The application of gibberellic acid increases berry size of 'Emperatriz' seedless grape

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Abstract

Gibberellic acid (GA_3) increases berry size of 'Emperatriz' seedless grape, the response depending on the phenological stage of vine at treatment date and on the concentration applied. From berry fruit set to 21 days later, 80 mg L^{-1} GA_3 increased commercial berry weight by 50%-90%, depending on the year, reaching similar size to that of 'Aledo' seeded grape, used as comparison. This effect takes place through: a) a larger berry growth rate; b) an early glucose, fructose and sucrose uptake; c) an increase of absolute glucose and fructose content (mg berry^{-1}) of seedless berries up to similar values to those of seeded berries; and d) an increase of absolute berry water content but not of relative content to fresh weight, thus water potential and osmotic potential are not significantly modified by treatments. GA_3 does not affect berry pericarp cell number but increases pericarp cell diameter.

Additional key words: fruit development, plant growth substances, sugars, *Vitis vinifera* L., water potential.

Resumen

La aplicación de ácido giberélico aumenta el tamaño de la baya de la vid apirena 'Emperatriz'

La aplicación de ácido giberélico (GA_3) aumenta el tamaño de la baya de la vid apirena 'Emperatriz', dependiendo de la época de aplicación y de la concentración aplicada. Desde el momento del cuajado y hasta 21 días más tarde, 80 mg L^{-1} de GA_3 aumentaron el peso de las bayas comerciales entre un 50% y un 90%, dependiendo del año, alcanzando un tamaño similar al de las bayas de la variedad con semillas 'Aledo', utilizada como comparación. Este efecto tiene lugar a través de: a) una mayor tasa de crecimiento; b) una anticipación en la acumulación de glucosa, fructosa y sacarosa; c) un aumento del contenido absoluto en glucosa y fructosa (mg baya^{-1}) en las bayas de la variedad apirena que alcanza valores similares a los de las bayas con semillas; y d) un aumento del contenido absoluto de agua de las bayas, aunque no relativo a su peso fresco, de modo que el potencial hídrico y el potencial osmótico no se ven modificados por los tratamientos. El GA_3 no modifica el número de células del pericarpo, pero sí su diámetro.

Palabras clave adicionales: azúcares, desarrollo del fruto, potencial hídrico, reguladores del desarrollo, *Vitis vinifera* L.

Introduction

Seedless grapes (*Vitis vinifera* L.) are of interest because they reach good prices and, thus, increase grower's returns. However, their small size represents a problem for commercialization. Gibberellic acid (GA_3) has been used on seedless table grapes to increase berry size (Weaver, 1976), however there is no solid guideline for

Abbreviations used: CCC (2-chloroethyl trimethylammonium chloride), DAA (days after anthesis), DAFS (days after fruit set), DW (dry weight), FPA (5% formaldehyde, 5% propionic acid and 90% ethanol), FS (fruit set), GA_3 (gibberellic acid), Ψ_p (pressure potential), Ψ_w (water potential), π (osmotic potential).

GA$_3$ application, the number of applications varying from one to five (Fallahi *et al*., 1995).

Berry size is affected by both endogenous (*e.g.* nutritional and hormonal factors) and exogenous factors (*e.g.* temperature, light and water availability) (Ojeda *et al*., 2001; Ollat *et al*., 2002). Seeds have been related to endogenous growth promoters (Coombe, 1960; May, 2000). Further, number of seeds per berry has been significantly correlated with endogenous gibberellins concentration (Lavee and Nir, 1986; Göktürk and Harmankaya, 2005), and growth retardants, such as 2-chloroethyl trimethylammonium chloride (CCC), increase fruit set but reduce berry size and sugar accumulation by inhibiting gibberellins synthesis (Coombe and Hale, 1973; Looney, 1981).

The involvement of gibberellins in berry development was first described by Coombe (1960). Seeded berry has a high gibberellins concentration at fruit set, that persists for, at least, three weeks, then falling to a very low values and reincreasing after that, giving rise to a second peak two weeks later; finally concentration diminishes and remains low during fruit ripening (Scienza *et al*., 1978). Unexpectedly, similar pattern is found in seedless grapes (Iwahori *et al*., 1968; Pérez *et al*., 2000).

Accordingly, it must be possible to increase berry size by means of GA$_3$ application. Although, there are many studies reporting the effect of GA$_3$ (Lavee and Nir, 1986; Williams, 1996; Zabadal and Dittmer, 2000; Hyunggook *et al*., 2008), the role of gibberellins on berry growth has not been completely elucidated.

