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The Effects of Bud Load and Applied Water Amounts on the Biochemical Composition of the 'Narince' Grape Variety (*Vitis vinifera* L.)

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Abstract

This study evaluated the effects of various bud load and water amounts (WA) on the berry composition of the 'Narince' white wine grape variety. Two water amounts (WA-I and WA-II) administered during growth stages or a non-irrigated (rain-fed) control treatment were paired with two different bud loads (K (normal) and 2K (two-fold buds) to assess their effects on sugar, organic acid and phenolic compound contents, as well as antioxidant capacity of the berries for a white wine grape variety 'Narince'. Despite a slight decrease observed only in sugars in the second year, increases in all phenolic compounds examined (especially in catechin and epicatechin) were detected in the WA-I application. The total phenolic compound values obtained for 2K in the same application was also high. The antioxidant capacity values were not significantly affected by the irrigation or bud load applications. Therefore, a 2K bud load with WA-I treatments in which 50% and 75% of the cumulative evaporation from the Class A pan during berry set to veraison and veraison to ripening, respectively, are recommended for irrigation in high plateau viticulture. By maintaining or increasing the fruit composition of 'Narince' grapes, these treatments can optimize grape yield and the earnings of growers. In all applications, glucose (among the sugars), tartaric acid (among the organic acids), and catechin and epicatechin (among the phenolic compounds) were higher than their counterparts.

Keywords: flavanols, flavonols, grapevine, irrigation, phenolics

Introduction

Grapes are among the most important and popular fruit species worldwide due to their economic importance and beneficial effects on human health and nutrition (Eshghi *et al.*, 2014; Rahmani *et al.,* 2015). Their popularity can be related to their biochemical composition. Sugars, organic acids and phenolic compounds are found in large amounts in grapes (Breksa *et al.,* 2010; Göktürk Baydar *et al.*, 2011; Zhu *et al.*, 2012; Kelebek *et al.*, 2013). Their adaptability and various uses (table grapes, wine, raisins, vinegar, molasses, grape juice, and others) contribute to the value of these grapes.

The importance of human health in nutrition and the protection of the body from carcinogens have been increasing. Protective compounds (Masa *et al.*, 2007; Beslic *et al.*, 2010; Kurtural *et al.*, 2013) and the antioxidant capacity of grapes have been the focus of recent viticulture studies (Iacopini *et al.*, 2008; Koyama and Goto-Yamamoto, 2008; Benmeziane *et al.*, 2014; Rahmani *et al.*, 2015). In many viticulture studies, phenolic compounds have been shown to demonstrate antioxidant, anti-allergic, anti-cancer, anti-microbial, anti-ageing and anti-inflammatory effects (Weston, 2005; Beslic *et al.*, 2010; Eftekhari *et al.*, 2012).

The biochemical contents of grapes are affected by many environmental and cultural factors such as the variety, training system, maturation time, biotic and abiotic stress conditions, crop loads, irrigation, fertilization, summer pruning, and others (Beslic *et al.*, 2010; Wessner and Kurtural, 2013).

Pruning is one of the most important practices in vineyards. Pruning affects the form and size of the vine and establishes a balance between shoot and fruit growth. The level of applied pruning greatly affects yield, quality, and fruit composition (Kurtural *et al.*, 2013; Wessner and Kurtural, 2013; Rahmani *et al.*, 2015) by altering the canopy size and numbers of shoots and clusters per vine.

Vine irrigation is another important practice that affects the content of the grapes. In locations with an annual rainfall of 500-600 mm, non-irrigated viticulture can be performed depending on the soil type. However, supplementary irrigation may be necessary to maintain high grape yield and quality when the seasonal rainfall is insufficient. Irrigation management as a tool

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Table 1. The number of retained buds by pruning weight in the water amount (WA) treatments and bud load treatments of 'Narince' grapevine

Year	Irrigation	Bud load	Pruning weight (g/vine)	K	2K
2013	Initial average weight		400	16	32
2014	No irrigation	K[1]	687	23	
		2K	703		48
	WA-I	K	1,003	30	
		2K	917		56
	WA-II	K	967	29	
		2K	907		56
	Mean	K	886	27	
		2K	842		52

[1] K (Control): 20 buds left for the first 500 g pruning weight and 10 additional buds left per additional 500 g. 2K: Double the buds of K

Table 2. Irrigation dates and water amount (WA) applied (mm) in irrigation treatments

