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# Gibberellins and Abscisic Acid Promote Carbon Allocation in Roots and Berries of Grapevines

**Daniela Moreno · Federico J. Berli · Patricia N. Piccoli · Rubén Bottini**

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**Abstract** Carbon allocation within grapevines may affect berry growth and development. The plant hormones gibberellins (GAs) and abscisic acid (ABA) control various processes across the plant life and both have been involved in assimilate production and transport in different species. Hence, this work examined the distribution of sugars (sucrose, fructose, and glucose) and starch in grapevines at *veraison* after foliar applications of $GA_3$, ABA, and an inhibitor of GA biosynthesis, paclobutrazol (PBZ). The results demonstrated that $GA_3$ increased total grapevine mass, with carbon allocated to the whole grapevine (as structural and soluble carbohydrates). Both $GA_3$ and ABA increased monosaccharide (glucose and fructose) levels in berries (up to tenfold) and roots (up to threefold). However, $GA_3$ increased the net carbon fixation whereas ABA did not. PBZ diminished most growth parameters except grapevine mass, and allocated more carbohydrates to roots (up to threefold more sucrose and starch). Such results indicate that GAs promote net carbon fixation and transport, whereas ABA as a stress signal only enhances sugar transport; notwithstanding the two hormones promoted carbon allocation toward roots and berries.

**Keywords** Abscisic acid · Gibberellic acid · *Vitis vinifera* · Sugars · Starch

D. Moreno · F. J. Berli · P. N. Piccoli · R. Bottini (✉)
Instituto de Biología Agrícola de Mendoza (IBAM), Facultad de Ciencias Agrarias, Consejo Nacional de Investigaciones Científicas y Técnicas-Universidad Nacional de Cuyo, Almirante Brown 500, M5528AHB Chacras de Coria, Argentina
e-mail: rbottini@fca.uncu.edu.ar

## Introduction

The allocation of resources in different tissues is a fundamental process where leaves are the sources that provide carbohydrates to the rest of the heterotrophic tissues, like roots, trunks, and fruits; and sucrose is the predominant metabolite for carbon transportation in grapevine, as in most higher plants (Avigad 1982; Kühn and others 1999; Zapata and others 2004), while starch is the carbon reserve form (Bouard 1966). Plant tissues compete for photoassimilates, and their distribution will determine if the plant favors vegetative growth, reproductive development, or starch accumulation.

Wine's excellence is directly related to fruit quality, so it is very important to assure high sugar concentration in grapes at harvest for winery purposes. This is associated with carbohydrate distribution during grapevine growth and development, which in berries is important not only as substrate for further ethanol production in the winemaking process but also for their metabolism in compounds related with the fruit and hence the wine's flavor (Hornsey 2007). Phytohormones may be key compounds in regulating carbon mass production and allocation (Davies 2005; Wheeler and others 2009).

Gibberellins (GAs) are acidic diterpenes with phytohormonal characteristics that control various processes across the plant's life (Crozier and others 2000). Although the most conspicuous function of GAs is to induce internode elongation, there are reports regarding promotion of sucrose accumulation in several species following $GA_3$ application, most noticeable in sugar cane shoots (Nickell 1988), but also in grape berries (Fidan and others 1981; Nakamura and Hori 1985). In *Sorghum bicolor*, however, $GA_3$ promoted glucose and fructose but not sucrose concentration in shoots along with decreases in starch

concentrations in leaves (Bastián and others 1999), which indicate that the hormone stimulates α-amylase activity like it does in germinating seeds (Woodger and others 2004). This implies that GAs facilitate transport and allocation of photoassimilates by favoring sugar discharge from the sources (Daie 1987) because it has been shown that photosynthesis is inhibited by accumulation of products in chloroplasts (Sawada and others 2001). The growth retardant paclobutrazol (PBZ) is an inhibitor of P450 monooxygenases involved in *ent*-kaurene oxidation (Hedden and Graebe 1985), a crucial step of GAs biosynthesis, which therefore reduces the tissue levels of "active" GAs, that is, $GA_1$, $GA_3$, and $GA_4$ (Crozier and others 2000). Therefore, PBZ application to the soil or by foliar sprays reduces shoot growth in different species (Williams and Edgerton 1983; Asin and Vilardell 2006), including grapevines (Wample and others 1987; Calissi and Eaton 1989; Reynolds and others 1992; Carreño and others 2007).