In this paper we studied the effect of GA$_3$ applied to both seedless and seeded berries on the pattern of sugar levels, in order to achieve a better understanding of the role of gibberellins in developing grapes.

Material and methods

The experiments were conducted over 3 consecutive years (2003-2005) on adult vines (>10 years old) of 'Emperatriz' (stenospermocarpic seedless cultivar obtained by 'Emperador' × 'Sultanina' breeding) and 'Aledo' cultivars (*Vitis vinifera* L.), grafted onto 1103 Paulsen rootstock, and grown at Novelda, Alicante, Spain (38°24'N; 00°45'W; altitude 260 m). Vines planted 2.8 × 2.8 m apart, grown in a loamy-clay soil with drip irrigation, trained on a Y-shaped trellis as bilateral cordon, pruned yearly to six 8-node canes per vine and thinned to 25 bunches per vine were used.

Cultural practices and pests and diseases control were carried out in accordance with local standards.

To determine the optimal date of treatment, GA$_3$ (80 mg L^{-1}) (1.6% w/v; Clemencuaje, Agrevo Ibérica, S.A., Alcasser, Valencia, Spain) was applied at FS (fruit set) and 7, 14 and 21 DAFS (days after fruit set). To determine optimal GA$_3$ concentration, a range of 40–400 mg L^{-1} GA$_3$ was applied at the onset of cell enlargement stage. Treatments were sprayed by handgun on whole vines to the point of runoff. A non-ionic wetting-agent (nonylphenyl polyethyleneglycol ether, 20% w/v; Ditene, Industrial Química Key, S.A., Tárrega, Lleida, Spain), at the rate recommend by the manufacturer, was added to the solution. The experiments were laid out in randomized complete-block design with one-vine plots of 6 replications each.

Time-course of berry fresh weight for control and 160 mg L^{-1} GA$_3$ treated berries at the onset of cell enlargement stage (20 DAA or days after anthesis) was evaluated from 20 berries per replica. At harvest, 20 commercial berries per date of treatment and concentration applied and replica were randomly selected and their fresh weight, length and diameter, and peduncle length and diameter, measured. Average bunch weight, length and width, and stalk weight, and number and weight of containing commercial berries, from 5 bunches per replica were also measured at harvest.

The procedure for carbohydrate analysis was previously described (Mehouachi *et al.,* 1995). In brief, 10 berries per vine from each treatment and cultivar were collected weekly from the onset of cell enlargement stage to maturation, frozen immediately in liquid N$_2$, lyophilised and stored as powders at –40°C. Powdered samples (100 mg DW) were extracted with 1.0 mL 80% (v/v) ethanol and purified sequentially by cation and anion exchange columns. The eluates were then passed through a C18 Sep-Pak cartridge (Waters-Millipore, Barcelona, Spain) and analysed using an HPLC Spectra System® (Spectra, San José, CA, USA) equipped with a differential refractometer (Spectra R150), vacuum pump (Spectra P2000) and ChromQuest® Chromatography Data System for Windows NT (Thermo Quest Inc., San José, CA, USA). Sucrose, glucose and fructose were identified by their retention times.

In 2005, six berries per vine and cultivar from control and 80 mg L^{-1} treated vines were collected on 67 DAA for measurement of water potential (Ψ_w) and osmotic potential (π) of flesh. Samples were taken at dawn when the soil, plant and atmospheric water potential were in equilibrium (Milad and Shackel, 1992).

To measure Ψ_w and π, 5 mm flesh square pieces 3-4 mm thick were excised with a blade from the equatorial area of the berry. Fresh tissue was used for Ψ_w, whereas frozen tissue was used for π. Pieces were placed in a sampler chamber (C-52, Wescor Inc., Logan, UT, USA) connected to a psychrometer switchbox (Ps-10) and to a dew point microvoltimeter (HT-33T). The dew point hygrometer was previously calibrated with NaCl solutions of known concentrations. To ensure initial water vapour equilibrium, Ψ_w and π were measured at least 4 h after setting the sample in the chamber. Pressure potential (Ψ_p) was calculated by subtracting osmotic potential from water potential (Milad and Shackel, 1992).