Phenological stages	Irrigation dates	2013		2014	
		WA-I	WA-II	WA-I	WA-II
Budbreak		22 April		21 April	
Bloom		4 June		14 June	
Berry set		13 June		21 June	
	26 July	57.2	85.7		
	1 August			43.4	65.1
	8 August			39.2	58.7
Veraison		7 August		14 August	
Total (Berry set to Veraison)		57.2	85.7	82.6	123.8
	12 August	233.6	155.7		
	16 August			42.5	28.4
	22 August			72.2	48.2
Maturity		21 August		29 August	
Total (Veraison to Maturity)		233.6	155.7	114.7	76.6
	Total (General)	290.8	241.4	197.3	200.4

for increasing the grape production has been receiving attention in many regions of the world. The economic and careful use of water is important due to reductions of water resources worldwide. Regulated deficit irrigation (RDI) is a strategy that has been intensively used in vineyards (Basinger and Hellman, 2006; Keller *et al.*, 2008; Bowen *et al.*, 2011) for mitigating water use. RDI further enables a water deficit on the grapevine primarily between the berry set and maturation periods (Kriedemann and Goodwin, 2003; Chaves *et al.*, 2007; Terry and Kurtural, 2011). In wine grape varieties, RDI may increase the berry and wine quality by controlling vegetative growth (Shellie, 2006), thereby providing more sunlight and optimum temperature conditions in the microclimate around the clusters. In this manner, RDI may increase the skin/pulp ratio, phenolic compounds, anthocyanins and antecedent aroma compounds (Ferreyra *et al.*, 2002; Romero *et al.*, 2010) compared with excessive water conditions.

'Narince' (*Vitis vinifera* L.) is an important Turkish grape cultivar grown for wine, juice and table grapes that originated from Tokat province in mid-eastern Anatolia in Turkey (Selli *et al.*, 2006; Göktürk Baydar *et al.*, 2011). Its fresh leaves also have commercial importance. However, there is lack of information and research on the commercial production of 'Narince' and the effects of irrigation and crop load management under high plateau conditions. This study assessed various water amounts (WA) applied between berry set and maturing time and bud load applications for their effects on the contents of sugars, organic acids, phenolic compounds and antioxidants in the 'Narince' grape variety. Thus, if left more buds on the vines, giving a little more water added in addition to the natural rainfall is aimed to increase yield while maintaining biochemical content of grapes.

Materials and Methods

Materials

Berries of sixteen-year-old wine grape plants *cv.* 'Narince' (*Vitis vinifera* L.) grown at the Pozanti Agricultural Research Center (Latitude: 37° 25' N, longitude: 34° 52' E and altitude 1,080 m) of Çukurova University (Adana, Turkey) in 2013 and 2014 were used. The rooted vines were cultivated under rain-fed conditions. The vine rows were planted in an east/west orientation, and the distances between rows and vines were 3x2 m. The vines were trained using a bilateral cordon system and were spur-pruned. The soil in the experimental field has a slightly alkaline character with a clay-loam texture to a depth of 0-60 cm.

Three irrigation treatments were used: non-irrigated (control) and two water amounts (WA-I and WA-II), which were each respectively paired with two bud load treatments (traditional (K) and twofold bud load (2K). In both WA treatments, the irrigation time was scheduled according to the midday leaf water potentials (LWP); the irrigation amount applied was calculated using the cumulative evaporation (Epan) values obtained from a Class A Evaporation Pan. The midday LWP values used for timing the irrigation for both WA treatments were the same; however, these values differed at each plant growth stage: -1.0 MPa prior to flowering and -1.3 MPa between berry set to veraison and veraison to ripening. The irrigation amounts applied to the WA-I plots were 50% and 75% of the cumulative evaporation from the Class A pan during the berry set to veraison and veraison to ripening growth stages, respectively; these percentages were 75% and 50% of the evaporation from the pan for the same growth stages, respectively. For the K treatment, 20 buds for the first 500 grams of pruning weight and 10 additional buds for every additional 500 grams thereafter were retained (Winkler *et al.*, 1974; Ahmedullah and Himmelrick, 1990; Çelik, 2011); for 2K, twofold the buds of K (Table 1) were retained. Prunings were performed in March 2013 and March 2014.

The irrigation water amount was calculated using the following equation based on cumulative Class A pan evaporation within the irrigation intervals.

$$I = A * Epan * Kpc * P$$

I: Irrigation water amount (mm), A: parcel area (m^2), Epan: Evaporation from the Class A pan (mm), Kpc: Crop and pan coefficients (0.6), P: Percentage of wetted area (50%).

Irrigation was applied using a drip irrigation system, and the drip lines were placed near a vine row. The applied water amounts are shown in Table 2. Fertilizers were applied in both experimental years at the beginning of February (20 kg ha^{-1} N; 30 kg ha^{-1} P_2O_5; 50 kg ha^{-1} K_2O) and at the end of May (30 kg ha^{-1} N).

