# Effects of water deficit regimes on yield components and berry composition of 'Black Kishmish' under Mediterranean region

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**Abstract.** The effects of different irrigation treatments on yield components and berry composition of 'Black Kismish' table grape were investigated in this study. Research was carried out in the experimental vineyards of Viticulture Research Institute, Manisa, Türkiye. Research took place for three consecutive (2015-2016-2017) years and three treatments were compared: T100 (control) irrigated to the field capacity, T65 and T35 irrigated 65% and 35% of T100 treatment, respectively. It was determined that yield value obtained by Full Irrigation was 11% and 18% higher than T65 and T35, respectively. In addition, some of the bio-active compounds such as total anthocyanin content, total phenolic compounds, total flavonoid content, antioxidant capacity and maturity parameters were increased with water stress. Also, color index of red grapes increased with deficit irrigation treatments. As a result, to ensure optimum yield and quality in Manisa conditions, it was determined that table grape growing without irrigation was not suitable and the irrigation requirement of the grapevine should be fully supplied during the growing season for high grape yield. When the water footprint and functional food concepts were taken into consideration, water deficit treatments (T65 and T35) became prominent due to the use of less water and higher nutrient content of the grapes obtained.

# **1** Introduction

Today, the distribution of main viticultural regions is expressed by the isotherms of the average growing season temperatures of 12°-13 °C (lower threshold) and 22°-24 °C (upper threshold) [1]. Viticultural activities are carried out successfully in 94 countries and 6.8 million ha area. Turkey ranks 5<sup>th</sup> among the world countries in terms of vineyard area with approximately 3.9 million decare and ranks 6<sup>th</sup> in terms of fresh grape production with 3.7 million tons [2].

It is very important to develop sustainable production techniques to increase food safety and quality in table grape production, which constitutes almost half of the total grape production in Turkey [3]. However, global climate change threatens sustainability by affecting grape production and quality attributes. In previous studies, it has been stated that climatic changes directly and indirectly affect many parameters such as grape yield, berry composition, quality, vine physiology, phenology, morphology and vegetative development [4-6]. In this regard, it has been stated in many studies that the countries in the Mediterranean basin will be adversely affected by the global climate change [7-9]. Also, it is indicated that climatic changes, with the increment in temperature and decrement in precipitation regime will significantly affect the irrigation-based Mediterranean agriculture [10]. Therefore, alternative modern irrigation strategies that increase water use efficiency are needed. Additionally, it is necessary to accurately estimate the water requirement and irrigation time of the vines [11,12].

For this reason, regulated deficit irrigation (RDI) and partial rootzone drying (PRD) techniques, which reduce water consumption according to the phenological stages of the vine, can increase water use efficiency and improve grape quality [13]. For instance, [14] expressed that RDI effects berry phenolic composition, soluble solids and anthocyanins positively. Furthermore, it is determined that PRD can help controlling excessive vine vigor and improving quality attributes [15]. On the other hand, it has been stated that the effects caused by water stress vary according to the stress level, duration, phenological period of the grapevine, climatic and soil conditions, variety-rootstock combination [16-18].

Therefore, it has become inevitable to take the necessary measures against the expected water scarcity because of climate change. In this regard, it is necessary to investigate the reactions of grape varieties under stress conditions, especially in countries located in the Mediterranean basin. Therefore, this study aimed to examine the effects of restricted irrigation practices on yield components and berry composition in Black Kishmish variety grown under field conditions in semiarid region of Turkey.

## 2 Materials and methods

#### 2.1 Plant material and site description

This study was performed in a Black Kishmish vineyard of Viticulture Research Institute, Manisa, Turkey during two consecutive seasons (2016 and 2017). The experimental area is located at lat. 38° 37'N; long. 27° 24' E, 40 m above sea level. The vineyard was planted in Y shaped trellis system 2011 and plant material correspond to Black Kishmish grafted on 1103 Paulsen rootstock. Vine spacing is 3 m between rows and 2 m between vines. All vines were cane-pruned with each cane approximately 10-12 nodes in length and the orientation is N to S. The experimental area is loamy textured with a depth of 0-90 cm. Additionally, the same agronomic activities were performed to all plots in an aim to table grape growing during the experimental years.