For a better understanding of the mode of action of the GA_3, histological studies of the pericarp tissue were made. At maturation stage, 10 'Emperatriz' seedless berries were harvested from not treated and 80 mg L^{-1} GA_3 treated vines at fruit set. Equatorial portions of these berries were sampled and fixed in FPA (5% formaldehyde, 5% propionic acid and 90% ethanol). The material was dehydrated in a graded ethanol and *t*-butanol series, and embedded in paraffin. Polymerization was at 60°C for 24 h. Sections (4-5 μm) were cut with a Microns HM 400 microtome using glass knives and fixed to microscope slides with

3-aminopropyl-triothoxysilane adherent. The sections were stained with safranin fast-green (Jensen, 1962) for general flesh tissue structure observation. Four section of each berry and eight replicates per sample were examined by using a Nikon Eclipse E600 light microscope.

Analysis of variance was performed using Statgraphics Plus for Windows, V.2.1 (Statistical Graphics Corp., MD, USA). Data were compared using the Duncan's multiple range test for means separation ($P \le 0.05$).

Results

Influence of date of treatment

At harvest, average berry fresh weight for the three years of experiments was 3.17 ± 0.13 g and 5.78 ± 0.78 g for 'Emperatriz' seedless and 'Aledo' seeded variety, respectively (Fig. 1).

The influence of date of treatment on berry mature and bunch characteristics of 'Emperatriz' seedless variety is shown in Table 1. GA_3 at a concentration of 80 mg L^{-1} was effective increasing berry size when applied between fruit set and 21 DAFS. Average berry weight was increased by 45% and 80%, with regard to
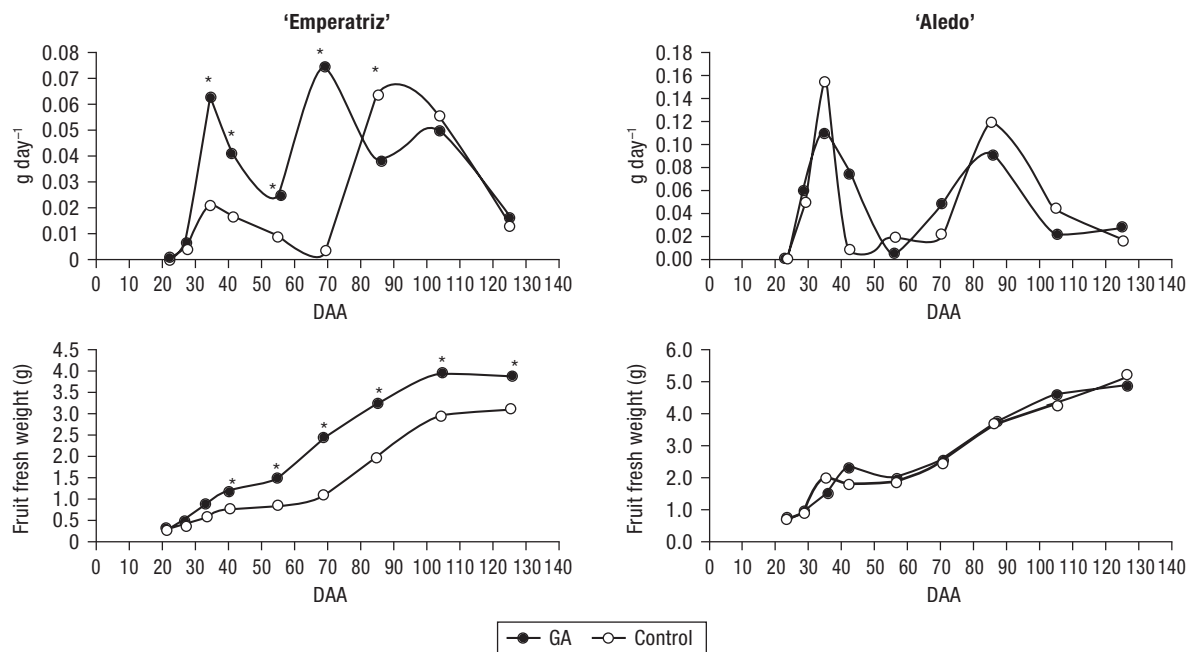
Figure 1. Effect of GA_3 application (160 mg L^{-1}) at the onset of cell enlargement stage of berry development on the time-course of growth rate and berry fresh weight in 'Emperatriz' seedless and 'Aledo' seeded grape. DAA: days after anthesis. Values, corresponding to 2003, are the mean of 120 berries. Standard errors are smaller than the symbols. * means significant differences ($P \le 0.05$).