The midday leaf water potentials (LWP) were measured in four leaves for each treatment using a pressure chamber device (Model 600 Pressure Chamber, PMS Instrument Company, Albany, Oregon, USA) between 11:30 h and 14:00 h.

During the grape ripening period (when the amount of total soluble solids (TSS) has reached approximately 22-23%), 500 g

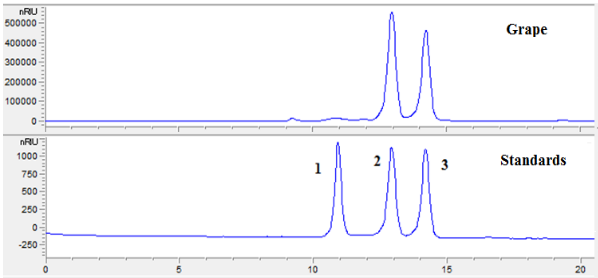

Fig. 1. HPLC_RID chromatograms of sugars. (1: Sucrose, 2: Glucose, 3: Fructose)

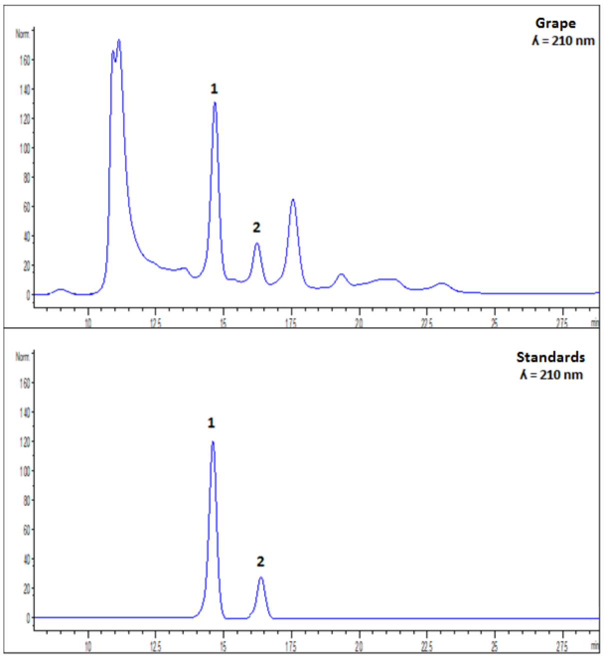

Fig. 2. HPLC-DAD chromatograms of organic acids recorded at 210 nm (1: Tartaric acid, 2: Malic acid)

of berry samples with pedicels were randomly taken from each replicate. These samples were maintained at -80 °C until the following analyses.

Sugar and organic acid analysis

To assess sugars (glucose, fructose and sucrose) and organic acids (tartaric and malic acid), grape samples were prepared according to the method of Sturm *et al.* (2003). Samples were prepared from a 100 gram grape sample from individual plants that were converted into pulp using a mixer and homogenized with Ultra-Turrax T-25 (IKA Laboratory Equipment). The liquid part of fruit puree (10 mL) was diluted to 100 mL with distilled water and clarified via centrifugation at 5,000 rpm for 15 min. The extract was filtered through 0.45-mm Millipore filters, and a 2 mL sample was used for an HPLC (High Performance Liquid Chromatography) analysis of sugars and organic acids.

An Agilent 1100 HPLC system (Agilent Technologies, Palo Alto, CA, USA) equipped with a pump system, a refractive index detector (RID) for sugar analysis, and a diode array detector (DAD) for the analysis of organic acids was used. Sugars and organic acids were simultaneously analyzed in an Aminex HPX-87H column (300 x 7.8 mm) (Bio-Rad, UK) at 55 °C. The analytical conditions were as follows: flow 0.3 mL/min, eluent 0.045 N H_2SO_4 with 6% acetonitrile (v/v). The chromatographic peak corresponding to each sugar (Fig. 1) and organic acid (Fig. 2) were identified by comparing the retention time with that of a standard A calibration curve using the standards to determine the relationship between the peak area and concentration.