#### 2.2 Experimental design and measurements

Subsurface drip irrigation system was used to impose three treatments: (i) full irrigation (T100), irrigated to the field capacity; moderate deficit irrigation (T65), irrigated at 65% of full irrigation; severe deficit irrigation (T35), irrigated at 35% of full irrigation. When the available water decreased 50% in the effective root depth, irrigation treatments started and performed weekly. All treatments were applied in a randomized block design with three replicates. Each replicate consisted of six vines.

Soil moisture was regularly monitored by AquaCheck Basic Wireless probe in the experiment. For this purpose, access tubes, which were previously installed in the plots, were used and soil moisture levels were observed from the soil surface to a depth of 90 cm. Also, soil water content (SWC) was determined weekly by gravimetry analysis before the irrigation day. The amount of irrigation water was calculated according to the following equation:

$$I = (FC-SWC) \times A \times P \tag{1}$$

In this equation, I is the amount of irrigation water (mm); FC is the soil water content at field capacity (mm); SWC is the pre-irrigation soil water content (mm); P is the wetting percentage (35%) and A is the surface area of the plot ( $m^2$ ). Crop water use or evapotranspiration (ET) was calculated with the water balance equation:

$$ET = I + P \pm \Delta SW - Dp - Roff$$
(2)

Where ET is evapotranspiration (mm); I is amount of irrigation water (mm); P is precipitation (mm);  $\Delta$ SW is the change in the soil water content (mm); Dp is deep percolation (mm) and Roff is amount of runoff (mm). Dp and Roff were assumed to be negligible. On the other hand, water use efficiency (WUE) was calculated as grapevine yield divided by seasonal ET [19].

Midday leaf water potential ( $\Psi_{md}$ ) was measured using the pressure chamber (Skye Instrument Co., UK) as described by [20]. Mature, healthy, and fully expanded leaves were selected from three vines from each replicate and measurements were carried out between 12:00 and 13:30 hr. All measurements were conducted before the irrigation day in each week.

Total yield values (kg vine<sup>-1</sup>), cluster weight (g), cluster number, cluster width and length (cm) were determined at harvest. Moreover, 100 berries were sampled from each replication to determine 100 berry weight (g). Additionally, berry width and berry length (mm) were measured by using caliper compass and color measurements (L, a, b, Chr, Hue) were made by Minolta Co (CR-300). CIRG Index (Color Index of Red Grapes) was estimated according to the [21]. Berries were squeezed for each replication and the total soluble solids (TSS,%) was measured by the Refractometer. On the other hand, juice pH was measured by pH meter and total titratable acidity (g L<sup>-1</sup>) was measured using official methods of the International Organisation of Vine and Wine [22]. Total phenolic content (mg kg<sup>-1</sup>) of the grapes was determined by the Folin-Ciocalteu method and total flavonoid (mg kg<sup>-1</sup>) was estimated using the aluminum chloride colorimetric assay. Antioxidant capacity (EC<sub>50</sub>) was determined according to the DPPH method.

The results were analyzed with SPSS statistical program and Duncan multiple comparison test was used to show the differences or similarities between the samples.

## **3 Results and Discussion**

#### 3.1 Phenological development stages and soilvine water status

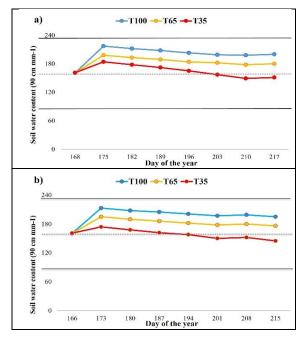
The dates of the phenological stages were recorded during the experimental years. According to the phenological observations (Table 1), bud break (EL-05), flowering (EL-23), berry set (EL-27) and veraison (EL-35) occurred earlier in the first year of the trial compared to the second year. However, harvest (EL-38) dates were recorded as 02 August in both 2016 and 2017 growing seasons.

**Table 1.** Phenological (EL) stages of Black Kishmishgrapevines during the experimental years.

Year	Bud break (EL-05)	Flowering (EL-23)	Berry set (EL-27)	Veraison (EL-35)	Harvest (EL-38)
2016	9 Mar	3 May	9 May	5 Jul	2 Aug
2017	18 Mar	11 May	20 May	11 Jul	2 Aug

Soil water content (SWC) varied depending on irrigation treatments in the study. SWC remained above 50% of the available water under T100 and T65 treatments while it decreased below 50% of the available water under T35 treatment in both growing seasons (Fig. 1). In the study, field capacity (FC) and wilting point (WP) values were found 233 mm and 88 mm, respectively. Also, the amount of available water was estimated 145 mm. In this regard, SWC values were found between FC and WP during the experimental seasons. Therefore, it shows that monitoring of SWC was performed accurately in the experiment.

