v/F_m	Mean	RWC (%)	Mean	EL (%)	Irrigation levels	Cultivar
	0.67±0.06c		86.71±3.18cd		10.63±0.07ij	FI	
	0.60±0.01e		82.27±0.84gi		10.64±0.07hij	75% FI	'Flame seedless'
	0.59±0.01f		80.19±2.21ijk		10.66±0.07hij	50 % FI	
0.59C	0.51±0.01g	80.63CD	73.37±1.40l	1.66CD	10.71±0.07ghi	25 % FI	
	0.69±0.07bc		86.11±1.98cde		10.46±0.03k	FI	
	0.69±.01bc		83.24±0.48fg		10.66±0.01hij	75% FI	'Perlette'
	0.65±0.03cd		79.17±1.47jk		10.80±0.06efg	50 % FI	
0.66A	0.62±0.01d	81.6C	77.88±1.36jk	1.75C	10.92±0.01e	25 % FI	
	0.74±0.03a		89.87±1.37b		10.25±0.11l	FI	
	0.71±.05b		87.89±0.69bc		10.54±0.08jk	75% FI	'Rotabi'
	0.60±0.07e		85.27±0.93c-f		20.50±0.05c	50 % FI	
0.67A	0.62±0.02d	86.03A	81.12±0.57i	1.98B	20.65±0.04b	25 % FI	
	0.70±0.07b		94.99±1.89a		20.19±0.11d	FI	
	0.62±0.07d		86.66±0.74cd		20.43±0.12c	75% FI	'Yaghouti'
	0.55±0.05fg		83.87±0.91efg		20.56±0.05bc	50 % FI	
0.58C	0.48±0.03gh	86.22A	79.38±2.19jk	2.48A	20.77±0.10a	25 % FI	
	0.67±0.03c		86.77±0.93cd		10.15±0.01l	FI	
	0.65±0.02cd		86.10±1.12cde		10.47±0.01k	75% FI	'Siah Samarghandi'
	0.61±0.04de		82.82±1.39g		10.77±0.03fgh	50 % FI	
0.62B	0.58±0.01f	82.51B	74.36±2.17l	1.56CD	10.86±0.01ef	25 % FI	
	0.6±0.02e		87.04±0.79cd		10.25±0.1l	FI	
	0.49±0.0gh		84.57±0.75d-g		10.43±0.1k	75% FI	'Thampson seedless'
	0.42±0.0i		79.69±1.43f-k		10.47±0.07k	50 % FI	
0.46D	0.37±0.01j	82.37B	74.21±0.86l	1.41D	10.49±0.03k	25 % FI	
	**		**		**	Cul	
	**		**		**	T	Significant
	*		**		**	Cul×T	
	6.78		1.77		4	CV	

*, **: significant at 5% and 1% probability respectively and ns: non-significant
In each column, the means with the same letter are not significantly different using Duncan's multiple range test. Each mean = ± SD, n = 3. FI, Full Irrigation; Cul, cultivar; T, treatment

Chlorophyll Fluorescence

Obviously, the progression of water stress decreased the maximum quantum efficiency of photosystem II (F_v/F_m ratio) in all cultivars. In FI and 75% FI, 'Rotabi' cultivar showed the maximum Fv/Fm index compared with the others. Under good water condition (FI), there was no significant difference in the F_v/F_m values between 'Flame Seedless', 'Perlette' and 'Siah Samarghandi'. In severe water stress (25% FI), 'Thompson Seedless' and 'Yaghouti' showed a reduction of 38 and 31% in the F_v/F_m values compared to their control respectively, whereas in 'Rotabi' 'Siah Samarghandi' and 'Perlette' cultivars this reduction was 16, 13 and 10 % respectively (Table 1).

