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Influence of post-véraison water deficit on berries yield and quality of three table grape cultivars

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- Key words: antioxidant activity, anthocyanin, drought, total phenol, water requirement.

Abstract: In order to investigate the effect of regulated deficient irrigation (RDI) on some quantitative and qualitative characteristics of the three commercial grapevine cultivars (i.e. Keshmeshi, Sahebi and Sharabi), an experiment was set in split plot arranged in an RCBD design with three replications. In this experiment, irrigation treatments including 100 (as control), 80%, 60% and 40% crop evapotranspiration (ETc) were implemented in a period between onset of berries color change (véraison) to harvest of fruits. At the end of experimental period, some traits such as berry weight, berry length, berry diameter and cluster weight as well as some fruit quality traits such as total soluble solids (TSS), titratable acidity (TA), flavonoids, anthocyanin, total phenol, and total antioxidant capacity were measured. The results of this experiment revealed that effect of RDI at different levels on yield, berry and cluster weight, berry length and diameter, TSS, TA, anthocyanin, phenol, and flavonoid was significant. Also, there was no significant difference between control and 80% ETc in terms of yield, berry diameter, TSS, and TA; and these treatment enhanced total phenol, anthocyanin and antioxidant activity in the three cultivars. In both years of experiment, RDI remarkably enhanced anthocyanin, flavonoids, antioxidant activity and phenolic concentration. Overall, the results indicated that 80% ETc might be sufficient to gain adequate yield in 'Keshmeshi', 'Sahebi' and 'Sharabi' without undermining the quality of fruit.

1. Introduction

Water deficiency is one of limiting factors influencing grape production throughout the world (Rabiei *et al.*, 2003). In order to improve water use efficiency (WUE), it is necessary to recognize factors restricting WUE and then introduce appropriate approaches for mitigating the harmful effects of such factors (McCarthy *et al.*, 2002). In this respect, balancing between vegetative and reproductive phases in plants in favor of hindering excess vegetative growth is one of these approaches being used to improve not only WUE but also fruit quality of grapevine, because excess growth of shoots may have adverse impact on fruit quality (Chalmers et al., 1981). Reduction in vegetative growth in favor of improving WUE can be implemented by many approaches. One of such approaches is to constrict the amount of water required for plant growth through reducing the number of irrigation or decline in amount of water in each irrigation (Chalmers et al., 1981; Goodwin and Jerie, 1992). For achieving this purpose, regulated deficit irrigation (RDI) was introduced (Santesteban and Mirandam Royo, 2011). As more than 82% of Iran territory is located in arid and semiarid zone with drought climate and extreme temperature changes in the world (Amiri and Eslamian, 2010), this approach may pave the way for planting crop in a vast majority of abandoned farmland deprived of adequate water distribution, although employing this approach may reduce crop production because of reduction in water use (Chalmers et al., 1981). Under drought condition, RDI abridges vegetative growth in favor of enhancing reproductive growth and this could improve yield (Santesteban and Mirandam Royo, 2011).

At critical stages of plant growth and development, change in water status certainly influences fruit quality by affecting vegetative growth and fruit development (Van Leeuwen et al., 2004; Ezzhaouani et al., 2007). Therefore, RDI improves cluster quality through reduction in berry drop (Ojeda et al., 2002). Under a mild drought stress, Acevedo-Opazo et al. (2010) proposed an appropriate threshold for managing RDI schedule to keep quality of grape fruits. The positive RDI effect on phenolic compounds, TSS, and anthocyanins has previously been documented (Ojeda et al., 2002; Van Leeuwen et al., 2004). The highest yield (48 Kg a tree) with high fruit quality was observed in seedless grape cultivars experiencing RDI under drought condition (Faci et al., 2014). According to the results obtained by Zabihi and Azarpajouh (2004) and Dolatibaneh and Norjo (2012), RDI probably decrease photosynthetic capability in leaves of grape; and as a result, it reduces (fruit) yield. In other research, it was shown that RDI increased berry quality and increase water use efficiency in grape trees Pinillos et al. (2016). Rabiei et al. (2003) pointed out that using RDI at the end of growth season significantly increased phenolic compounds in Merlot cultivar. With respect to mentioned issues, the current experiment was designed to investigate effects of different RDI treatments on yield and fruit quality of the three grape cultivars (i.e. Keshmeshi, Sahebi and Sharabi) during the onset of véraison to harvest under climate of Khorramabad province, Iran.