Amount of irrigation water for Black Kishmish varied between 82 mm and 285 mm in the experimental years. In the case of the T100 treatment, highest water applied to the vines and irrigation water ranged between 233 mm and 285 mm depending on climatic conditions (Table 2).



**Figure 1.** Variation of soil water content under different irrigation treatments in 2016 (a) and 2017 (b) seasons.

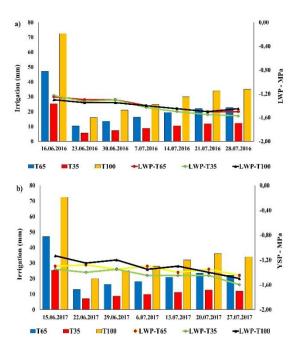
According to the (Table 2), higher evapotranspiration (ET) values were obtained with the increment of irrigation water. Therefore, highest ET values were determined under T100 treatment and it ranged between 396 mm to 444 mm during the study. Moreover, highest ET values were found between berry set (EL-27) and veraison (EL-35) stages for all treatments during the experiment. The reason of higher ET values between these stages may be due the higher temperature values in the growing season. Additionally, the highest water use efficiency (WUE) value was found under T35 in 2016 (5.07 kg m<sup>-3</sup>) and 2017 (5.57 kg m<sup>-3</sup>) seasons.

Table 2. Amount of seasonal irrigation water (mm), evapotranspiration (mm) and water use efficiency (kg  $m^{-3}$ ) in experimental years.

Seasonal irrigation (mm)								
	T100	T65	T35					
2016	233	152	82					
2017	285	186	100					
Average	259	169	91					
	ET (mm)							
	T100	T65	T35					
2016	444	330	232					
2017	396	315	245					
Average	420	322.5	238.5					
	WUE (k	g m <sup>-3</sup> )						
	T100	T65	T35					
2016	4,13	4,47	5.07					
2017	3,56	4,56	5.57					
Average	3.85	4.52	5.32					
ET: evapotranspiration; WUE: water use efficiency								

During the study, more negative midday leaf water potential  $(\Psi_{md})$  values were obtained under reduced

irrigation treatments (Fig. 2). In 2016 and 2017, the lowest midday leaf water potential values (highest stress level) were determined in T35 treatment with -1.57 MPa and -1.60 MPa, respectively. In addition, the more negative  $\Psi_{md}$  values were found at the end of the season for all irrigation regimes compared to the beginning of the season. This situation can be explained by the higher evaporative demand of the environment at the end of the season [23]. On the other hand, previous studies expressed that more negative  $\Psi_{md}$  values were observed during post-veraison in comparison to the pre-veraison period [24, 25].



**Figure 2.** Variation of midday leaf water potential ( $\Psi_{md}$ ) values according to the seasonal irrigation water (mm) in 2016 (a) and 2017 (b) seasons.

In the study, hourly measurements were carried out throughout the day in veraison period with a view to reveal the variation of leaf water potential values of Black Kishmish variety. For this purpose, leaf water potential measurements were started in the early hours of the day with pre-dawn leaf water potential ( $\Psi_{pd}$ ) measurements, and continued throughout the day at one-hour intervals until last measurements at around 17:00 hours. In this regard, in 2016, maximum stress levels were determined between 11:00-12:00 hours under T100 and T65 treatments while it was between 14:00-15:00 hours under T35 treatment. On the other hand, in 2017, the highest stress level occurred between 13:00 and 14:00 hour under all irrigation treatments (Fig. 3).

Significant differences ( $p \le 0.05$ ) were determined for the yield in both 2016 and 2017 seasons. In 2016, highest yield was obtained under T100 with 9.79 kg vine<sup>-1</sup>. In 2017, T100 had the highest yield and was statistically in the same level group with T65. Also, T35 had the lowest yield values in both experimental years. Therefore, current study showed that decrement of irrigation amount had negative impacts on yield attributes of Black Kishmish table grape variety in Aegean Region, Turkey. Among all treatments, there were no significant differences for the cluster number, cluster weight, cluster width and length in the experimental years (Table 3).