The use of chlorophyll fluorescence technique can be considered a promising tool, in order to rapidly quantify the plant responses to water stress in higher plants (Faraloni et al., 2011). In this research, it seems that severe water stress affected the photosynthetic system of 'Thompson Seedless' and 'Yaghouti' more than those of 'Rotabi', 'Siah Samarghandi' and 'Perlette' cultivars. It has been reported that *Vitis* hybrid Richter-110 showed similar value of F_v/F_m in water-stressed plants compared to its control (Flexas et al., 2009). However, genetic differences exist in the reaction of the photosynthetic apparatus to drought and in drought tolerant species the photosynthetic processes have developed various mechanisms to safeguard against water stress (Ow et al. 2011)

Total Chlorophyll

Total chlorophyll content in the leaves of different cultivars (except 'Siah Samarghandi') was significantly affected by different water availability. Severe water stress (25% FI) decreased total chlorophyll (TCh) of the 'Flame seedless', 'Yaghouti', Rotabi' and 'Thompson seedless' by 40.38, 76.91, 39.26 and 17.96% compared to their controls respectively (Fig 3). In 'Siah Samarghandi', there was no significant difference in TCh content in all levels of irrigation.

One of the major chloroplast components is chlorophyll, which has a positive relationship with photosynthetic rate. The reduction in chlorophyll content under water-stress condition is a common symptom of oxidative stress, which may be due to photo-oxidation of pigments and chlorophyll degradation (Anjum et al., 2011; Shirbani et al., 2013). In addition, water deficit induced reduction in chlorophyll content has been attributed to the chloroplast membrane damage, deformation of the lamellae vesiculation, and the appearance of lipid droplets (Anjum et al., 2011). In this research, grapevine cultivars reacted differently to the reduction of chlorophyll under intense stress conditions. For example, 'Flame Seedless' showed 55% reduction in TCh content, but there was no reduction in TCh in the case of 'Siah Samarghandi'. This indicates that the light dissipation and antioxidant systems may prevent the degradation of chlorophyll molecules (Niu et al., 2008). Sircelj et al. (2005) also reported that no reduction in the chlorophyll content of apple leaves 'Elastar' under water stress was due to a strong antioxidant system and an efficient light dissipation system.

Proline

The results showed high interaction between cultivars and irrigation levels on leaf proline content. In all studied cultivars, with increasing water stress, proline content increased. 'Yaghouti' in 25% FI showed the highest proline concentration (241.9 µM g^{-1} FW) which was not significantly different compared to 'Flame Seedless' and 'Rotabi' in the same condition (Table 2). The 'Flame Seedless' and 'Siah Samarghandi' exhibited the lowest proline content in FI.

A large number of compounds can contribute to osmoregulation: sugars, organic acids, sugar alcohols (polyols, mannitol, sorbitol), amino acids (proline, glutamic and aspartic acid). These compounds have a large amount of hydroxyl that helps facilitate hydrogen bonds with molecules of water, thereby maintaining the functionality of macromolecules in solution (González-Chavira et al., 2018). In addition to regulating osmosis,