2. Materials and Methods

Vineyard site and experimental design

This experiment was conducted during the 2015 and 2016 growing seasons at semiarid climate in Lorestan province, Iran (47°E, 51°N, 1400 M). Ownrooted *Vitis vinifera* L. cvs. Keshmeshi, Sahebi and Sharabi were planted in 2007 by spacing of 3×2 m in N-S oriented row. Vines were trained to spur-pruned bilateral cordon with 6, 4 and 4 nodes per vine in 'Keshmeshi', 'Sahebi' and 'Sharabi', respectively according to flowering type. The vineyard was dripirrigated using pressure-compensated emitters (flow rate 4 Lh⁻¹). The vines were fertigated from June with a nutrient compound containing 150 kg ha⁻¹ N, 25 kg ha⁻¹ K, 6 L ha⁻¹ S and 4 kg ha⁻¹ Fe chelate.

Three RDI strategies were compared with the full irrigation practice (Control). Specifically, the control received full irrigation over fruit growth and development (ETc 100), whereas RDI treatments were: 80 % of ETc, 60% of ETc and 40% of ETc. RDI treatments were applied at the onset of berries color change (véraison) to harvest time. Daily metrological data were obtained from a database of Khoramabad's meteorology station located closely to the experimental vineyard and water requirement for each vine was calculated according to CROPWAT 8.0 software. Furthermore, irrigation scheduling was based on soil water holding capacity, daily crop water requirement, effective depth of root, percentage of wetted soil surface according to defined treatments (100%, 80%, 60% and 40% ETc) using the following relations.

$$In = \frac{FC - PWP}{100} . \rho_b . Dr. Pw. F$$
$$I_i = \frac{In}{K(ETc)}$$

where *In*= Net irrigation water content (mm), FC= Field capacity (%), PWP= Permanent wilting point (%), *pb*= Soil bulk density (gr.cm⁻³), *Dr*= effective depth of root (120 Cm) *I*= Irrigation interval (day), *K*= Operation Coefficient of RDI treatments, ETc= Plant water requirement.

All vines were equally irrigated based on their water requirement before starting RDI treatment. In this study, the drip irrigation system was equipped with two droplets adjusted manually for 4 Lh⁻¹ per vine. In order to access required pressure, a floating pump was used and the amount of water allocated to each irrigating turn was measured by water counter with 0.1 L accuracy. Directing water into each plot containing three grapevines was performed through stopcock water equipped in drip irrigation.

For each irrigation treatment, three replications were established according to a randomized block design. Each replication consisted of five lines of 60 vines each, and the experimental measurements were performed on 20 homogeneous vines from the 3 central lines chosen at the beginning of the study according to their trunk cross sectional area (TCSA).

Sampling and analysis

The berries were harvesting from 10 vines per each treatment and three samples for each vine, based on minimum maturity index of sugar content and berry color change. The mean total yield, clusters weight, number of berry per cluster and berry weight was measured from 10 vines for each treatment and three samples for vines. Soluble solid content (SSC) was measured by a digital refractometer (Model Eurromex RD). Titratable acidity (TA) was measured with a digital titrator in presence of 0.1 NaOH and pH was determined by a digital pH meter (Model Sclto TT).

Total phenolic was determined by using the Folin-Ciocalteau method (Brand-Williams *et al.*, 1995) with slight modification in a UV-Vis spectrophotometer (T80+, PG instruments Ltd). Briefly, 0.5 g of extract was mixed with 3 ml of methanol 85%, and then centrifuged for 10 min by 10000 per min. Then, 300 µl of diluted extract was mixed with 1.5 ml of diluted Folin-Ciocalteau reagent (1:10 in distilled water). After vortexing, 1.2 ml of 7.5% sodium carbonate was added and allowed to stand for 90 min at room temperature. The absorption at 760 nm was measured and results were expressed as gallic acid equivalents (GAE) per 100 g fresh weight.

Total flavonoids content were determined spectrophotometerically according to the Zarrouk *et al.* (2012). Catechin was used as a standard. The flavonoids content were expressed as mg catechin equivalents (RE) per 100 g FW.