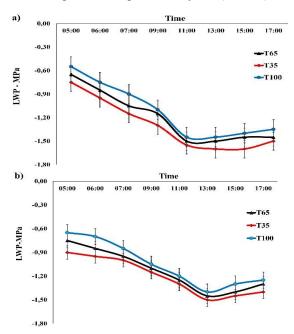


Figure 3. Variation of midday leaf water potential ( $\Psi_{md}$ ) values throughout the day in veraison period of 2016 (a) and 2017 (b) seasons.

there were significant (p < 0.05)However. enhancement of 100 berry weight in 2017. Therefore, an increment of berry weight with the increasing of irrigation amount was determined especially in 2017. [16] indicated that most irrigated treatment had higher yield and berry weight values compared to the deficit irrigation (Table 3). Irrigation regimes had statistically significant effect ( $p \le 0.05$ ) on TSS and pH values in both 2016 and 207 seasons. The highest TSS values were obtained under T35 treatment with 22.23% in 2016 and 20.46% in 2017. In addition, significant differences were found among irrigation treatments on the TA and MI values in 2017 season. The highest TA value was observed under T100 while highest MI value was determined under T35 treatment (Table 4). In the current study, the deficit irrigation leading to water stress gradually in the vines, decreased the TA but caused the TSS to increase. These findings were found similar with previous studies [25, 26].

According to the (Table 5), color parameters were affected differently by irrigation treatments in both years. Particularly color index of red grapes (CIRG index) was influenced statistically significant by irrigation regimes in both 2016 and 2017 seasons. T35 had the highest CIRG index value with 6.73 in 2016. But T35 and T65 was statistically in the same level group in 2017 with 7.08 and 7.80, respectively. It was observed that better coloration was obtained with deficit irrigation treatments in both growing seasons. According to the [21], red grapes were categorized in different groups regarding to their external color such as green-yellow (CIRG<6) and blue-black

(CIRG>6). As can be seen in (Table 5), CIRG index values were found in the blue-black group (CIRG>6) with the deficit irrigation treatments.

Total anthocyanin, total phenolic content, total flavonoid and antioxidant capacity of grapes under different irrigation regimes are given in (Table 6). Total anthocyanin values were significantly different at  $p \le 0.05$ in 2016 and 2017. Also, total anthocyanin values varied between 304.61-401.97 mg kg-1 in 2016 and 423.33-500.31 mg kg<sup>-1</sup> in 2017. As water stress increased, anthocyanin values also increased, with the highest values under T35. The effects of treatments on total phenolic content were significant at the level of 5% least significant difference in 2016 while non-significant in 2017. In this regard, T35 had 4.44% and 12.12% higher total phenolic content compared to the T65 and T100 in 2016 season. On the other hand, deficit irrigation regimes showed statistically higher total flavonoid values in the experiment. In the first year, T35 had the highest value with 554.41 mg kg<sup>-1</sup>. But in the second year, T35 and T65 were statistically in the same level group with 395.44 and 373.48 mg kg<sup>-1</sup> which were higher in comparison to the T100. Furthermore, the effects of irrigation treatments on antioxidant capacity were statistically significant  $p \le 0.05$ during the experiment. EC<sub>50</sub> values represent the antioxidant substance concentration that inhibits 50% of DPPH radicals, and low EC<sub>50</sub> value show that the amount of antioxidant substances is high. In this regard, the lowest values were determined under T35 in 2016 and 2017 seasons. Therefore, the highest antioxidant levels were obtained in T35 treatment. Our findings show that deficit irrigation treatments stimulate total anthocyanins, total phenolic content, total flavonoid and antioxidant capacity. These results were in agreement with [26-29].

## 4 Conclusion

The results of the experiment revealed that deficit irrigation regimes affected yield components and berry composition of Black Kishmish table grape. The study showed the yield values were increased with the increment of irrigation amount but T100 and T65 were found in the same level group in the second year of the study. On the other hand, deficit irrigation regimes had higher TSS values. Therefore, early maturation (3-7 days) in harvest were observed under deficit irrigations. Regarding that earliness is an advantage in terms of marketing, this result is considered to be important. Moreover, deficit irrigation treatments induced enhancement of berry composition particularly in terms of bioactive compounds such as anthocyanins, phenolic content, flavonoids and antioxidant capacity. Consequently, it was understood that deficit irrigations improved berry composition and quality attributes of Black Kishmish while causing lower yield components. However, considering that the T100 and T65 treatments were statistically at the same level in terms of yield parameter in the second year of the study, it was determined that the T65 could be more applicable due to the improving quality attributes and compensating yield loss.