Phenolic compounds analysis

Extracts for phenolic compound analysis were prepared according to the method of Breksa *et al.* (2010). Samples were prepared from 100 gram of grape sample. Twenty grams of grape puree was diluted with 80 mL 50:45:5 (v/v/v) methanol: formic acid: distilled water. The samples were blended with shaker with incubator at 24°C 180 rpm during 3 hours. At the end of blend process, liquid phase was centrifuged in 5000 rpm at 4°C during 10 minutes. After the centrifugation the supernatant was filtered by 0.20 mm Millipore filters. An Agilent 1100 HPLC system (Agilent Technologies, Palo Alto, CA, USA) with a diode array detector operated using Windows NT-based ChemStation software was used for the phenolic compounds analysis. Separation was performed on a Beckman Ultrasphere ODS column (Roissy, France; 4.6 mm x 250 mm, 5 μm). The mobile phase consisted of water with 5% formic acid for solvent A (v/v) and methanol/formic acid (95/5, v/v) for solvent B. The elution program was performed as previously described (Kelebek *et al.*, 2013). The identification and assignation of each compound was performed by comparing their retention times and UV spectra with authentic standards; these were confirmed using an Agilent 6430 LC-MS/MS spectrometer equipped with an electrospray ionization source. Electrospray ionization mass spectrometry detection was performed in negative ion mode and MRM (Multiple Reaction Monitor) mode (Kelebek *et al.*, 2013).

Antioxidant capacity analysis

The electron donation ability of the obtained extract was measured by bleaching the purple-colored solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical according to the method of Kelebek *et al.* (2013). For the analyses, the samples, prepared for the phenolics, were used in diluted form with distilled water (1:40, v/v). An aliquot of 0.1 mL of diluted sample was added to 3.9 mL of DPPH solution in methanol (6×10-5 M). The mixture was shaken vigorously and left standing at room temperature for 30 min. The absorbance of the resulting solution was then measured at 515 nm using an Agilent Carry 60 UV-Vis Spectrophotometer (Agilent Technologies, Palo Alto CA-USA) A Trolox calibration curve was used to calculate the antioxidant activity of the grape extracts and to express the antioxidant capacity of the mM Trolox equivalent per kg of berry.

Statistical analysis

The experimental design was 'Split - Plot' with two replicates (each consisting of 5 vines). Samples were taken from the inner two vines. The irrigation treatments were performed on the main plots, and the bud load treatments were performed on the sub plots. Analysis of variance (ANOVA) was performed using an SAS based JMP statistical programmer. The significance level was set at $\alpha = 0.05$ and the means were separated using a Least Significant Difference (LSD) test.

Table 3. Total rainfall between September 2012 and August 2014 in the experimental area (Adana Sixth Regional Directorate of Meteorology, Pozantı Climate station)

Year	Month	Total rainfall (mm)	Total (mm)	Year	Month	Total rainfall (mm)	Total (mm)
2012	September	0.4	432.6	2013	September	1.8	62
	October	107.0			October	30.4	
	November	93.4			November	10.4	
	December	231.8			December	19.4	
2013	January	31.4	234.8	2014	January	94.8	360
	February	47.2			February	10.6	
	March	65.6			March	124.8	
	April	67.6			April	42.6	
	May	23.0			May	87.2	
	June	11.8	12.2		June	76.8	78.8
	July	0.4			July	0.6	
	August	0			August	1.4	
	Total	679.6	679.6		Total	500.8	500.8

Table 4. Effects of two water amounts (WA) and two bud load levels on sugar and organic acid contents of 'Narince' cultivar (g/kg)

Source of variance	Sugars (g/kg)								Organic acids (g/kg)					
	Glucose		Fructose		Sucrose		Total		Tartaric acid		Malic acid		Total	
	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
Irrigation														
No irrigation	92.00 a[1]	104.52 a	88.51 a	93.77 a	0.00 b	2.63 b	180.51 a	200.92 a	5.27 a	4.26	2.55 b	2.87 a	7.82 a	7.12
WA-I	90.71 a	91.11 c	86.16 a	81.06 c	0.51 a	2.80 a	177.37 a	174.96 c	5.16 b	4.28	2.61 a	2.50 c	7.76 a	6.78
WA-II	81.94 b	96.22 b	79.28 b	86.42 b	0.00 b	2.36 c	161.23 b	185.00 b	4.50 c	4.10	2.41 c	2.74 b	6.91 b	6.83
LSD 5%	1.88	0.73	3.07	0.95	0.005	0.10	4.85	1.61	0.04	NS	0.03	0.004	0.08	NS
Bud load level														
K[2]	92.21 a	96.99	88.07 a	86.94	0.00 b	2.63	180.28 a	186.55	4.79 b	4.22	2.59 a	2.69 b	7.39 b	6.91
2K	84.22 b	97.57	81.23 b	87.22	0.34 a	2.57	165.79 b	187.36	5.16 a	4.20	2.45 b	2.72 a	7.60 a	6.91
LSD 5%	1.53	NS	2.50	NS	0.004	NS	3.96	NS	0.04	NS	0.03	0.003	0.06	NS
Interaction														
No irr.[3] × K	91.57 b	99.19 b	89.81 a	89.16 b	0.00 b	2.47 c	181.38 b	190.82 b	4.65 d	4.10	2.67 a	2.73 c	7.32 d	6.83 b
No irr. × 2K	92.44 b	109.85 a	87.20 ab	98.37 a	0.00 b	2.79 b	179.64 b	211.01 a	5.88 a	4.41	2.44 c	3.00 a	8.33 a	7.41 a
WA-I × K	98.46 a	95.26 d	92.92 a	84.97 d	0.00 b	3.06 a	191.38 a	183.28 c	4.70 d	4.39	2.62 ab	2.57 e	7.32d	6.96 b
WA-I × 2K	82.95 c	86.96 e	79.39 c	77.15 e	1.02 a	2.53 c	163.36 cd	166.64d	5.61 b	4.17	2.60 b	2.44 f	8.20 b	6.61 b
WA-II × K	86.60 c	96.52 c	81.48 bc	86.68 c	0.00 b	2.35 d	168.08 c	185.55 c	5.03 c	4.18	2.50 c	2.77 b	7.53 c	6.95 b
WA-II × 2K	77.29 d	95.91 c	77.09 c	86.16 c	0.00 b	2.37 d	154.38 d	184.44 c	3.97 e	4.01	2.31 d	2.71 d	6.28 e	6.72 b
LSD 5%	2.65	1.04	4.34	1.35	0.008	0.15	6.86	2.28	0.62	NS	0.05	0.006	0.11	0.36