Total antioxidant capacity (TAC) was measured

using the 2, 2 diphenyl-1-pic-rylhydrazyl (DPPH) radical scavenging method described by Brand-Williams *et al.* (1995) with slight modification. The amount of 75 μ l of fruit extract was mixed with 2925 μ L 0.1 mol L⁻¹ DPPH in methanol. After incubating at room temperature for 30 min in the dark, the absorbance of the mixture was measured at 517 nm using UV-Vis spectrophotometer. For each sample, three separate determinations were recorded. Antioxidant activity was expressed as the percentage decline in absorbance in comparison with the control, corresponding to the percentage of DPPH scavenged (% DPPHsc), which was calculated as:

%DPPHsc = [(Acontrol-Asample)/Acontrol]× 100

Data analysis

The results obtained for each irrigation treatment were compared using ANOVA and when significant differences were found, Duncan's test was used to identify differences in mean values. Data from each year were analyzed separately; all analyses were performed with SAS 17.0.

3. Results and Discussion

Fruit yield

The results of ANOVA showed that simple effect of RDI on total yield at first year was significant (p<0.01). Compared to controls, 80% ETc did not show a significant effect on yield at first year, whereas applying 40% and 60% ETc reduced fruit yield by 33% and 48%, respectively. Also, it was found that Sahebi in comparison to Sharabi gained higher fruit yield at first year (Table 1). The results of this experiment showed that the interaction effect of Cultivar × RDI had a significant effect on yield in second year of experiment (Fig. 1). In 'Keshmeshi' and 'Sharabi', using 40% ETc significantly resulted in a yield reduction, while 40% and 60% ETc decreased fruit yield in Sahebi. Santesteban and Mirandam Royo (2011) and Di Vaio et al. (2001) showed that RDI brought about a reduction in grape yield, although they proposed RDI as a useful approach to enhance quality and quantity of fruits in the grapes grown especially in subtropical climate. On the contrary, Dolatibane and Norjo (2012) and Zabihi and Azarpajouh (2004) stated that RDI could reduce grape yield. The negative effect of RDI on fruit yield is associated with its harmful effect on photosynthesis activities and consequently supplying required assimilates for berry's formation

Season	Treatment	Levels	Yield (kg vine ⁻¹)	Berry weight (g)	SSC (%)	TA (%)	рН
2015	RDI	Control	23.70 a	1.11 a	17.55 a	0.68 a	3.51 a
		80% ETc	21.75 a	1.08 a	17.18 a	0.69 a	3.40 a
		60% ETc	15.90 b	0.89 ab	17.24 a	0.72 a	3.48 a
		40% ETc	12.24 d	0.72 b	17.12 a	0.66 a	3.48 a
		Significance	**	**	NS	NS	NS
	Cultivar	Keshmeshi	19.47 ab	0.60 b	19.84 a	0.78 a	3.35 b
		Sahebi	22.77 a	1.28 a	14.15 b	0.6 2b	3.59 a
		Sharabi	15.20 b	0.98 a	17.83 a	0.67 b	3.46 ab
		Significance	**	**	**	**	**
2016	RDI	Control	24.40 a	0.96 a	20.25 a	0.66 a	3.50 a
		80% ETc	23.56 ab	0.92 a	21.31 a	0.64 a	3.47 a
		60% ETc	21.82 b	0.78 b	20.33 a	0.68 a	3.45 a
		40% ETc	18.71 c	0.62 c	21.67 a	0.67 a	3.42 a
		Significance	**	**	NS	NS	NS
	Cultivar	Keshmeshi	17.31 b	0.57 b	24.50 a	0.75 a	3.37 b
		Sahebi	29.02 a	0.99 a	17.95 c	0.59 b	3.59 a
		Sharabi	20.04 b	0.90 a	20.22 b	0.65 ab	3.41 b
		Significance	**	**	**	**	**

Table 1 - The effects of regulated deficit irrigation (RDI) on fruit yield and quality of grape cultivars

NS= not significant, ** significant at p<0.01, * significant at p<0.05.

(Conesa *et al.*, 2016). Also, a remarkable increase in transpiration rate of berries under severe drought stress can be accounted for losing berries weight and lowering grape yield. Conesa *et al.* (2016) reported that RDI had not a significant effect on grape yield and RDI-experiencing plants had the same yield as controls. In this study, we also didn't find any significant differences between 80% RDI and control during two consecutive growing seasons.