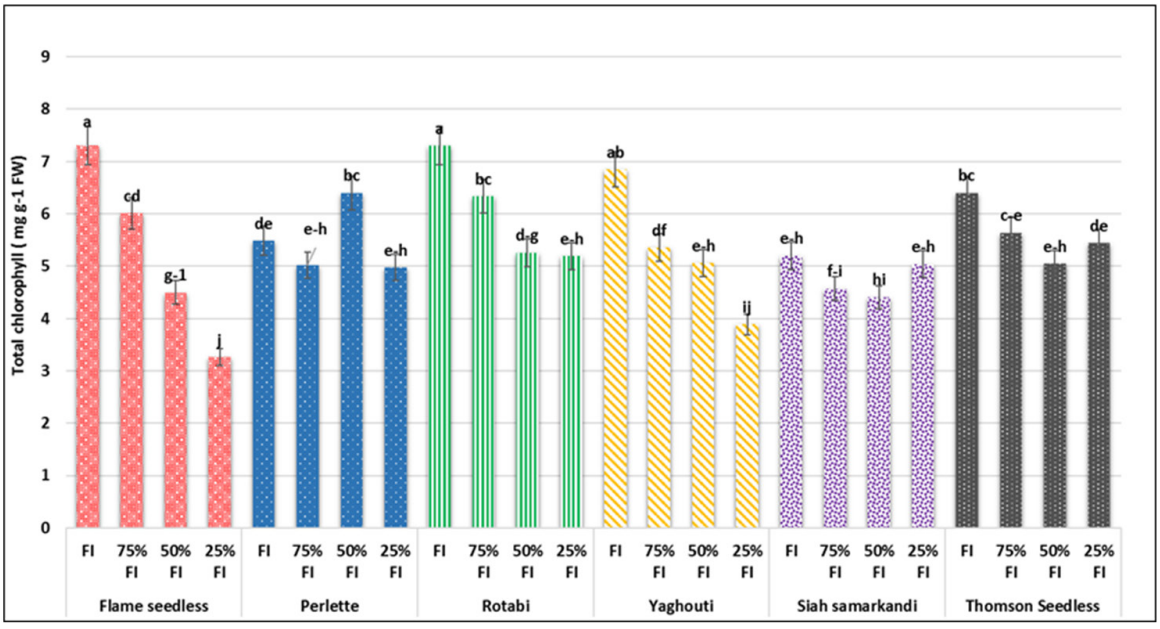

Figure 3. Interaction between different grape cultivars and irrigation levels on total chlorophyll. Means with the same letter are not significantly different using Duncan's multiple range test at 5% of probability. Each = ± SD; n = 3; FI= Full Irrigation

Table 2. Interaction between different grape cultivars and irrigation levels on the starch, Soluble carbohydrate (SC) and proline

Mean	Proline (µM g^{-1} FW)	Mean	SC (mg. g^{-1} DW)	Mean	Strach (mg. g^{-1} DW)	Irrigation levels	Cultivar
	70.2±22.4i		46.67±1.3d-f		5.15±0.01e	FI	
	143.8±23.3gh		43.96±5.01d-f		4.95±0.03ef	75% FI	'Flame seedless'
	186.5±6.8de		21.87±5.96f		4.57±0.02fg	50 % FI	
156.6C	226.1±14.6ab	35.78E	30.62±4.37d-f	4.34B	2.71±0.01kl	25 % FI	
	128.9±22.1h		73.75±12.29ab		4.17±0.01gh	FI	
	143.3±29.4gh		49.38±15c-e		3.89±0.01hi	75% FI	'Perlette'
	169.8±15.3e-g		38.33±26.34d-f		3.50±0.01ij	50 % FI	
157.6C	188.3±14.0de	46.98B	26.46±8.77ef	3.78C	3.58±0.03ij	25 % FI	
	142.3±13.7e		45.42±1.57d-f		7.74±0.00a	FI	
	158.7±11.5e		52.29±1.90b-d		7.21±0.02b	75% FI	'Rotabi'
	232.2±8.2ab		54.58±4.16b-d		7.14±.06bc	50 % FI	
193.6A	241.3±27.3a	43.90C	23.33±5.20f	7.12A	6.39±0.03d	25 % FI	
	109.1±26.7gh		26.87±15.47ef		3.60±0.00ij	FI	
	128.9±13.2c-e		36.66±5.63d-f		3.41±0.01ij	75% FI	'Yaghouti'
	232.0±17.4ab		42.29±6.06d-f		3.17±0.01jk	50 % FI	
178.0B	241.9±7.5a	35.31E	35.42±4.52d-f	3.26D	2.89±0.03kl	25 % FI	
	76.3±18.0 i		38.12±0.62d-f		2.76±0.01kl	FI	
	166.6±26.1 e-g		35±5.96d-f		2.77±0.01kl	75% FI	'Siah Samarghandi'
	177.6±4.0 e-f		39.79±0.59d-f		2.42±0.0ll	50 % FI	
159.8C	218.5±15.1 bc	38.0D	39.38±1.87d-f	2.42E	1.74±0.04m	25 % FI	
	149.7±14.4fh		70.63±23.75a-c		4.06±0.00g	FI	
	188.6±9.3de		87.26. ±8.4a		3.71±0.05h-i	75% FI	'Thampson seedless'
	211.6±17 4cd		89.58±3.73a		3.46±0.04ij	50 % FI	
191.2A	214.7±23.5bc	84.5A	90.62±3.73a	3.65C	3.43±0.03ij	25 % FI	
	**		**		**	Cul	
	**		ns		**	T	Significant
	**		**		**	Cul×T	
	10.95		0.19		25.63	CV	

*, **: significant at 5% and 1% probability respectively and ns: non-significant
In each column, the means with the same letter are not significantly different using Duncan's multiple range test. Each mean = ± SD, n = 4. FI, Full Irrigation, Cul, cultivar; T, treatment

accumulation of proline plays an important role in stabilizing the biological macromolecular structure, declining acidity of cell and the release of ammonia toxicity and as a pool of energy to regulate cell redox potential (Szabados and Savoure, 2010).