Abscisic acid (ABA) is a plant hormone generally involved in plant responses to stress (Dood and Davies 2005) and possibly in the control of berry development by triggering the initiation of ripening (Antolín and others 2003; Wheeler and others 2009). Berry skin ABA levels increase markedly during *veraison* (when berries soften, accumulate sugars actively, and the pigment development in the skin is initiated) and then decline as ripeness approaches (Nambara and Marion-Poll 2005; Gagné and others 2006; Deytieux-Belleau and others 2007). The supposition that ABA controls grape berry maturation has been substantiated by the demonstration that sprayed ABA enhances several processes involved in berry ripening, such as anthocyanin biosynthesis and sugar accumulation (Pan and others 2005). It has also been reported that ABA is involved in assimilate transport in other plant species, like cereals (Brenner and Cheikh 1995; Yang and Zhang 2006; Travaglia and others 2007) and soybean (Travaglia and others 2009). However, the suggestion that ABA is involved in the regulation of assimilate partitioning toward developing tissues has remained in dispute (Schussler and others 1991; de Bruijn and Vreugdenhil 1993; Sharp and LeNoble 2002; Gazzarini and McCourt 2003).

Cultural practices include the use of $GA_3$ sprays either at anthesis, which reduces the number of flowers that set, or shortly before *veraison*, which will increase berry size. ABA applications to the berries on pre-*veraison* improve the skin coloring and sugar accumulation in anticipation of the onset of *veraison*.

Based on the above, this work studied the distribution of sugars (sucrose, fructose, and glucose) and starch at *veraison*, the moment of the supposedly most "active" transportation, in different tissues of grapevines cv. Malbec after foliar applications of $GA_3$, ABA, and PBZ.

## Material and Methods

### Plant Material and Treatments

The experiment was carried out at Facultad de Ciencias Agrarias, Universidad Nacional de Cuyo, Mendoza, Argentina (33°0′S, 68°52′W) during the 2007–2008 season. Two-year-old grapevines of a selected clone of *Vitis vinifera* L. cv. Malbec were planted in 2.5-L plastic pots with grape compost as substrate. Grapevines were pruned to the fifth node from the base of the shoot and allowed to break buds and grow under field conditions without water deficit in a random design with four treatments and five replicates (experimental unit = one grapevine). When the lower leaves were fully expanded (ca. 15 days after sprouting), the following solutions were sprayed weekly with a hand-held sprayer onto the grapevines until runoff (ca. 25 mL per grapevine). The spraying was done in the late afternoon to minimize photodegradation of ABA. Treatments were ABA (±-S-*cis*, *trans* abscisic acid, 90%, Kelinon Agrochemical Co., Beijing, China, 250 mg $L^{-1}$), $GA_3$ (Sigma-Aldrich, St. Louis, MO, USA, 500 mg $L^{-1}$), paclobutrazol (PBZ, as Crestar, Zeneca Agricultural, Wilmington, DE, USA, 200 mg $L^{-1}$), and control (water). All solutions, including the control, contained a minimum amount of 96% aqueous ethanol to initially dissolve the growth regulators (ca. 10 μL $mg^{-1}$) and 0.1% (v/v) Triton X-100 as a surfactant. The treatment doses were chosen based upon previous work by our group with different species (Bastián and others 1999; Travaglia and others 2007, 2009), including grapevine (Quiroga and others 2009; Berli and others 2010).

At *veraison* (stage 35, Coombe 1995), 50 days after the imposition of the treatments, three replicates were randomly selected around 10:00 a.m. and each grapevine was separated into the following tissues: (1) shoot apex, (2) fifth fully expanded leaf from the shoot apex (considered a new leaf), (3) berries, (4) basal leaf (second leaf from the base of the shoot, considered a mature leaf), (5) basal stem (first internode from the base of the shoot), (6) woody stem (previous year's woody shoot's main axis), and (7) roots. The tissues were weighed and the total grapevine mass calculated. Intermediate leaves were used for destructive measurement and then their soluble carbohydrate (sugars and starch) concentration was estimated based on the leaf weight. The tissues were then frozen in liquid nitrogen and stored at −20°C until processing.

### Sugars and Starch

Each tissue sample was homogenized in a mortar with liquid nitrogen, and 100 mg of the powdered tissue was placed into a 1.5-mL microfuge tube and extracted with