[1] Means (n=2) followed by different letters on the same column are significantly different according to LSD test at $P < 0.05$.
NS: Not significant [2] K (Control): 20 buds left for the first 500 g pruning weight and 10 additional buds left per additional 500 g. 2K: Double buds of K. [3] irr.: Irrigation.

Table 5. Effects of two water amounts (WA) and bud load levels on the flavanol contents (mg/kg) of 'Narince' cultivar

Source of variance	Catechin		Epicatechin	
	2013	2014	2013	2014
Irrigation				
No irrigation	80.31 c[1]	87.18 c	108.01 c	108.48 c
WA-I	131.15 a	142.38 a	123.75 a	124.28 a
WA-II	114.99 b	124.83 b	120.34 b	120.86 b
LSD 5%	0.64	1.42	0.72	1.49
Bud load level				
K[2]	89.06 b	96.69 b	96.25 b	96.67 b
2K	128.57 a	139.57 a	138.48 a	139.08 a
LSD 5%	0.52	1.16	0.59	1.21
Interaction				
No irr.[3] × K	56.67 f	61.52 f	75.15 f	75.47 f
No irr. × 2K	103.95 d	112.84 d	140.88 b	141.49 b
WA-I × K	135.34 b	146.92 b	133.46 c	134.03 c
WA-I × 2K	126.97 c	137.84 c	114.03 d	114.53 d
WA-II × K	75.19 e	81.62 e	80.15 e	80.50 e
WA-II × 2K	154.79 a	168.04 a	160.52 a	161.22 a
LSD 5%	0.90	2.00	1.02	2.10

[1] Means (n=2) followed by different letters on the same column are significantly different according to LSD test at $P < 0.05$. [2] K (Control): 20 buds left for the first 500 g pruning weight and 10 additional buds left per additional 500 g. 2K: Double buds of K. [3] irr.: Irrigation.

Results and Discussion

The amounts of water applied at different periods according to the applications are given in Table 2. The LWP values prior to flowering were <-1.0 MPa; thus, irrigation was not applied until berry set (Table 2). The rainfall between September and December (and until berry set in 2013 and 2014) were sufficient. Total rainfalls of 679.6 mm and 500.8 mm were recorded in the experimental field between September and August in 2013 and 2014 (Table 3), respectively.

Effects of water amounts and bud load levels on sugars and acids

There was a clear effect for the treatments on sugars and organic acids (Table 4). The glucose, fructose and total sugar values for 2014 in the non-irrigated vines were higher than those of the other treatments. However, there were no differences between the non-irrigated and WA-I vines in 2013. Tartaric acid, malic acid and their total in the non-irrigated vines and WA-I vines were higher than those in the WA-II vines in 2013. However, there were no significant differences for these in 2014. The glucose and fructose ratios were slightly higher than 1, as

Table 6. Effects of two water amounts (WA) and two bud load levels on the phenol acid (mg/kg) contents of 'Narince' cultivar