Table 1. Effect of GA$_3$ (80 mg L^{-1}) treatment date on mature commercial berry and bunch characteristics of 'Emperatriz' seedless grape at harvest for years 2003 and 2004

Treatment date	Bunch characteristics				Commercial berry	
	Fresh weight (g)	Length (cm)	Width (cm)	Stalk (g)	Fresh weight (g)	Number
Year 2003						
FS	1,514.5^b	34.5	27.1^b	34.0	5.4^c	250.6
7 DAFS	1,914.7^b	39.9	27.5^b	36.4	5.8^c	290.5
14 DAFS	1,525.4^b	39.5	24.5^{ab}	41.7	5.7^c	266.7
21 DAFS	1,646.3^b	39.5	27.9^b	40.3	4.8^b	304.1
Control	1,177.5^a	36.2	22.1^a	32.8	3.7^a	305.1
Year 2004						
FS	1,582.6^b	34.1^b	26.1^b	38.5^b	5.8^c	263.0
7 DAFS	1,622.5^b	32.5^{ab}	26.1^b	38.9^b	6.1^c	251.6
14 DAFS	1,562.2^b	31.6^{ab}	26.9^b	38.0^b	5.7^c	266.2
21 DAFS	1,440.4^b	33.8^b	26.9^b	30.2^b	4.6^b	302.8
Control	780.8^a	28.8^a	19.3^a	13.8^a	3.1^a	231.5

Values are the mean of 30 bunches and 20 commercial berries per bunch. FS: fruit set. DAFS: days after fruit set. Different letters in the same column and year indicate significant differences ($P \leq 0.05$).

control, for 2003 and 2004, respectively. Nevertheless, response for treatments applied between FS and 14 DAFS was significantly higher than that for 21 DAFS, irrespective of the year of experiment. Number of commercial berries was not significantly altered ever. Bunch weight and width were also significantly increased by GA$_3$, irrespective of the date of treatment and year. The effect increasing bunch length and stalk weight depended on the year (Table 1). No effect was observed on 'Aledo' berry and bunch characteristics, irrespective of the date of treatment and year, for 80 mg L^{-1} GA$_3$.

Effect of concentration applied

The effect of GA$_3$ increasing concentrations is shown in Table 2. In the first year of experiments, 'Emperatriz' seedless commercial berry fresh weight at harvest increased with increasing GA$_3$ concentration up to 160 mg L^{-1}, reaching 5.8 g compared to 3.3 g in the controls. In the second year, berry weight reached its maximum value (5.2 g) for 160 mg L^{-1}, compared to control (3.3 g), as well. On the third year, maximum value was for 240 mg L^{-1} (4.4 g), compared to control (3.1 g), but 160 mg L^{-1} GA$_3$ was not applied this year. Taking together the three years results, the response show a saturating effect for 160 mg L^{-1} (Table 2). And similar results were obtained for diameter and length

of commercial berry (Table 2). GA$_3$ also modified commercial berry shape, 160 mg L^{-1} treated berries being slightly longer than control ones; this effect, however, depended on the year (Table 2). Berry peduncle length and diameter also increased with GA$_3$ concentration up to 160 mg L^{-1} (Table 2). In 'Aledo' seeded cultivar, the response to GA$_3$ was erratic, berry fresh weight, length and diameter, and peduncle length and diameter increasing significantly for 200 mg L^{-1} GA$_3$ or higher concentration depending on the year (data not shown).

Berry growth changes

Daily berry growth rate has typically two peaks in the course of the development for both seeded and seedless berry, those of the first one being eight times and twice higher, respectively, than those of the second one (Fig. 1). For 'Emperatriz' seedless cultivar, the application of 160 mg L^{-1} GA$_3$ at the onset of cell enlargement stage (20 DAA) increased value of the first peak by more than 300% and anticipated the second one 15 days, approximately (Fig. 1). As a result of that, berry fresh weight at harvest increased by 40%, reaching a commercial size similar to that of 'Aledo' seeded grape (Fig. 1). Differences became significant 20 days after treatment (40 DAA) and reached maximum 67 DAA.