800 μL of 80:20 (v/v) ethanol:twice-distilled water for 90 min at 70°C. The samples were then centrifuged for 10 min at 15,000×*g*, the supernatants were collected, and the pellets were extracted twice, as described above. A 500-μL aliquot of the supernatants was evaporated in a rotary evaporator with vacuum at room temperature. The dried extracts were added with 40 μL of 20-mg $mL^{-1}$ methoxyamine hydrochloride into dry pyridine (both from Fluka, Steinheim, Germany) and maintained for 15 min at 70°C. Then, 10 μL of dry pyridine and 80 μL of MSTFA (*N*-methyl-*N*-trimethylsilyltrifluoroacetamide; Sigma-Aldrich, St. Louis, MO, USA) were added for derivatization and left for 30 min at 37°C. Finally, a 1-μL aliquot of the mixture was injected into a splitless mode in a PerkinElmer Clarus 500 capillary gas chromatograph–electron impact mass spectrometer (GC-EIMS, PerkinElmer, Shelton, CT, USA) equipped with an AS 2000 autoinjector sampler. The column used was a PerkinElmer 5-MS of 0.25-μm film thickness, 0.25-mm internal diameter, and 30 m long. The injection temperature was 220°C, and the column was kept at 100°C for 30 s, and then changed from 100 to 200°C at a rate of 16°C $min^{-1}$, then up to 280°C at a rate of 4°C $min^{-1}$, and finally kept at 280°C for 10 min. The flow of the gas carrier (He) was 1 mL $min^{-1}$, the interface temperature was 250°C, and the flow of electrons was set at 70 eV. The total ion chromatogram was monitored from 6 min up to the end of the run. The identification of glucose, fructose, and sucrose was made by comparison with the mass spectra of standards purchased from Sigma-Aldrich (St. Louis, MO, USA). For quantification of glucose, fructose, and sucrose in the samples analyzed, standard curves were obtained (ca. $R^2 = 0.99$) by successive injections of increasing amounts of sugars from a stock solution of sugar in dry pyridine, that is, 0.1, 0.5, 1, 5, and 10 ng $\mu L^{-1}$ after derivatization as described above. The concentrations of sugars were calculated from the values of the integration area of the chromatographic peaks of the most abundant ion for the corresponding sugar. Sucrose, glucose, and fructose were summed to calculate total sugars (TS).

Starch was quantified from the pellets obtained after the extraction of sugars described above. The pellets were desiccated for 24 h at 35°C, and then 1.5 mL of dimethylsulfoxide (DMSO) was added and allowed to extract for 45 min at 60°C. The samples were cooled to room temperature and centrifuged for 10 min at 10,000×*g*. A 150-μL aliquot of the supernatant was treated with 150 μL of iodine reagent (aqueous solution of 5% $I_2$ and 10% KI, w/v) and diluted with 3 mL of twice-distilled water, and then the absorbance was measured at 610 nm against a blank of reagent with a Cary-50 UV–Vis spectrophotometer (Varian Inc., Palo Alto, CA, USA). The concentrations were calculated from a standard curve using known concentrations of starch (Merck KGaA, Darmstadt, Germany) ($R^2 = 0.99$). Then, starch and TS were summed to calculate total soluble carbohydrates and used to determine starch concentration relative to total carbohydrates.

## Growth Parameters

Shoot and internode lengths and the number of leaves were measured in the five replicates every 15 days, beginning the fifth day after the first application (DAFA) of the treatments. At the end of the experiment (50 DAFA), all the leaves of each shoot were collected in nylon bags and kept in ice to prevent dehydration. The weight of all leaves from a single grapevine replicate and the weight of a 1-$cm^2$ leaf disc were measured and the leaf area (LA) was calculated according to the relationship between these parameters.

## Photosynthesis and Stomatal Conductance

Leaf net photosynthesis (NP, μmol $CO_2$ $m^{-2}$ $s^{-1}$) and stomatal conductance ($g_s$, mmol $H_2O$ $m^{-2}$ $s^{-1}$) were measured in the five replicates at 50 DAFA using an infrared gas analyzer (IRGA CIRAS-2, PP System, Amesbury, MA, USA). Measurements were taken on the first fully expanded leaf from the shoot apex between 09:00 and 12:00 a.m. The NP per area basis was multiplied by the grapevine LA to calculate the NP per grapevine.

## Photosynthetic Pigments

Fifty days after treatment imposition, chlorophyll (Chl) *a* and *b*, carotenoids, and total Chl concentration (Chl *a* + Chl *b*) were determined in the five replicates using a Cary-50 UV–Vis spectrophotometer (Varian) and the extraction technique described by Chappelle and others (1992) and the formulas of Wellburn (1994). A 1-$cm^2$ single leaf disc taken from the seventh fully expanded leaf from the shoot apex was placed in 5 mL of DMSO and allowed to extract for 45 min in darkness at 70°C. Absorbance was read at 665, 649, and 480 nm against a blank of reagents with 10-mm optical path cells. The photosynthetic pigments per area basis were multiplied by the grapevine LA to calculate these values per grapevine.

## Statistical Analysis

One-way ANOVA and Fisher's multiple comparison of means were used to discriminate between the averages by the minimum difference, with a significance level of $p \leq 0.05$. Analyses were performed with Statgraphics Centurion XV ver. 15.0.10 (Statpoint Technologies Inc., Warrenton, VA, USA).