Fig. 1 - Effect of regulated deficit irrigation (RDI) on fruit yield of three grape cultivars in the second year. Bars represent standard error of three replicates. Values with the different letters are significantly different according to Duncan's Multiple Range Test at P<0.05.</p>

Berry weight and cluster weight

The results of ANOVA showed that berry weight was significantly affected by simple effects of cultivar and RDI (p<0.01). The interactional effect of Cultivar × RDI had a significant effect on cluster weight in

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both years of experiment (p<0.01). In general, the weight of grape berry and cluster was significantly decreased as the percentage of RDI was gradually decreased (from 100% to 40% ETc) in both experimental years. Comparing to controls, although applying 40% and 60% ETc reduced cluster weight of 'Keshmeshi' at first year, all RDI treatments did not render a reduction in cluster weight of Keshmeshi in second year of experiment. In 'Sahebi', cluster weight was diminished by applying 40% and 60% ETc at first year, whereas by just 40% ETc in second year of experiment. In this regard, all RDI treatments reduced berry and cluster weight of Sharabi while 80% ETc did not significantly reduce cluster weight in Sharabi only in second year (Fig. 2). The results of this research revealed that effect of RDI on berry and cluster weight is strongly correlated to cultivars. According to Coombe (1992), change in berry's water status under drought is directly related with change in berry weight. Rabiei et al. (2003) figured out that RDI may be responsible for a reduction in berry weight in Merlot cultivar. In this regard, Dolatibaneh and Norjo (2012) stated that the highest berry weight was obtained at full irrigation; and, in spite of finding non-significant difference between 50% and 75% ETc treatments, they concluded that berry weight is often depressed under RDI performance. As compared to controls, the cell size of fruit experienced different RDI regimes reduced due to reduction in vegetative growth, leaf area, and photosynthesis products, and this probably led to reduction in berry and cluster weight (Ezzhaouani *et al.*, 2007). In current research, the grape cultivars had different responses to RDI especially at 40% and 60% ETc regimes. Regarding these results, different responses to RDI treatments suggests the sensitivity of different cultivars to drought stresses.



Fig. 2 - Effect of regulated deficit irrigation (RDI) on berry weight of three grape cultivars in the second year. Bars represent standard error of three replicates. Values with the different letters are significantly different according to Duncan's Multiple Range Test at P<0.05.</p>

Berry length and diameter

The data presented in figure 3 showed a significant interaction effect of cultivar × RDI on traits of berry length and diameter. In both years of experiment, 40% ETc reduced berry length and diameter, whereas 80% ETc did not have a significant effect on



Fig. 3 - Effect of regulated deficit irrigation (RDI) on rachis weight of three grape cultivars in two growing seasons. Bars represent standard error of three replicates. Values with the different letters are significantly different according to Duncan's Multiple Range Test at P<0.05.</p>

both of them. Accordingly, the cultivar response to RDI was found different (Fig. 3). Under RDI performance, the cell size was lower than that of at full irrigation. Due to reduction in the vegetative growth and leaf area of RDI-subjected plants, the size of berry and photosynthetic products were reduced (Deluc *et al.*, 2009). Zabihi and Azarpajouh (2004) reported that water deficiency resulted in reduction in the size of berry, which is in agreement with our findings.

Total soluble solid

The results of this research revealed that only simple effect of cultivar on TSS was significant in both years of experiment, and the highest and lowest TSS were found in 'Keshmeshi' and 'Sahebi', respectively (Table 1). Contrary to Dolatibaneh and Norjo (2012) who stated that RDI had a positive effect on TSS of grape fruit, Lanari et al. (2014) figured out a non-significant effect of RDI of TSS in grape fruit. According to previous research, mild drought may stimulate ABA production in favor of increasing some sugar production through different ways in order to cope with stressful condition. In this respect, some researchers pointed out that water deficiency could reduce photosynthesis activities and consequently constrict sugar production, but whenever water deficiency is exacerbated even fruits may not ripe properly and this probably affects TSS status in fruits (Dolatibaneh and Norjo, 2012).