Table 2. Effect of increasing GA_3 concentrations applied on peduncle and commercial berry characteristics of 'Emperatriz' seedless grape at harvest for the three years of experiments

	Year	GA_3 concentration (mg L^{-1})								
		0	40	80	120	160	200	240	320	400
Peduncle										
— Length (mm)	2003	6.4[a]	6.7[b]	7.2[c]	7.0[c]	7.8[d]	7.1[c]			
	2004	7.2[a]		7.9[d]		8.0[c]		7.8[c]	7.58[b]	8.3[c]
	2005	6.4a		7.3[c]				7.0[b]		7.0[b]
— Diameter (mm)	2003	1.4[a]	1.8[b]	1.7[b]	2.0[c]	2.2[d]	1.8[b]			
	2004	1.5[a]		1.6[b]		1.8[d]		1.7[cd]	1.7[bc]	1.8[d]
	2005	1.3[a]		1.7[b]				1.8[bc]		1.8[c]
Berry										
— Fresh weight (g)	2003	3.3[a]	4.2[b]	4.5[c]	5.1[d]	5.8[e]	5.0[d]			
	2004	3.3[a]		4.3[b]		5.2[d]		5.4[d]	4.7[c]	4.9[c]
	2005	3.1[a]		4.1[b]				4.4[c]		4.0[b]
— Length (L; mm)	2003	19.7[a]	20.6[b]	21.0[b]	23.0[d]	24.5[e]	22.3[c]			
	2004	19.9[a]		22.1[b]		23.5[c]		23.8[c]	22.2[b]	23.3[c]
	2005	18.7[a]		21.0[c]				21.0[c]		20.1[b]
— Diameter (D; mm)	2003	15.7[a]	17.5[b]	17.8[bc]	18.0[cd]	18.8[e]	18.3[d]			
	2004	15.9[a]		17.3[b]		18.3[d]		18.4[d]	17.8[c]	17.8[c]
	2005	15.6[a]		17.1[b]				17.5[c]		17.4[bc]
— Shape (L/D)	2003	1.26[c]	1.18[a]	1.18[a]	1.28[c]	1.30[d]	1.22[b]			
	2004	1.25[a]		1.27[ab]		1.28[abc]		1.29[bc]	1.25[a]	1.31[c]
	2005	1.20[b]		1.23[c]				1.19[b]		1.16[a]

GA_3 was applied at the onset of cell enlargement stage. Values are the mean of 120 berries and peduncles. Different letters in the same column indicate significant differences ($P \le 0.05$).

Daily berry growth rate and berry fresh weight at harvest of 'Aledo' seeded vine were not significantly altered by GA_3 (Fig. 1).

Water changes

In accordance with the increase of berry fresh weight (Fig. 1), 160 mg L^{-1} GA_3 also increased absolute berry water content at harvest with regard to not treated berry; values averaged 2.6 g and 3.3 g water per berry, respectively. In contrast, 'Aledo' did not alter absolute berry water content as a result of treatment (4.1 g and 4.0 g, respectively). However, treatment did not increase significantly relative to fresh weight water content for both 'Emperatriz' (81.8% and 82.1%, respectively) and 'Aledo' (80.1 and 80.0, respectively). Interestingly, both varieties accumulate same quantity of water in relative value to fresh weight and irrespective of the treatment.

These results agree with those of Ψ_w and π (Table 3). Sixty seven days after anthesis, that is, when differences between control and treated berry became larger, water potential and osmotic potential were not significantly modified by GA_3.

The application of 160 mg L^{-1} GA_3 at the onset of cell enlargement stage also increased significantly 'Emperatriz' average berry dry weight (0.71 g) with regard to control (0.60 g). However, berry dry weight of 'Aledo' seeded berries, were not significantly altered by GA_3 (data not shown).

Carbohydrate changes

GA_3 anticipates glucose uptake in the 'Emperatriz' seedless berries compared to untreated berries (Fig. 2). Glucose concentration increased faster in GA_3 160 mg L^{-1} treated berries, reaching maximum concentration 67 DAA, whereas control berries reached maximum value 85 DAA. Similar results were obtained for fructose and sucrose (Fig. 2). Interestingly, peaks for control berries were of the same magnitude of those for treated

Table 3. The effect of 80 mg L^{-1} GA_3 applied at the onset of cell enlargement stage on water (Ψ_w), osmotic (π) and pressure potential (Ψ_p) of the berry flesh 67 DAA (47 days after treatment) in 'Emperatriz seedless and 'Albedo' seeded grape

	Ψ_w (MPa)		π (MPa)		Ψ_p (Mpa)	
	GA_3	Control	GA_3	Control	GA_3	Control
'Emperatriz'	−0.92	−0.88	−1.97	−1.69	1.05	0.81
'Aledo	−0.85	−0.79	−1.85	−1.66	1.00	0.87