Both intra- and intercellular proline transport is critical for maintaining of cellular homeostasis, but the intracellular movement of proline plays a more important role in augmenting stress tolerance. The reduction of proline oxidase activity may be the cause for increasing accumulation of proline (Manivannan et al., 2008). Proline protects plants by functioning as a regulator of cellular osmosis between vacuole and cytoplasm, by detoxifying of ROS, thus membrane integrity protection and stabilizing antioxidant enzymes. Other researchers suggested that proline accumulation in grapevine was due to the increase in proline transport as no increase in the gene expression of P5CS was observed (Stines et al., 1999).

Soluble Carbohydrate and Starch

The results showed that different cultivars exhibited the diverse starch concentrations. In this regard, in all levels of irrigation regimes, 'Rotabi' showed higher starch content than that of other cultivars. However, all cultivars showed a reduction in their starch content under water stress condition, whereas the extent of this reduction differed from one cultivar to another (Table 2). Regardless of irrigation levels, Thompson Seedless indicated the maximum amount of soluble carbohydrate (84.5 mg g^{-1} DW). In all cultivars, soluble carbohydrate content decreased in 25 % FI treatment. However, this reduction was significant in 'Perlette' and 'Rotabi'. Generally, some photosynthetic products such as starch are stored in source leaves and the sucrose is mostly transported to the sink through the phloem (Lalonde et al., 1999). It has been reported that water deficit negatively affects the photosynthesis and synthesis of carbohydrates by decreasing the pigment contents of leaf and damages the leaf ultrastructure. Different sugar accumulation may be involved in osmotic adjustment of leaves during water stress that makes photosynthesis more efficient (Xu et al., 2007). However, convert metabolism of starch (stored carbohydrate) to soluble carbohydrate and its accumulation in leaves is very sensitive to environmental stress such as drought and salinity. Soluble carbohydrates that accumulate in reaction to water shortage can function as osmolytes to maintain turgor of cells and have the ability to keep biological membranes and proteins from stress damages (Kaplan and Guy, 2004).

Antioxidant Enzymes Activity

Regardless of irrigation levels, 'Perlette' showed the highest SOD activity (181.5 U g^{-1} FW min^{-1}), which had no significant difference compared with 'Siah Samarghandi' and 'Thamson Seedless' and 'Flame Seedless' showed the lowest SOD activity (125.8 U g^{-1} FW min^{-1}) (Table 3). The results showed high interaction between different cultivars and water stress on SOD activity. A mild water stress (75% FI) on the cultivars of 'Perlette', 'Siah Samarghandi' and 'Thompson Seedless' triggered SOD activity, while in 'Rotabi' SOD activity was not significantly affected by 25% FI.

In all cultivars, with increasing water stress, peroxidase activity significantly increased. The highest activity of POD was observed in 'Perlette' 0.069 U g^{-1} FW min^{-1}) and the lowest one in 'Thamson Seedless' (0.023 U g^{-1} FW min^{-1}) (Table 3). In relation to CAT activity, different cultivars responded differently to water stress. 'Siah Samarghandi' showed the highest level of CAT (0.173 U g^{-1} FW min^{-1}) and SOD (190 U g^{-1} FW min^{-1}) acitvities in high-stress condition (25% FI). On the other hand, this cultivar showed no reduction in chlorophyll content in the same condition. It seems that 'Siah Samarghandi' with the increase of CAT and SOD activities and detoxification of reactive oxygen species (ROS) protected its leaf chlorophyll content. However, in 'Thompson Seedless', CAT activity increased in mild and moderate (75% and 50% FI respectively) water stress and decreased in severe water stress, but in 'Rotabi' CAT activity decreased in mild and moderate and increased in severe condition (Table 4).