Source of variance	Gallic acid		Protocatechuic acid		Caftaric acid		Coutaric acid		Caffeic acid		p-coumaric acid	
	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
Irrigation												
No irrigation	10.52 c[1]	9.07 c	12.92 b	9.82 b	38.61 b	60.74 b	9.57 b	11.37 b	0.17 c	0.19 c	0.43 b	0.46 b
WA-I	12.47 a	10.75 a	26.86 a	20.43 a	72.59 a	114.19 a	14.12 a	16.77 a	0.34 a	0.36 a	0.45 a	0.48 a
WA-II	12.06 b	10.39 b	11.12 c	8.46 c	30.92 c	48.64 c	8.77 c	10.41 c	0.31 b	0.32 b	0.34 c	0.36 c
LSD 5%	0.03	0.05	0.18	0.29	0.48	0.32	0.13	0.32	0.002	0.005	0.002	0.005
Bud load level												
K[2]	11.77 b	10.15 a	22.27 a	16.94 a	61.22 a	96.29 a	14.99 a	17.79 a	0.22 b	0.23 b	0.49 a	0.52 a
2K	11.59 a	10.00 b	11.66 b	8.87 b	33.54 b	52.76 b	6.65 b	7.90 b	0.33 a	0.35 a	0.33 b	0.35 b
LSD 5%	0.02	0.04	0.15	0.23	0.39	1.25	0.11	0.26	0.002	0.004	0.002	0.004
Interaction												
No irr.[3] × K	9.10 f	7.85 f	22.27 c	16.94 c	63.20 c	99.42 c	16.39 a	19.46 a	0.00 d	0.00 d	0.53 b	0.57 b
No irr. × 2K	11.94 c	10.29 c	3.56 e	2.71 e	14.03 e	22.06 e	2.76 e	3.28 e	0.35 a	0.37 a	0.33 d	0.35 e
WA-I × K	13.94 a	12.02 a	24.12 b	18.35 b	65.81 b	103.53 b	13.29 d	15.78 d	0.35 a	0.37 a	0.59 a	0.63 a
WA-I × 2K	11.00 e	9.48 e	29.60 a	22.51 a	79.37 a	124.85 a	14.96 c	17.76 c	0.33 b	0.36 b	0.32 e	0.34 f
WA-II × K	12.26 b	10.57 b	20.42 d	15.53 d	54.63 d	85.94 d	15.29 b	18.15 b	0.31 c	0.32 c	0.35 c	0.37 c
WA-II × 2K	11.85 d	10.21 d	1.82 f	1.39 f	7.22 f	11.35 f	2.24 f	2.66 f	0.31 c	0.32 c	0.33 d	0.36 d
LSD 5%	0.04	0.07	0.26	0.41	0.67	2.17	0.18	0.45	0.003	0.007	0.003	0.007

[1] Means (n= 2) followed by different letters on the same column are significantly different according to LSD test at $P < 0.05$.
[2] K (Control): 20 buds left for the first 500 g pruning weight and 10 additional buds left per additional 500 g. 2K: Double buds of K.
[3] irr.: Irrigation.

Table 7. Effects of two water amounts (WA) and two bud load levels on the content of flavonols and total phenolic compounds (mg/kg) of 'Narince' cultivar

Source of variance	Ferulic acid		Rutin[1]		Myricetin 3-glucoside		Isoramnetin 3-glucoside		Quercetin 3-glucoside		Kaempferol 3-gllucoside		Total phenols	
	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
Irrigation														
No irrigation	0.65 c[1]	0.69 c	1.38 c	1.47 c	4.28 c	4.56 c	1.17 b	1.29 b	0.00	3.24 b	0.00	0.01 a	268.03 c	298.57 c
WA-I	0.92 a	0.98 a	1.80 a	1.91 a	9.62 a	10.24 a	1.22 a	1.35 a	0.00	3.38 a	0.00	0.00 b	395.29 a	447.51 a
WA-II	0.83 b	0.88 b	1.58 b	1.69 b	7.98 b	8.50 b	0.77 c	0.85 c	0.00	2.14 c	0.00	0.00 b	310.00 b	338.33 b
LSD 5%	0.007	0.01	0.005	0.01	0.08	0.18	0.005	0.01		0.03		0.0003	0.59	0.85
Bud load level														
K[2]	1.02 a	1.09 a	1.69 a	1.80 a	8.64 a	9.21 a	1.15 a	1.27 a	0.00	3.17 a	0.00	0.01 a	308.76 b	351.81 b
2K	0.57 b	0.61 b	1.49 b	1.59 b	5.94 b	6.33 b	0.97 b	1.07 b	0.00	2.67 b	0.00	0.00 b	340.12 a	371.13 a
LSD 5%	0.005	0.01	0.004	0.008	0.07	0.15	0.004	0.009		0.02		0.0002	0.48	0.69
Interaction														
No irr.[3] × K	0.67 d	0.72 d	1.22 f	1.30 f	1.22 e	1.30 e	1.03 c	1.14 c	0.00	2.85 c	0.00	0.02 a	247.47 f	288.56 f
No irr. × 2K	0.62 e	0.66 e	1.54 d	1.64 d	7.34 c	7.82 c	1.31 b	1.45 b	0.00	3.62 b	0.00	0.00 b	288.60 d	308.58 e
WA-I × K	1.11 b	1.18 a	2.02 a	2.15 a	13.92 a	14.83 a	1.54 a	1.71 a	0.00	4.27 a	0.00	0.00 b	405.48 a	455.75 a
WA-I × 2K	0.73 c	0.77 c	1.57 c	1.67 c	5.31 d	5.66 d	0.90 d	1.00 d	0.00	2.50 d	0.00	0.00 b	385.10 b	439.27 b
WA-II × K	1.28 a	1.36 b	1.82 b	1.93 b	10.78 b	11.49 b	0.86 e	0.95 e	0.00	2.38 e	0.00	0.00 b	273.33 e	311.12 d
WA-II × 2K	0.38 f	0.40 f	1.35e	1.44 e	5.17 d	5.51 d	0.68 f	0.76 f	0.00	1.89 f	0.00	0.00 b	346.67 c	365.55 c
LSD 5%	0.009	0.02	0.007	0.02	0.12	0.25	0.007	0.02		0.04		0.0004	0.84	1.20