Values corresponding to 2005. Values are the mean of four replicates of four berries per treatment. There were no significant differences in the water relations of the flesh.
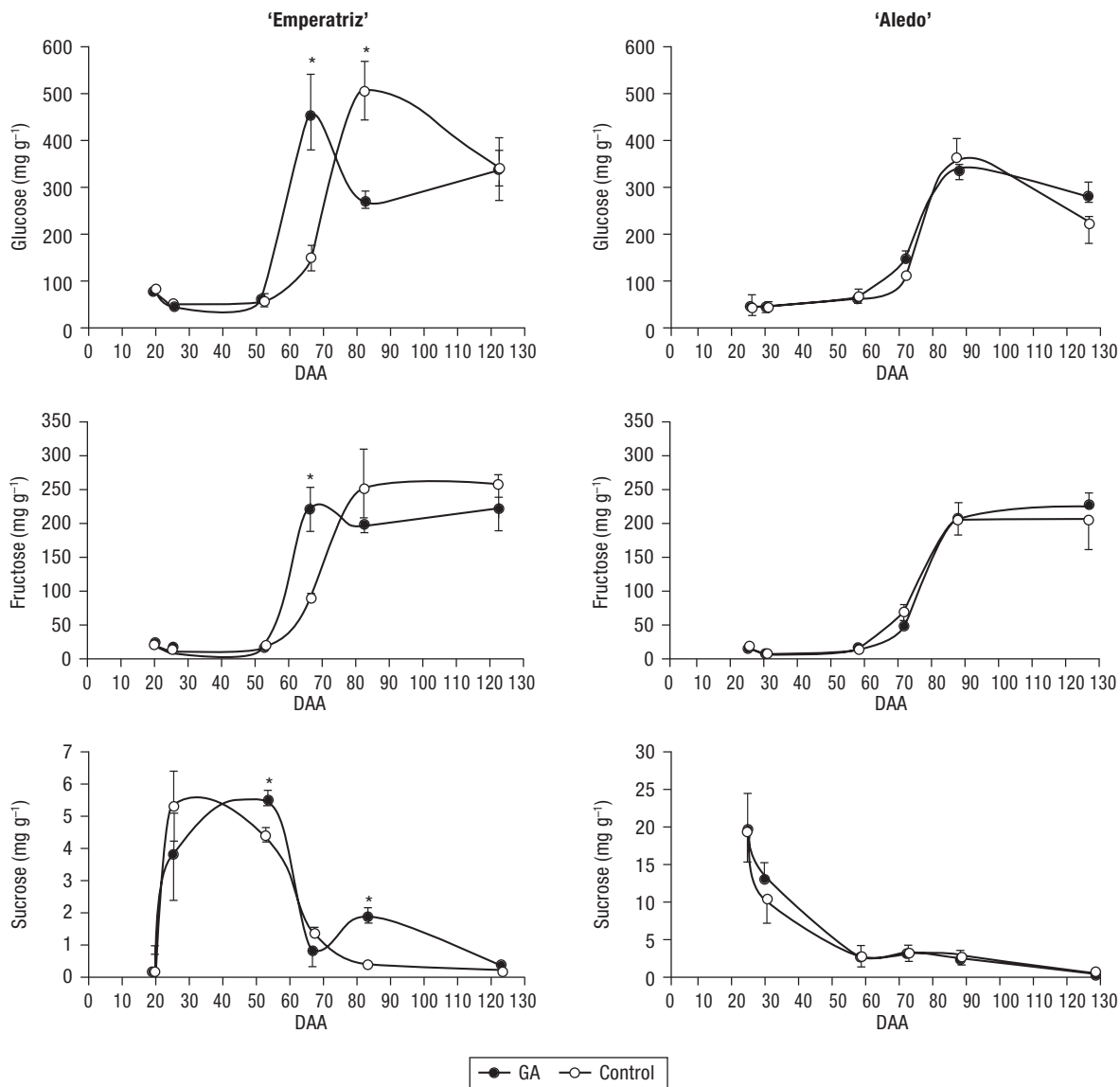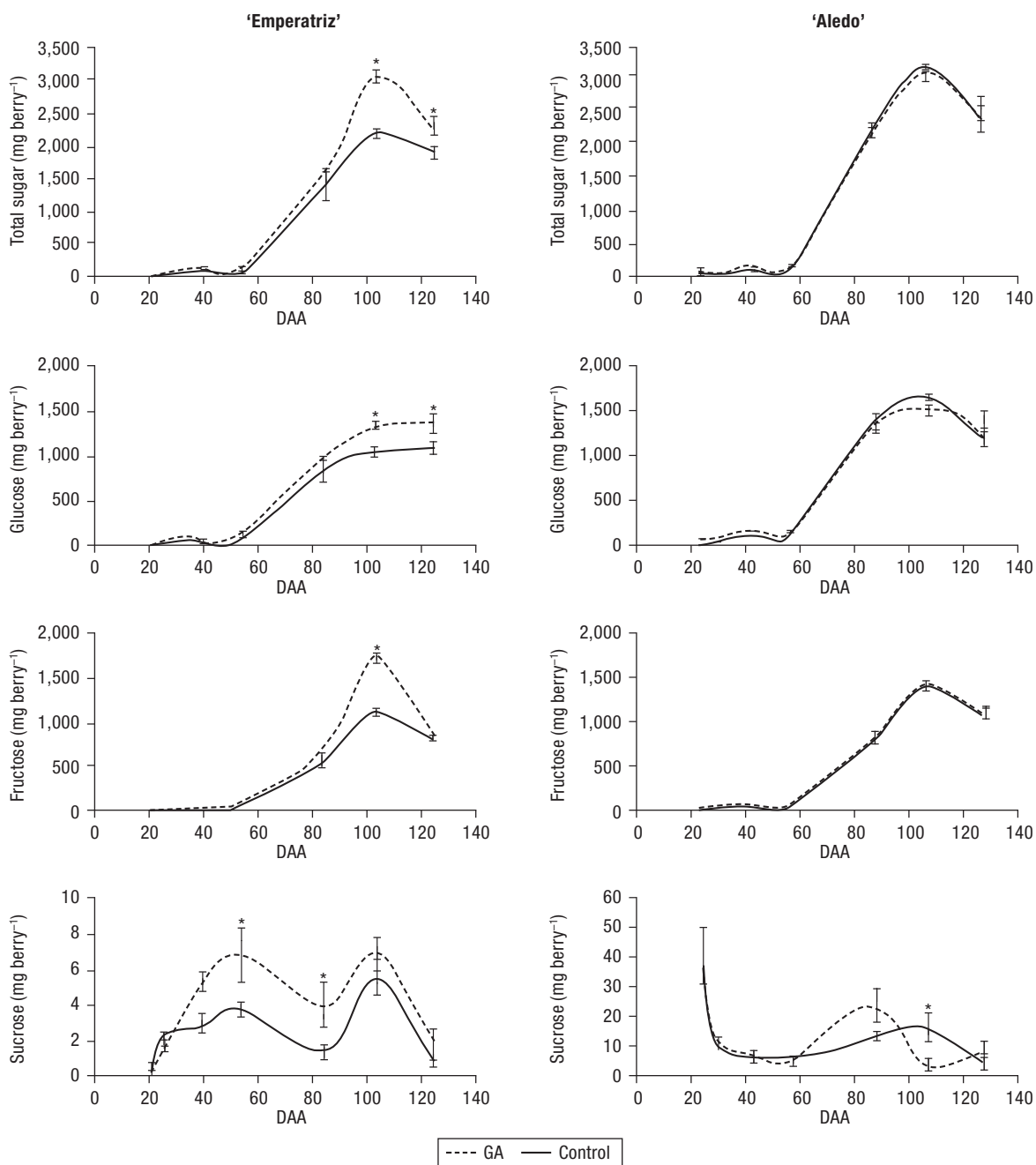