It has been reported that SOD operates as the first line of defence against oxidation by decomposition of O^{2-} into H_2O_2 and O_2 with high efficiency at the membrane boundaries consequently as protection of cells (Sadeghi and Shekafandeh, 2014). CAT that is known as stress marker is a heme-containing enzyme, which operates in the hydrogen peroxide (H_2O_2) scavenging into oxygen and water (Asada, 2006). Based on our findings, there were significant differences among the different cultivars ('Flame Seedless' and 'Siah Samarghandi' have the same amount of CAT) in catalase activity ($P = 0.05$), which is in accordance with other study (Jia et al., 2003). However, the activities of CAT and SOD increased and then decreased in reaction to water deficit, and these results are consistent with the findings of other studies (Jua et al., 2018). This response might be due to the antioxidant system destruction (Boo and Jung, 1999). There are many investigations which have proven peroxidase activity increase under stress of drought (Manivannan et al., 2008; Pompelli et al., 2010). Yaghooti cultivar in well-watered condition (high RWC) showed the minimum SOD enzyme activity. However, SOD activity of 'Rotabi' was non-significant at all irrigation levels. So, it seems that rate of SOD activity is genetically dependent.

Protein Content

Data analysis showed that there was a significant interaction between cultivars and irrigation levels. In all cultivars, with increasing in water deficit the protein content decreased significantly. Irrespective of irrigation levels, 'Rotabi' and 'Perlette' showed the highest soluble protein content and 'Siah Samarghandi' showed the lowest level of protein (Table 4).

The synthesis of protein is an important process during early growth of seedling that is affected by water deficit. This lower protein level could be due to reduction in the protein synthesis, the lack of amino acid availability, accelerated proteolysis and enzyme denaturation involving protein synthesis (Sadeghi and Shekafandeh, 2014).

Table 3. Interaction between different grape cultivars and irrigation levels on the activity of Superoxide dismutase (SOD), Peroxidase (POD)

Cultivar	Irrigation levels (%)	(SOD) (Ug^{-1} FW min^{-1})	Mean	(POD) (Ug^{-1} FW min^{-1})	Mean
'Flame seedless'	FI	103.6±6.8i		0.019±0.002h	
	75% FI	107.2±3.2i		0.024±0.008gh	
	50 % FI	125.3±4.7h		0.027±0.012f-h	
	25 % FI	167.1±4.1de	125.8C	0.049±0.017c-e	0.029CD
'Perlette'	FI	170.4±9.2cd		0.031±0.011e-h	
	75% FI	176.5±9.8a-d		0.041±0.008c-g	
	50 % FI	192.4±5.6a		0.053±0.005b-d	
	25 % FI	186.7±7.5ab	181.5A	0.069±0.006ab	0.048A
'Rotabi'	FI	165.4±8.0cd		0.016±0.001h	
	75% FI	169.3±7.6cd		0.035±0.007d-h	
	50 % FI	166.4±13.2d-f		0.030±0.001e-h	
	25 % FI	173.1±7.2b-d	168.6B	0.054±0.007b-d	0.033C
'Yaghouti'	FI	108.1±3.7i		0.029±0.007f-h	
	75% FI	123.0±14.4h		0.042±0.005c-g	
	50 % FI	144.8±5.4g		0.045±0.025c-f	
	25 % FI	151.8±7.2fg	131.9C	0.057±0.011a-c	0.043AB
'Siah Samarghandi'	FI	153.0±6.8e-g		0.027±0.006f-h	
	75% FI	189.9±3.2a		0.031±0.005e-h	
	50 % FI	190.1±4.7a		0.044±0.03c-f	
	25 % FI	190.6±4.1a	180.9A	0.052±0.05b-d	0.038B
'Thampson seedless'	FI	151.8±8.2fg		0.042±0.013f	
	75% FI	181.6±10.0a-d		0.047±0.006c-f	
	50 % FI	183.2±12.7a-c		0.032±.011e-h	
	25 % FI	189.3±4.6a	176.5AB	0.023±0.008gh	0.036BC
Significant	Cul	**		**	
	T	**		**	
	Cul×T	**		**	
	CV	5.11		25.63	