Titratabe acidity and pH

The results of this experiment demonstrated that only cultivar treatment had a significant effect on TA at first year of experiment, and additionally Sahebi and Sharabi obtained the lower TA as compared to Keshmeshi cultivar. Contrary to the results obtained at first year of experiment (Table 1), an interaction effect of cultivar × RDI had a significant effect on TA in second year of experiment. Although, RDI did not have effect on TA in 'Keshmeshi', RDI at 60% ETc significantly increased TA in Sahebi cultivar. In addition, applying 40% and 60% ETc caused a reduction in TA of Sharabi cultivar (Fig. 4).

In both years of experiment, pH was only affected by cultivar; and highest pH was observed in Sahebi as compared to the two other cultivars. Dolatibaneh and Norjo (2012) pointed out that RDI could decline the rate of organic acid of grape fruit. During cluster ripping, coinciding with rising environmental temperature, drought stress is able to decrease acidity of berry and accordingly paves the way for minimizing the rate of TA in grape berries. Lanari *et al.* (2014) reported that RDI did not have a significant effect on TA, whereas findings of Zabihi and Azarpajouh (2004) showed water availability increased organic acids in grape berries.



Fig. 4 - Effect of regulated deficit irrigation (RDI) on titratable acidity percentage of three grape cultivars in second year. Bars represent standard error of three replicates. Values with the different letters are significantly different according to Duncan's Multiple Range Test at P<0.05.</p>

Total phenol

The results of this experiment revealed that the interaction effect of cultivar × RDI had a significant impact on total phenol (Fig. 5). Comparing to controls, applying only 80% ETc increased total phenol in 'Keshmeshi' while applying all RDI treatments in Sahebi enhanced total phenol in both years of experiment. In Sahebi, the highest amount of phenol was found by employing 40% (at first year) and 80% (in



Fig. 5 - Effect of regulated deficit irrigation (RDI) on total phenol of three grape cultivars during two seasons. Bars represent standard error of three replicates. Values with the different letters are significantly different according to Duncan's Multiple Range Test at P<0.05.</p>

second year) ETc treatments. The results also revealed that using 40% and 60% ETc (at first year) as well as 60% and 80% ETC (in second year) had significantly affected total phenol in Sharabi.

Rabiei et al. (2003) reported that applying RDI at the end of growth season significantly increased phenol content of Merlot comparing to controls, which is in agreement with our findings. A prolonged drought stress was found to significantly decrease total phenol and other phenolic compounds such as frolic acid, coumaric acid, and caffeic acid (Krol et al., 2014). By investigating effects of different RDI regimes on phenolic compounds in Shirazi cultivar, Ojeda et al. (2002) demonstrated that RDI, dependent on type of phenolic compounds, may change the amount of phenol in grape berries. In this regard, some factors including type of cultivar, climate, soil, media culture, maturely period, and yield are effective on the amount of phenols and other biocompounds in grapes (Deluc et al., 2009; Bindon et al., 2011; Zarrouk et al., 2012). In our research, RDI treatments had different impacts on phenol; and the results of this research, dependent on type of cultivar, suggest that an appropriate RDI can increase total phenol in grape berries.

Flavonoids

The results of this research revealed that flavonoid in berries was influenced by RDI treatments. In general, applying 60% and 80% ETc significantly increased flavonoid in all the three cultivars. As compared to other cultivars, 'Keshmeshi' was lowly affected by RDI, in a way that 60% ETc at first year and 80% ETc in second year of experiment significantly enhanced flavonoid content in berries. In contrast, applying 80% and 60% ETc at first year and 40%, 60%, and 80% ETc in second year significantly increased flavonoid content in Sahebi. As compared to the second year of experiment, the rate of flavonoid in Sharabi was significantly elevated by all RDI treatments at first year of experiment relative to controls (Fig. 6). In grape berries, flavonoids are the abundant secondary metabolites influencing wine's physical features (especially color and astringency) (Brillante et al., 2017). The biosynthesis of such secondary metabolites is highly affected by environmental stresses (Kuhn et al., 2013). The previous research has demonstrated that regulating vineyard irrigation is efficiently able to increase these metabolites (Kennedy et al., 2002). Similarly, findings of Zarrouk et al. (2012) revealed that RDI significantly increased flavonoid compounds in grape's Aragonez cultivar in

the two subsequent years, which are in consistent with ours. Deluc *et al.* (2009) reported that effect of RDI on flavonoid content of Chardonnay and Cabernet Sauvignon cultivars was different, in a way that its content in former cultivar increased while in latter one decreased. In current research, drought stress at 60% and 80% ETc raised flavonoid content in the all grape cultivars and this manifests the positive effect of RDI on increasing grape quality.