Figure 2. Effect of GA_3 application (160 mg L^{-1}) on sugar content (glucose, fructose and sucrose) in relative value (mg g^{-1} dried weight) in 'Emperatriz' seedless and 'Aledo' seeded grape. GA_3 was applied at the onset of cell enlargement stage of berry development. Values are the mean of six replicates of 10 berries each. Standard errors are given as vertical bars. * means significant differences ($P \leq 0.05$).

berries, irrespective of the sugar (Fig. 2). Despite of differences in berry growth rate and final fruit size, both control and GA_3 treated fruits reached similar glucose, fructose and sucrose concentration. Moreover, berry sugars concentration was similar for both 'Emperatriz' seedless and 'Aledo' seeded varieties, except for sucrose, which had a lower concentration in 'Emperatriz'

with respect to 'Aledo'. Nevertheless, the very low concentration of sucrose compared with glucose and fructose concentration, makes the difference irrelevant.

In absolute value (mg berry^{-1}), 'Aledo' seeded berry had higher glucose and fructose content than 'Emperatriz' seedless berry because of its larger weight (Fig. 3). Also here, sucrose content was lower for 'Emperatriz'

Figure 3. Effect of GA_3 application (160 mg L^{-1}) on sugar content (glucose, fructose and sucrose) in absolute value (mg berry^{-1}) in 'Emperatriz' seedless and 'Aledo' seeded grape. GA_3 was applied at the onset of cell enlargement stage of berry development. Values are the mean of six replicates of 10 berries each. Vertical bars denotes standard error. * means significant differences ($P \le 0.05$).

seedless berry and lower than glucose and fructose content. However, GA₃ increased absolute glucose and fructose content of 'Emperatriz' seedless grape up to values similar to those of 'Aledo' seeded berries. Treatment also increased significantly total content per berry of sucrose, but values remained very low compared to those of glucose and fructose and with 'Aledo' values (Fig. 3). GA₃ had no effect on total sugar content in 'Aledo' seeded berries either (Fig. 3).

Cell changes

The application of 80 mg L^{-1} GA₃ to 'Emperatriz' seedless cultivar at the onset of cell enlargement stage did not affect berry pericarp cell number (data not shown). However, treatment increased pericarp cell diameter. At harvest, pericarp cells of 'Emperatriz' treated berries averaged 124 μm diameter, whereas those from not treated berries averaged 116 μm ($P \leq 0.05$).

Discussion

The effect of GA₃ on 'Emperatriz' seedless berry characteristics and sugar concentration depends on date of treatments and concentration applied. Optimal period of sensitivity coincides with the second peak of gibberellins concentration found in 'Aledo' seeded berries (Scienza *et al*., 1978). It is logical since, in seedless grape, the application of GA₃ compensates for the low gibberellins berry concentrations (Pérez *et al*., 2000), which is correlated with seed number (Lavee and Nir, 1986). Optimal GA₃ concentration varies with cultivar and year, and can be established between 160 and 240 mg L^{-1} GA₃ as a single application. Korkas *et al*. (1999) recommended 320 mg L^{-1} GA₃ to increase bunch weight and 650 mg L^{-1} GA₃ to increase berry weight in 'Sultanina' seedless grape, whereas Bhujbal and Chaudhari (1973) recommended 100 mg L^{-1} and Dass and Radhawa (1972) 75 mg L^{-1}. Environmental factors may be responsible for differences.

The most outstanding result of our experiments is that GA₃, applied at the onset of cell enlargement stage, increases 'Emperatriz' seedless berry weight up to weight similar to that of 'Aledo' seeded grape. This effect is due to an increase on the total sugars content, which, in turn, increases total water content. Indeed, because of the absence of seeds, seedless grape has a lack of suitable sink capacity and the concentration of sucrose that reaches the berry is lower than that of seeded ones, but GA₃-treated-seedless berry increase sink activity (Zhenming *et al*., 2008), measured as sugar uptake, and, thus, increase berry weight. It agrees with changes of source-sink relationship affecting berry development (Wu *et al.,* 2001).

Sugars accumulation is of relevance in wine and table grape cultivar, since high level of sugars at ripening is required for a better berry quality. Besides, sugars bear osmotic driving force for cellular expansion (Stadler *et al*., 1999) and modulation of gene expression (Koch, 1996) through signalling mechanisms (Lalonde *et al*., 1999). In vine, sugars accumulation begins just after veraison (Saito and Kasai, 1978; Hrazdina *et al*., 1984; Possner and Kliever, 1985) and continues slowly, but constantly, up to maturity. In our experiments, GA₃ anticipated and increased in absolute value sugars uptake in 'Emperatriz' seedless grape vine, thus increasing sugars content up to similar values of 'Aledo' seeded berries, indicating that seeds may regulate fruit development by means of gibberellins, as suggested by Pérez *et al.* (2000) and Göktürk and Harmankaya (2005).

Although GA₃ treated 'Emperatriz' seedless berries accumulate higher amounts of sugars and water, not significant differences in osmotic potential and water potential were found with regard to not treated berries. It is due to the effect of GA₃ increasing cell size that allows accumulating larger amounts of water and sugars without changing pressure potential.

In conclusion, GA₃ applied to 'Emperatriz' seedless grape at the onset of cell enlargement stage increases berry fruit weight up to values similar to that of 'Aledo' seeded berries suggesting that GA₃ compensates for the absent of seeds. This effect takes place to a higher accumulation of both sugars and water provided by its effect increasing cell size, all together resulting in a larger 'Emperatriz' seedless berry size at harvest.

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