## Results and Discussion

The application of $GA_3$ and ABA did not affect the number of leaves and the shoot and internode lengths when compared to the control treatment (Fig. 1). The application of PBZ decreased the aforementioned growth parameters at 35 and 50 DAFA compared to the other two plant growth regulators and control treatments. It appears that the amount of endogenous GAs in those tissues was enough to sustain "normal" growth, so that the $GA_3$ applications did not promote vegetative growth. This is similar to reports from Pires and Botelho (2002) and Todic and others (2005) who found no increase in shoot length after $GA_3$ application to the foliage of grapevines. Notwithstanding, when endogenous GAs levels were apparently reduced by PBZ, the growth parameters decreased, in agreement with other authors who applied PBZ to the soil or by foliar sprays to grapevines (Buban 1986; Reynolds and Wardle 1990; Reynolds and others 1992; Carreño and others 2007). Thompson and others (2007) reviewed several studies of ABA application and with ABA-deficient mutants, establishing that ABA can have both positive and negative effects on growth, depending on tissue, the concentration applied, and interactions with the environment. In fact, Sansberro and others (2004) showed that ABA promoted vegetative growth in *Ilex paraguariensis*, which was positively correlated with a decrease in stomatal aperture and increases in the relative water content in leaves. Therefore, ABA promoted the higher cell turgor required for cellular expansion (Acevedo and others 1971). Besides, our results corroborate previous findings showing that ABA administered at a physiological dose has no direct effect on visible growth in several plant species (Travaglia and others 2007, 2009; Cohen and others 2009), including grapevine (Quiroga and others 2009).

The application of $GA_3$ increased total grapevine LA, whereas the application of PBZ reduced it (Fig. 2a). Also, individual LA was increased by $GA_3$ and reduced by PBZ (Fig. 2b), indicating that GAs enhanced leaf expansion, even if endogenous GAs were "normal," whereas PBZ repressed it, as found in other studies (Intrieri and others 1986; Métraux 1988; Hunter and Proctor 1990; Christov and others 1995; Mostafa and Saleh 2006; Steffens and others 2006). Total Chl concentration per area basis was decreased by $GA_3$ application and increased by PBZ (Fig. 2c), whereas carotenoids were increased by PBZ and ABA (Fig. 2d). These results seem to reflect an indirect effect of pigment concentration; that is, an apparently similar amount of photosynthetic pigments were diluted or concentrated in larger or smaller leaves due to the application of $GA_3$ and PBZ, respectively (Fig. 2b). This was confirmed when total Chl and carotenoid pigments were multiplied by the total grapevine LA and the differences between treatments disappeared (data not shown). ABA had no effect on either total grapevine LA or Chl concentration (Fig. 2a, c). However, as it has been reported in other plant species and also in grapevine (Travaglia and others 2007, 2009; Berli and others 2010), carotenoid concentrations were enhanced by ABA applications (Fig. 2d). It would appear that ABA activates the

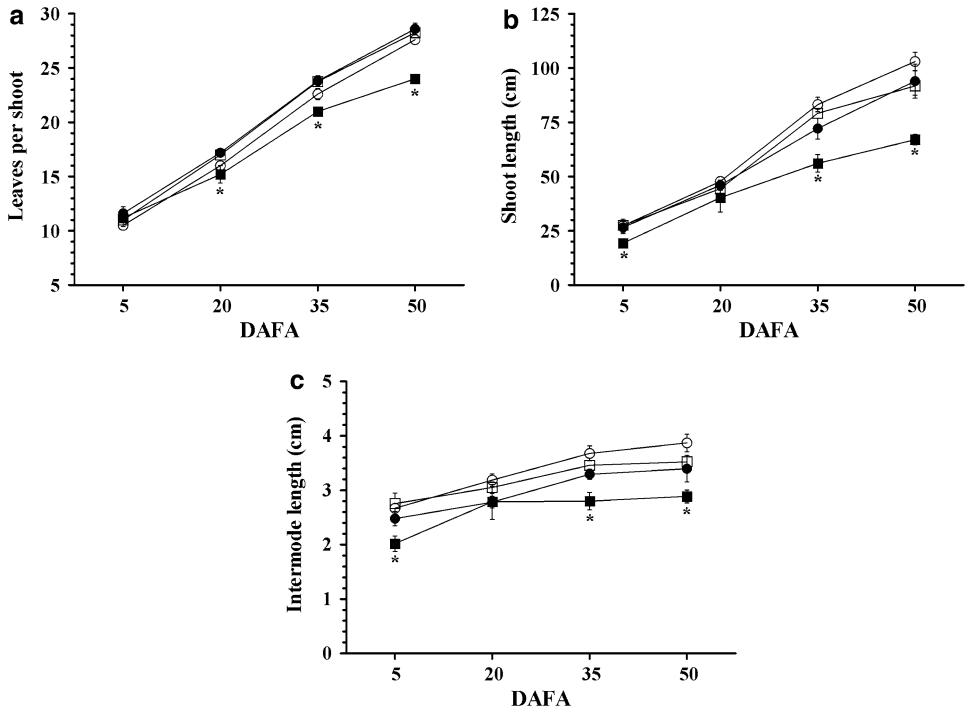


**Fig. 1** Growth characteristics of grapevines treated with ABA (*filled circle*), $GA_3$ (*open circle*), PBZ (*filled square*), and control (*open square*) for number of leaves per shoot (**a**), shoot length (**b**), and internode length (**c**). Measurements commenced 5 days after the first application (DAFA) of the growth regulators. Values are means ± SE and * indicates days in which treatment means were significantly different from the control ($p \leq 0.05$)