Fig. 6 - Effect of regulated deficit irrigation (RDI) on flavonoid content of three grape cultivars on both seasons. Bars represent standard error of three replicates. Values with the different letters are significantly different according to Duncan's Multiple Range Test at P<0.05.</p>

Total antioxidant capacity

The results of ANOVA revealed that interactional effect of cultivar × RDI had a significant effect on total antioxidant capacity (TAC) in both years of experiment (Table 1). As compared to full irrigation, applying 60% and 80% ETc at first year of experiment significantly increased TAC in 'Keshmeshi' and 'Sahebi'. In second year of experiment, all RDI treatments interestingly enhanced TAC in Sharabi relative to controls. Moreover, TAC was increased by using 60% and 80% ETc in Keshmeshi, as well as 60% and 80% ETc in Sahebi, and just 80% ETc in Sharabi (Fig. 7). In a similar way, Soukhtesaraee et al. (2017) figured out that drought stress significantly increased TAC in Chefta and Yaghoti cultivars, but did not affect Bidanehsefid. Tangolar et al. (2015) showed that RDI increased TAC at different growth stages of Razaki cultivar.

Comparing to full irrigation, RDI depending upon some factors such as time of applying RDI and type of cultivar diminished TAC in leaf and root of Kiszmisz cultivar (Krol *et al.*, 2014). Plant materials possessing antioxidant properties surely contain different phenolic compounds. The antioxidant properties of these materials, due to their redox ability and chemical structures are able to neutralize free radicals through forming a complex with metal ions (Krol *et al.*, 2014). In many previous research, the strong correlation between TAC and phenolic content were reported (Tangolar *et al.*, 2015). In our research, a positive relationship was found between phenol and TAC.



Fig. 7 - Effect of regulated deficit irrigation (RDI) on antioxidant capacity of three grape cultivars on two seasons. Bars represent standard error of three replicates. Values with the different letters are significantly different according to Duncan's Multiple Range Test at P<0.05.

Anthocyanin

The results of this research showed that the interaction effect of cultivar × RDI had a significant effect on anthocyanin content in both years of experiment (Table 1). Comparing to controls, all the three RDI treatments increased anthocyanin content in Keshmeshi cultivar. In other words, applying 40%, 60%, and 80% ETc at first year and 60% and 80% ETc in second year increased anthocyanin contents in Sahebi (Fig. 8). Anthocyanins are a class of phenolic compounds responsible for generating black and red colors in grape epidermis. These compounds possess powerful antioxidant property and also play a significant role in physiological process. According to previous study, water shortage can strongly influence anthocyanin accumulation in grape cultivars (Ozden et al., 2010; Kyraleou et al., 2016). Also, Bindon et al. (2011) reported that an increase in time of irrigation could remarkably hinder anthocyanin biosynthesis. So far, the effect of drought stress on reduction in canopy's density in favor of entrance more light radiation into tree canopy has been reported. Through this, the light quantitatively required to anthocyanin biosynthesis is supplied (Tangolar et al., 2015). Previous research showed that RDI, through influencing physiological and hormonal responses, changed the pathway of anthocyanin biosynthesis (Shellie, 2011; Nelson et al., 2015). In present research, it was found that RDI significantly increased anthocyanin accumulation of Iranian grape cultivars in both years of experiment, although the cultivar responses towards RDI were various.



Fig. 8 - Effect of regulated deficit irrigation (RDI) on anthocyanin content of three grape cultivars on two seasons. Bars represent standard error of three replicates. Values with the different letters are significantly different according to Duncan's Multiple Range Test at P<0.05.

4. Conclusions

Overall, the results of this research revealed the positive effect of RDI (especially 80% ETc) on yield and some fruit qualities in Keshmeshi, Sahebi, and Sharabi cultivars. In this regard, RDI significantly increased not only yield but also some fruits' qualitative features like anthocyanin, phenol, flavonoid, and total antioxidant capacity in comparison to controls. Therefore, 80% ETc can be advised to maintain and increase fruit qualities of Keshmeshi, Sahebi, and Sharabi cultivars under regional conditions.

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