*, **: significant at 5% and 1% probability respectively and ns: non-significant

In each column, the means with the same letter are not significantly different using Duncan's multiple range test. Each mean = ± SD , n = 4. FI, Full Irrigation; Cul, cultivar; T, treatment

Table 4. Interaction between different grape cultivars and irrigation levels on the activity of catalase (CAT) and protein content

Cultivar	Irrigation levels (%)	(CAT) (Ug^{-1} FW min^{-1})	Mean	Protein (mg. g^{-1} FW)	Mean
'Flame seedless'	FI	0.055±0.008e-h		91.43±1.4bc	
	75% FI	0.105±0.004b		90.16±0.4c	
	50 % FI	0.097±0.007bc		88.97±1.7cd	
	25 % FI	0.088±0.010b-d	0.086A	87.63±0.9d	89.54C
'Perlette'	FI	0.022±0.008j		93.14±0.7ab	
	75% FI	0.030±0.008ij		92.66±0.6ab	
	50 % FI	0.036±0.011g-j		90.10±0.1c	
	25 % FI	0.056±0.012e-g	0.036D	89.32±0.5cd	91.30A
'Rotabi'	FI	0.085±0.008b-d		94.12±0.8a	
	75% FI	0.047±0.010f-i		91.50±1.2bc	
	50 % FI	0.033±0.009h-j		90.82±0.6c	
	25 % FI	0.081±0.011cd	0.061B	89.72±1.6cd	91.54A
'Yaghouti'	FI	0.043±0.007g-j		92.44±0.7ab	
	75% FI	0.048±0.006f-i		91.33±0.3bc	
	50 % FI	0.053±0.010e-h		88.74±0.6d	
	25 % FI	0.079±0.010cd	0.055C	85.98±0.3e	89.62C
'Siah Samarghandi'	FI	0.046±0.011g-i		89.93±0.1cd	
	75% FI	0.054±0.013e-h		88.71±1.0d	
	50 % FI	0.071±0.006de		88.00±1.6d	
	25 % FI	0.173±0.029a	0.086A	85.85±2.6e	88.12D
'Thampson seedless'	FI	0.046±0.007g-i		93.64±0.4ab	
	75% FI	0.069±0.015d-f		90.18±0.2c	
	50 % FI	0.084±0.010cd		89.93±0.1cd	
	25 % FI	0.034±0.011g-j	0.053C	87.43±1.7d	90.29B
Significant	Cul	**		**	
	T	**		**	
	Cul×T	**		**	
	CV	17.88		5.11	

*, **: significant at 5% and 1% probability respectively and ns: non-significant
In each column, the means with the same letter are not significantly different using Duncan's multiple range test. Each mean = ± SD, n = 4. FI, Full Irrigation, Cul, cultivar; T, treatment

Conclusion

According to our findings, grapevine cultivars reacted differently in confronting severe water stress condition. The cultivars 'Rotabi' 'Siah Samarghandi' and 'Perlette' with a high F_v/F_m ratio in severe water stress experienced a lower injury to PSII performance. The highest amount of starch and soluble carbohydrate was observed in cultivar 'Rotabi' and 'Thompson Seedless', respectively. The results showed that cultivars 'Perlette' had the highest activity of POD, SOD and protein content. The cultivars 'Rotabi' and 'Thompson Seedless' indicated the highest accumulation of proline in different irrigation levels. The highest total chlorophyll (TCh) degradation occurred in 'Yaghouti' and 'Flame Seedless' and the lowest one in 'Rotabi' and 'Thompson Seedless' and no chlorophyll degradation in 'Siah Samarghandi'. It seems that cultivars 'Siah Samarghandi', 'Rotabi' and 'Perlette' are more tolerant in response to progressive water deficit. It remains to be demonstrated how performances of un-grafted genotypes are maintained and support the scion when tolerant genotypes are used as rootstocks.

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