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Table 3. Yield components of Black I	Kishmish grapevines under diffe	rent irrigation regimes	during the experimental years.
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Years	Treatments	Yield (kg vine <sup>-1</sup> )	Cluster number	Cluster weight (g vine <sup>-1</sup> )	Cluster width (cm)	Cluster length (cm)	100 berry weight (g)
	T65	8.42ab	19.56	431.65	10.83	17.33	216.02
2016	T35	7.43b	18.67	398.71	10.50	16.67	211.59
2010	T100	9.79a	20.83	469.41	12.00	18.33	223.98
	р	*	ns	ns	ns	ns	ns
	T65	8.28a	19.33	432.16	12.00	18.43	164.2c
2017	T35	7.25b	18.78	386.88	12.36	17.74	198.03b
2017	T100	8.60a	20.22	427.60	11.04	19.64	247.71a
	р	*	ns	ns	ns	ns	*
*: signi	*: significant effect at $p \le 0.05$ . ns: non-significant						

Table 4. Values of total soluble solids (TSS). titratable acidity (TA). pH and maturity index (MI) determined in a berry must under different irrigation regimes during two growing seasons.

Years	Treatments	TSS (%)	TA (g L <sup>-1</sup> )	pН	Maturity Index		
	T65	20.93b	4.46	3.45b	47.38		
2016	T35	22.23a	4.52	3.46b	49.32		
2010	T100	19.43c	4.54	3.58a	42.78		
	р	*	ns	*	ns		
	T65	18.33b	5.05b	36.26b	3.52ab		
2017	T35	20.46a	5.17ab	39.55a	3.59a		
2017	T100	17.96b	5.29a	33.93c	3.46b		
	р	*	*	*	*		
*: significant effect at $p \le 0.05$ . ns: non-significant							

Table 5. Effects of irrigation treatments on L. a. b. CHR. HUE and CIRG Index values during 2016 and 2017 growing seasons.

Years	Treatments	L	a	b	CHR	HUE	CIRG Index
	T65	30.26a	3.08	-1.88	4.91a	329.90	6.01b
2016	T35	27.63b	3.35	-1.93	3.60b	329.37	6.73a
2010	T100	30.32a	3.68	-1.57	4.72a	332.27	5.79b
	р	*	ns	ns	*	ns	*
	T65	29.35	0.71a	0.70	1.77	126.71	7.80a
2017	T35	29.89	-0.89b	0.77	1.56	143.63	7.08a
2017	T100	28.96	-0.73b	1.16	1.67	122.03	4.10b
	р	ns	*	ns	ns	ns	*
*: significant effect at $p \le 0.05$ . ns: non-significant							

**Table 6.** Effects of different irrigation regimes on total anthocyanins. Total phenolic content. Total flavonoid and antioxidant capacity of Black Kishmish in both experimental years.

Years	Treatments	Total anthocyanin (mg kg <sup>-1</sup> )	Total phenolic content (mg kg <sup>-1</sup> )	Total flavonoid (mg kg <sup>-1</sup> )	Antioxidant capacity (EC50)
	T65	359.23b	1405.87b	495.09b	13.47a
2016	T35	401.97a	1468.26a	554.41a	11.49b
2010	T100	304.61c	1309.53c	459.21c	13.73a
	р	*	*	*	*
	T65	449.52b	1238.67	373.48a	10.71b
2017	T35	500.31a	1302.93	395.44a	9.09c
2017	T100	423.33c	1202.41	315.83b	13.42a
	р	*	ns	*	*
*: sign	ificant effect a	t <i>p</i> ≤0.05. ns: non-sigi	nificant		