[1] Means (n= 2) followed by different letters on the same column are significantly different according to LSD test at $P < 0.05$.
[2] K (Control): 20 buds left for the first 500 g pruning weight and 10 additional buds left per additional 500 g. 2K: Double buds of K.
[3] irr.: Irrigation.

mentioned in previous studies (Winkler *et al.,* 1974; Kelebek, 2009). Other studies found that berry growth in grapevines to which excess bud load was applied was lower than that for grapevines with less load due to reductions in the available sugar per berry and water absorption (Winkler *et al.,* 1974; Çelik, 2011); however, this study found no consistent effect for bud level on sugars or acids. Although no significant effect was observed between bud load applications in 2014, sugars (with the exception of sucrose) decreased and acids increased in the 2K treatments in 2013. The sucrose content was very low compared with other sugars. Tartaric acid values were higher than those for malic acid in all applications. These results were also obtained by other researchers (Winkler *et al.,* 1974; Çelik, 2011; Kelebek, 2009). Irrigation and bud load applications did not change this situation. Generally, sugars increased and acids decreased in 2014. This may be due to increased rainfall in 2014. The effects of water amounts and bud loads on sugars and acids have been examined in other studies, which tend to agree with our results. Some studies indicated that ripening accelerates in conditions with less water, especially between fruit set and veraison (Rain-fed and WA-I treatments in our research) (Kennedy *et al.,* 2002). However, Stevens *et al.* (2008) found no significant differences in grape maturity associated with irrigation treatments. Cus (2004) also stated that most sugar and acid contents were not clearly affected by crop load. Irrigation and bud load interactions were statistically significant for sugars and organic acids. The total sugar values changed from 154.38 to 191.38 g/kg for 2013 and from 166.64 to 211.01 g/kg for 2014. The total acidity levels ranged from 6.28 to 8.33 g/kg in 2013 and 6.61 to 7.41 g/kg in 2014.

Table 8. Effects of two water amounts (WA) and bud load levels on the antioxidant capacity (mM Trolox/kg) of 'Narince' cultivar

Source of variance	Antioxidant capacity	
	2013	2014
Irrigation		
No irrigation	311.64 a[1]	464.71
WA-I	304.61 b	369.04
WA-II	307.09 b	357.71
LSD 5%	2.21	NS
Bud load level		
K[2]	307.83	415.53
2K	307.73	378.78
LSD 5%	NS	NS
Interaction		
No irr[3] × K	312.85 a	451.75
No irr. × 2K	310.43 ab	477.67
WA-I × K	302.88 c	371.58
WA-I × 2K	306.35 bc	366.50
WA-II × K	307.78 b	423.25
WA-II × 2K	306.40 bc	292.17
LSD 5%	3.13	NS

[1] Means (n=2) followed by different letters on the same column are significantly different according to LSD test at $P < 0.05$. NS: Not significant
[2] K (Control): 20 buds left for the first 500 g pruning weight and 10 additional buds left per additional 500 g. 2K: Double buds of K. [3] irr.: Irrigation.