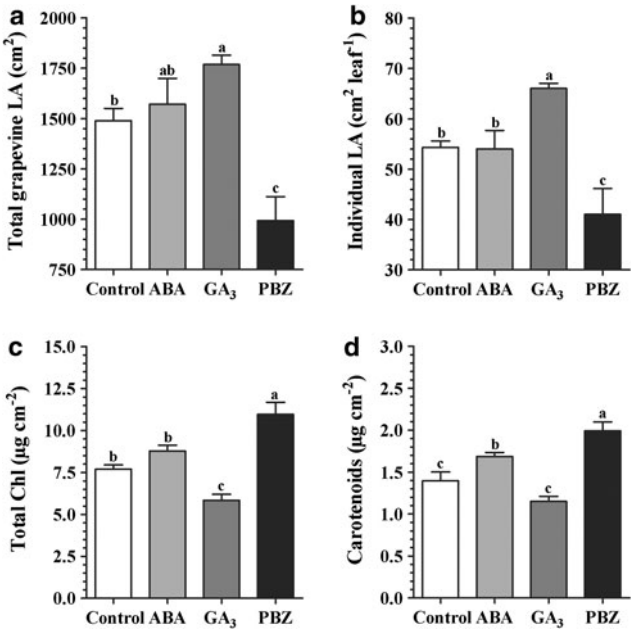


**Fig. 2** Total grapevine leaf area (LA) (**a**), individual LA (**b**), total chlorophylls (Chl) (**c**), and carotenoids (**d**) of grapevines treated with ABA (*light gray*), $GA_3$ (*dark gray*), PBZ (*black*), and control (*white*), measured at *veraison*, 50 days after the first application (DAFA) of the growth regulators. Values are means ± SE and the treatment means are significantly different if a *different letter* is placed atop the *treatment bars* ($p \leq 0.05$)

antioxidant system where carotenoids play an important role (Berli and others 2010). The higher concentration of photosynthetic pigments in the PBZ-treated grapevines thus correlated with enhanced NP and $g_s$ (Fig. 3a, b). The smaller LA (Fig. 2a, b) obtained due to the application of PBZ increased the concentration of the photosynthetic machinery to include more stomatal pores (data not given), light-capturing pigments (Fig. 2c, d), and $CO_2$-fixing activities per unit area (Fig. 3). Others have found that PBZ reduced LA and produced thicker leaves, thus increasing the photosynthetic capacity in grapevines (Wample and others 1987; Calissi and Eaton 1989) and the leaf gas exchange parameters (Abdul Jaleel and others 2007).

Another effect attributable to "active" GAs was the differential organ growth. Figure 4 shows that the ratio between the aerial part and roots was increased after $GA_3$ application, whereas the application of PBZ decreased it, showing a preferential growth of roots as was found in apples by El Hodairi and Canham (1990) and in grapevine by Todic and others (2005). Enhanced root formation is related to reduced shoot elongation and a larger partitioning of assimilates to the roots (Davis and others 1988). The total grapevine mass was augmented by $GA_3$ and decreased by PBZ (Fig. 5a), showing that although the photosynthetic activity may be higher on a per-area basis in the latter treatment, "active" GAs stimulated total grapevine net photosynthesis due to a higher LA (data not given). The increase in the NP activity with the application of $GA_3$ correlated with a major total grapevine mass; this was also found by others in wheat (Ashraf and others 2002). Superior growth may be the result of a higher efficiency and/or capacity in carbon gain, transport and allocation, and a general stimulation of growth. Sink strength can regulate the photosynthetic activity of the source leaves (Kaitaniemi and Honkanen 1996) and such increases can stimulate photosynthesis (Bazzaz and others 1987). Subrahmanyam and Rathore (1992) found that $GA_3$-treated mustard plants increased the export of assimilates out of source organs into reproductive organs due to the stimulation of sink activity. $GA_3$ stimulated α-amylase activity, which hydrolyzes starch to glucose (Kinet 1993; Woodger and others 2004), and decreased starch concentration in leaves (Bastián and others 1999), favoring sugar discharge from the sources (Daie 1985). In fact, sugars (calculated as TS or separated into sucrose, glucose, and fructose) were markedly enhanced by the treatment with $GA_3$ (Fig. 5b–e).