## References

- 1. H.R. Schultz, and G.V. Jones, J. Wine Res. **21**(2-3), 137-145 (2010)
- 2. FAOSTAT, Food and Agriculture Organization of the United Nations Statistics Division (2021)
- 3. A. Altındişli, 1. Ulusal Sarıgöl İlçesi ve Değerleri Sempozyumu Bildiriler Kitabı (2011)
- H. Fraga, M. Amraoui, A.C. Malheiro, J. Moutinho-Pereira, J. Eiras-Dias, J. Silvestre and J.A. Santos, Eur. J. Remote Sens. 47(1), 753-771 (2014)
- H. Fraga, I. García de Cortázar Atauri, A.C. Malheiro and J.A. Santos, Glob. Chang Biol. 22(11), 3774-3788 (2016)
- J.A. Santos, H. Fraga, A.C. Malheiro, J. Moutinho-Pereira, L.T. Dinis, C. Correia, M. Moriondo, L. Leolini, C. Dibari, S. Costafreda-Aumedes, T. Kartschall, C. Menz, D. Molitor, J. Junk, M. Beyer and H.R. Schultz, Appl. Sci. 10(9), 3092 (2020)
- H. Fraga, A.C. Malheiro, J. Moutinho-Pereira and J.A. Santos, Int. J. Biometeorol. 57(6), 909-925 (2013)
- 8. H.R. Schultz, J. Wine Econ. 11(1), 181-200 (2016)
- C. Van Leeuwen and P. Darriet, J. Wine Econ. 11(1), 150-167 (2016)
- 10. IPCC, Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, 1535 (2013)
- D.S. Intrigliolo, V. Lizama, M.J. García-Esparza, I. Abrisqueta, I. Álvarez, Agric. Water Manag. 170, 110-119 (2015)
- M. Permanhani, J.M. Costa, M.A.F. Conceição, R.T. de Souza, M.A.S. Vasconcellos, M.M. Chaves, Theor. Exp. Plant Physiol. 28(1), 85–108 (2016)
- Buesa, D. Pérez, J. Castel, D.S. Intrigliolo, J.R. Castel, Austral. J. Grape Wine Res. 23(2), 251-259 (2017)
- C. Van Leeuwen, P. Friant, X. Chone, O. Tregoat, S. Koundouras, and D. Dubourdieu, Am. J. Enol. Vitic. 55(3), 207-217 (2004)

- 15. D.S. Intrigliolo and J. R. Castel, Agric. Water Manag. 96(2), 282-292 (2009)
- 16. A, Ezzahouani and L.E. Williams, Am. J. Enol. Vitic. 58(3), 333–340 (2007)
- M.M. Chaves, O. Zarrouk, R. Francisco, J.M. Costa, T. Santos, A.P. Regalado, M.L. Rodrigues, C.M. Lopes, Ann Bot 105, 661-676 (2010)
- U. Hochberg, A. Degu, G.R. Cramer, S. Rachmilevitch, A. Fait, Plant Physiol. Biochem. 88, 42-52 (2015)
- T.A. Howell, A. Yazar, A.D. Schneider, D.A. Dusek, K.S. Copeland, Trans ASAE 38(6), 1737-1747 (1995)
- L.E. Williams, P. Baeza, P. Vaughn, Irrig Sci 30(3), 201-212 (2012)
- J. Carreño, A. Martínez, L. Almela and J.A. Fernández-López, Color Res. Appl. 21(1), 50-54 (1996)
- 22. OIV, Des vins et des mouts. Office Internationale de la Vigne et du Vin, 179 (1990)
- J.J. Cancela, E. Trigo-Córdoba, E.M. Martínez, B.J. Rey, Y. Bouzas-Cid, M. Fandiño, J.M. Mirás-Avalos, Agric. Water Manag. 170, 99-109 (2016)
- M.R. Conesa, J.M. de la Rosa, F. Artés-Hernández, I.C. Dodd, R. Domingo, A. Pérez-Pastor, J. Sci. Food Agric. 95(12), 2510-2520 (2014)
- J.M. Mirás-Avalos, D.S. Intrigliolo, Front. Plant Sci. 8, 851 (2017)
- 26. O. Soltekin and A. Altındisli, Acta Sci. Pol. Hortorum Cultus **21**(1), 89-102 (2022)
- H. Ojeda, C. Andary, E. Kraeva, A. Carbonneau and A. Deloire, Am. J. Enol. Vitic. 53, 261-267 (2002)
- N.C. Nascimento and A.G. Fett-Neto, Methods Mol. Biol. 643, 1–13 (2010)
- 29. S. Savoi, D.C.J. Wong, P. Arapitsas, M. Miculan, B. Bucchetti, E. Peterlunger, A. Fait, F. Mattivi, S.D. Castellarin, BMC Plant Biol. **16**, 67 (2016)