Effects of water amounts and bud load levels on phenolic compounds

Thirteen different phenolic compounds (PC), including two flavanols, six phenolic acids, and five flavonols, were identified in 'Narince' berries (Tables 5, 6 and 7). Considering all these compounds (including total phenolic values), WA-I values were the highest in each study year. These values are primarily followed by those obtained from WA-II vines related to catechin, epicatechin, gallic acid, caffeic acid, ferulic acid, rutin and myricetin. The total phenol value was also higher in the WA-II vines than the rain-fed treatments.

Studies on the influence of water amounts on the concentration of phenolics in grape berries have produced variable results. Phenolic concentrations increased (Ojeda *et al.*, 2002) or decreased (Kennedy *et al.*, 2002) in response to deficit irrigation conditions. In Bindon *et al.* (2011), increases were reported in the concentrations of grape anthocyanins and flavonols in response to a lack of water.

The concentrations of catechin and epicatechin (Table 5) and the total phenolic value of berries (Table 7) were higher in the 2K treatments, whereas flavonol values (with the exception of gallic acid and caffeic) acid were higher in the K bud load vines. Contrary to our study, Kurtural *et al.* (2013) found that increased berry skin phenolics were attributed to lower crop levels and a concentration effect resulting from a berry size reduction in an unthinned treatment. Similarly, Terry and Kurtural (2011) reported that increased yield (>27 t ha^{-1}) decreased berry skin phenolics. This difference was thought to be because the plants (both K and 2K vines) were irrigated according to their leaf water potential. The total PC values ranged from 247.47 (Non irrigated K) to 405.48 mg/kg (WA-I K) for 2013 and from 288.56 (Non irrigated K) to 455.75 mg/kg (WA-I K) for 2014. The total values of the berry skin phenolics of 'Syrah' were between 1,878 and 2,478 µg/g in Wessner and Kurtural (2013) and between 2,170 and 2,548 µg/g in Kurtural *et al.* (2013). Kelebek (2009) detected non-colored phenolic compounds for the wines obtained from 'Kalecik Karası', 'Öküzgözü' and 'Bogazkere' grapes. In his study, the phenolic amounts of the varieties varied between 142 mg/L and 349 mg/L. According to Göktürk Baydar *et al.* (2011), the total phenolic contents varied from 217 to 1,336 mg/L in wines from the 'Cabernet Sauvignon', 'Kalecik Karası' and 'Narince' grape cultivars. In Cantos *et al.* (2002), the total phenolics ranged from 115 (Dominga) to 361 (Flame Seedless) mg/kg fresh weight of grapes. Catechin and epicatechin were the most important elements among phenolic compounds (Montealegre *et al.*, 2006; Rusjan and Korosec_Koruza, 2007). Weston (2005) also indicated that simple polyphenols present in a relatively high proportion in mature grapes and grape juice include catechins, epicatechins, gallic acid, flavonols, cinnamic acids, hydroxycinnamates, and quercetin.

Effects of water amounts and bud load levels on antioxidant capacity

The antioxidant capacity was higher in the WA-I and 2K treatments (Table 8) in 2013. However, there were no significant differences between the treatments in 2014. Applying different water amounts during deficit water settings did not consistently change the antioxidant activity of the grapes.

According to Kriedemann and Goodwin (2003), the effect of WA changes according to phenological stage and the level of applied irrigation stress. Bowen *et al.* (2011) and Keller *et al.* (2008) stated that fruit composition, anthocyanin and berry content underwent slight or inconsistent changes due to deficit irrigation and yield load balance and that this effect varied according to the year or seasonal changes. Benismail *et al.* (2007) assessing Cardinal and Fawzi *et al.* (2010) assessing Seedless grapes determined that the vegetative growth decreased depending on bud load increases per grapevine; however, there were no changes in the biochemical contents of the grapes.

Conclusions

According to the common view, the relationship between grape yield and quality is that of the quality decreases linearly as yield increases. Irrigation and increased bud load applications are among the tools that can be used to achieve the maximum grape yield while maintaining the fruit quality. This study assessed the interactive effects of various water amounts and pruning levels on the sugars, organic acids, flavanols, phenol acids, flavonols and antioxidant capacity of the 'Narince' white wine grape variety grown in high plateau conditions. In the WA-I treatment, the concentrations of all phenolic compounds increased in both study years. The sugar and acid values were also at sufficient levels compared with vines with WA-II and rain-fed treatments. The application of 2K bud load together with WA-I did not significantly alter the quality-related attributes of the berries. There were no consistent effects on the antioxidant capacity related to the irrigation or bud load applications. Therefore, 2K bud load with a WA-I treatment (irrigation at 50% and 75% Epan during berry set to veraison and veraison to ripening, respectively) can be recommended for irrigation practice in high plateau viticulture. This practice can optimize grape yield and the earnings of growers by maintaining or increasing the fruit composition of 'Narince'.

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