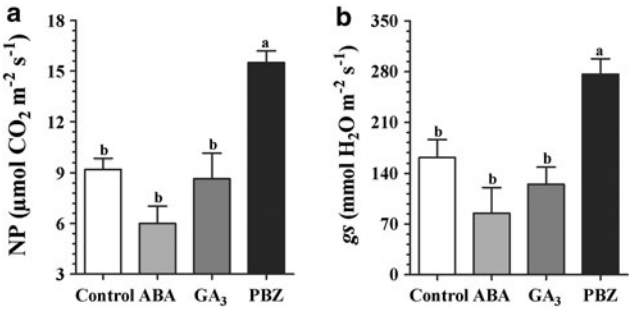


**Fig. 3** Leaf net photosynthesis (NP) (**a**) and stomatal conductance ($g_s$) (**b**) of grapevines treated with ABA (*light gray*), $GA_3$ (*dark gray*), PBZ (*black*), and control (*white*), measured at *veraison*, 50 days after the first application (DAFA) of the growth regulators. Values are means ± SE and the treatment means are significantly different if a *different letter* is placed atop the *treatment bars* ($p \leq 0.05$)

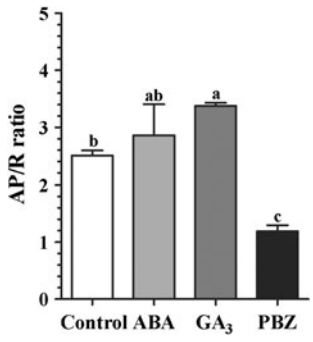


**Fig. 4** Ratio between aerial part and root weights (AP/R ratio) of grapevines treated with ABA (*light gray*), $GA_3$ (*dark gray*), PBZ (*black*), and control (*white*), measured at *veraison*, 50 days after the first application (DAFA) of the growth regulators. Values are means ± SE and the treatment means are significantly different if a *different letter* is placed atop the *treatment bars* ($p \leq 0.05$)

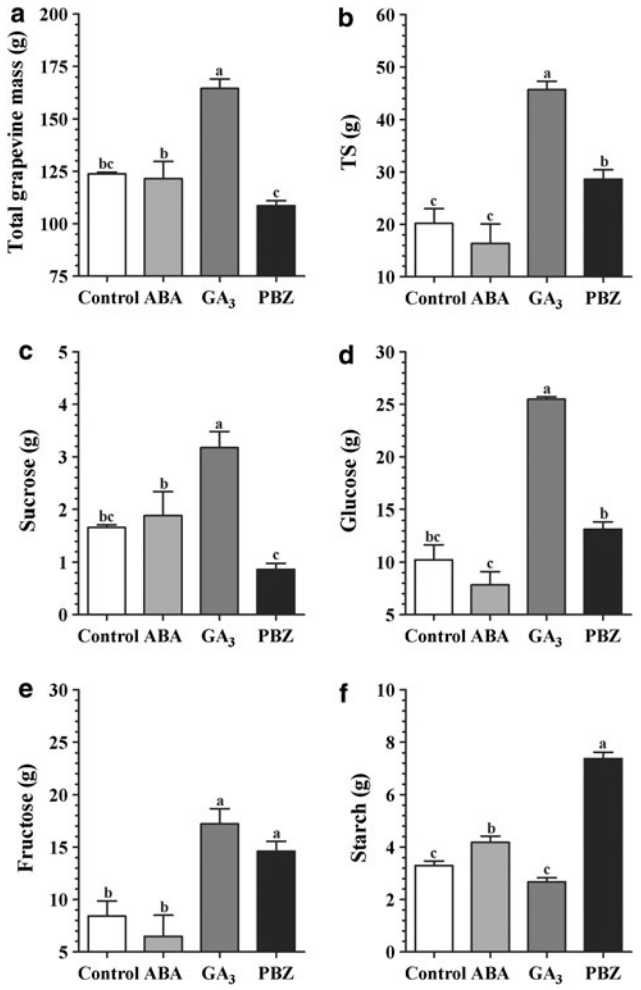


**Fig. 5** Total grapevine mass (**a**), total sugars (TS) (**b**), sucrose (**c**), glucose (**d**), fructose (**e**), and starch (**f**) of grapevines treated with ABA (*light gray*), $GA_3$ (*dark gray*), PBZ (*black*), and control (*white*), measured at *veraison*, 50 days after the first application (DAFA) of the growth regulators. Values are means ± SE and the treatment means are significantly different if a *different letter* is placed atop the *treatment bars* ($p \leq 0.05$)

Concurrently, $GA_3$-treated grapevines allocated less starch in the whole plant (Fig. 5f) compared with what occurs with ABA and PBZ treatments. Moreover, the total carbon allocation deduced from total grapevine mass reached the maximum in $GA_3$-treated grapevines (Fig. 5a). PBZ increased TS mainly because of the effect on fructose (Fig. 5b–e) and starch (Fig. 5f). ABA-treated grapevines did not show differences with respect to controls, except that starch accumulation was favored overall (Fig. 5a–f). The total grapevine starch concentration relative to total carbohydrates was reduced by $GA_3$ (mainly in root and leaves) and increased by PBZ (in roots) and ABA (in stems and basal leaves) (Table 1). Accumulation of starch, the carbohydrate reserve form, seems to be favored by PBZ and ABA, whereas $GA_3$ promoted accumulation of sugars,

**Table 1** Starch concentration relative to total carbohydrates (in %) of the whole grapevine and the different tissues of plants treated with ABA, $GA_3$, PBZ, and Control, measured by GC-EIMS at *veraison*, 50 days after the first application (DAFA) of the growth regulators

| Tissues | Starch concentration relative to total carbohydrates | | | |
|---|---|---|---|---|
| | Control | ABA | $GA_3$ | PBZ |
| Whole grapevine | 14.2 b | 21.7 a | 5.5 c | 20.7 a |
| Shoot apex | 3.2 a | 3.3 a | 1.3 a | 1.6 a |
| New leaves | 11.6 a | 8.4 b | 1.0 c | 2.2 c |
| Berries | 0.3 ab | 0.3 ab | 0.2 b | 0.7 a |
| Basal leaves | 8.1 b | 12.1 a | 2.1 c | 7.2 b |
| Basal stem | 8.4 b | 19.1 a | 6.9 b | 7.2 b |
| Woody stem | 23.0 b | 41.3 a | 23.6 b | 32.2 ab |
| Roots | 24.8 b | 24.9 b | 4.9 c | 39.1 a |

Values are means, and the treatment means are significantly different for each tissue if a different letter is indicated ($p \leq 0.05$)

**Table 2** Relative abundance of carbohydrates (in %) of the whole grapevine and the different tissues of control plants, analyzed by GC-EIMS at *veraison*, 50 days after the first application (DAFA) of the growth regulators

| Tissues | Relative abundance of carbohydrates | | | |
|---|---|---|---|---|
| | Sucrose | Glucose | Fructose | Starch |
| Whole grapevine | 7.3 | 43.2 | 35.3 | 14.2 |
| Shoot apex | 2.7 | 68.8 | 25.3 | 3.2 |
| New leaves | 8.0 | 38.9 | 41.5 | 11.6 |
| Berries | 1.4 | 66.0 | 32.6 | 0.0 |
| Basal leaves | 9.4 | 42.0 | 40.5 | 8.1 |
| Basal stem | 8.2 | 49.1 | 34.3 | 8.4 |
| Woody stem | 8.0 | 31.2 | 37.7 | 23.0 |
| Roots | 8.6 | 36.8 | 29.8 | 24.8 |

including sucrose, the migratory form of carbon. Table 2 shows that carbohydrates are differentially distributed in the tissues of the control grapevine. The starch concentration was very low in the shoot apex and berries, but it was relatively high in the leaves and the basal stem, and especially abundant in woody stem and roots.

Table 3 gives the distribution of TS, sucrose, glucose, fructose, and starch along the grapevine tissues. Both hormones ($GA_3$ and ABA) stimulated the presence of sugars as glucose and fructose in berries up to tenfold and in roots up to threefold, implying enhancement of sugar transport toward organs involved in the grapevine's survival. Coincidentally, total sugars present in the stem (both the basal portion of the current year and the woody portion from the previous year's growth) were reduced by almost half in the $GA_3$- and ABA-treated grapevines. The hampering of active GA synthesis by PBZ allocated less sugars as glucose and fructose in the new portion of the shoot

**Table 3** Distribution of total sugars (TS), sucrose, glucose, fructose, and starch (in %) along the different grapevine tissues, measured by GC-EIMS at *veraison*, 50 days after the first application (DAFA) of the growth regulators

| Tissues | Treatments | Distribution of carbohydrates | | | | |
|---|---|---|---|---|---|---|
| | | TS | Sucrose | Glucose | Fructose | Starch |
| Shoot apex | Control | 16.2 a | 6.1 a | 22.9 ab | 10.3 a | 3.2 a |
| | ABA | 16.0 a | 4.6 a | 26.0 a | 6.1 b | 1.9 ab |
| | $GA_3$ | 11.3 a | 3.8 a | 16.6 b | 4.7 b | 2.5 ab |
| | PBZ | 5.1 b | 3.9 a | 9.4 c | 1.3 c | 0.3 b |
| New leaves | Control | 8.5 a | 9.1 a | 7.2 ab | 9.5 a | 6.6 a |
| | ABA | 10.9 a | 10.9 a | 11.0 a | 12.5 a | 4.1 a |
| | $GA_3$ | 4.7 b | 2.9 b | 4.3 b | 5.6 b | 1.0 b |
| | PBZ | 5.1 b | 10.5 a | 6.0 b | 3.6 b | 0.5 b |
| Berries | Control | 2.0 b | 0.3 a | 2.6 c | 1.6 b | 0.1 a |
| | ABA | 15.7 a | 0.7 a | 19.1 ab | 15.5 a | 0.1 a |
| | $GA_3$ | 21.5 a | 0.9 a | 28.4 a | 17.0 a | 0.7 a |
| | PBZ | 6.2 b | 0.9 a | 9.4 bc | 3.6 b | 0.2 a |
| Basal leaves | Control | 18.1 a | 22.0 b | 17.0 a | 20.2 a | 9.8 a |
| | ABA | 6.8 c | 14.8 c | 4.6 c | 7.5 c | 3.3 c |
| | $GA_3$ | 17.0 a | 27.2 a | 11.2 b | 23.0 a | 6.0 b |
| | PBZ | 12.4 b | 12.2 c | 11.3 b | 13.5 b | 3.7 c |
| Basal stem | Control | 16.4 b | 14.1 a | 17.3 b | 15.4 b | 9.2 ab |
| | ABA | 8.1 c | 12.6 a | 7.2 c | 7.5 c | 6.6 b |
| | $GA_3$ | 11.3 bc | 15.8 a | 11.8 bc | 9.2 bc | 11.7 a |
| | PBZ | 30.0 a | 13.3 a | 28.6 a | 32.3 a | 8.9 ab |
| Woody stem | Control | 30.4 a | 37.1 ab | 25.0 a | 35.1 a | 54.3 a |
| | ABA | 23.3 b | 42.4 a | 16.5 a | 27.7 b | 61.6 a |
| | $GA_3$ | 11.1 c | 34.1 ab | 5.6 b | 15.1 c | 58.1 a |
| | PBZ | 24.2 ab | 27.9 b | 22.9 a | 25.1 b | 44.0 a |
| Roots | Control | 8.3 b | 11.3 b | 8.0 c | 8.0 b | 16.8 b |
| | ABA | 19.4 a | 14.0 b | 15.6 ab | 23.2 a | 22.4 b |
| | $GA_3$ | 23.1 a | 15.3 b | 22.1 a | 25.3 a | 20.0 b |
| | PBZ | 17.2 ab | 31.3 a | 12.4 bc | 20.7 ab | 42.4 a |

Values are means, and the treatment means are significantly different for each tissue and carbohydrate if a different letter is indicated ($p \leq 0.05$)

(shoot apex and new leaves). PBZ also augmented glucose and fructose in the basal stem and sucrose and starch in the roots. The latter statement is in agreement with the preferential growth of roots stimulated by PBZ treatment. It is worth noting that sugar concentration in berries at this time was quite low because the experiment was concluded at *veraison*. Therefore, the picture obtained was a "snapshot" of sugar transport and metabolism at that time and not at the time of final accumulation of sugars in the berries one would expect at maturity.

The increase of sugar transport toward organs involved in grapevine survival may be explained by the enhancement of amylase and invertase activities by $GA_3$, which in turn could stimulate sugar transport and allocation, as is suggested to occur in grapevines (Zhang and others 2006; Deluc and others 2007). Notwithstanding, the overall stimulation of cell expansion by GAs should not be discounted, because general increases in both sugar production and total grapevine mass were found (Fig. 5a, b). In the case of stimulation by ABA of sugar transport toward berries and roots, the situation may be explained through the enhancement of ABA stress and ripening-induced proteins (ASR, Çakir and others 2003) that facilitate sugar transport across the membranes, the increase of cell wall-modifying enzymes (Koyama and others 2010), and the promotion of invertases and hexose transporters (both expression and activity) by ABA application in grapevine (Çakir and others 2003; Pan and others 2005; Conde and others 2006; Hayes and others 2010). An augmentation of ABA concentration in the grape berry preceding the accumulation of soluble solids has been described (Pan and others 2005), achieving a maximum at the end of *veraison* (Antolín and others 2006), which possibly enhances berry sink strength. Others have found the enhancement in photoassimilate allocation in developing grains in both gramineae [wheat (Travaglia and others 2007); rice (Yang and Zhang 2006)] and dicots [soybean (Travaglia and others 2009)] when the stress signal was enhanced via ABA application. It is also worth noting that the increase in carbohydrate allocation toward reproductive organs caused by ABA was accomplished without further increases in overall carbon accumulation. Additionally, ABA was able to increase berry set in field-grown grapevines, therefore augmenting yield without net increases in the total grapevine mass (Quiroga and others 2009; Wheeler and others 2009).

In conclusion, the final picture of the experiments related here is that GAs, as growth promoters, are responsible for superior overall growth assessed as total grapevine mass and which was correlated with a major carbon fixation per grapevine. The carbon allocation stimulated by $GA_3$ is then directed toward the whole plant as structural and soluble carbohydrates, although some preference in monosaccharides (glucose and fructose) is related to the aerial and reproductive parts, that is, berries. The application of ABA also increases such monosaccharide orientation toward berries and also roots but without increasing the overall carbon fixation. The two hormones promoted carbon allocation toward organs involved in plant survival: roots and berries. The accumulation of carbohydrates in roots may be needed for plant survival (root and shoot growth), whereas the allocation to berries may favor the species survival (reproduction).

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