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Increasing the Phenolic Compound Content of Grapes by Preharvest Application of Abcisic Acid and a Combination of Methyl Jasmonate and Benzothiadiazole

Yolanda Ruiz-García, Rocío Gil Muñoz, Jose María López Roca, Adrián Martínez Cutillas, Inmaculada Romero Cascales, and Encarna Gómez Plaza*

Food Science and Technology Deparment, Faculty of Veterinary Science, University of Murcia, Campus de Espinardo, 30071 Murcia, Spain

Instituto Murciano de Investigación y Desarrollo Agroalimentario, Ctra. La Alberca s/n, 30150, Murcia, Spain

ABSTRACT: Benzothiadiazole (BTH) and methyl jasmonate (MeJ) have been described as exogenous elicitors of some plant defense compounds, polyphenols among them. Given that they activate different arrays of biochemical reactions to induce resistance, the objective of this study was to determine whether the joint application of BTH and MeJ to grape clusters affects the level of the main flavonoid compounds in grapes and in the resulting wines. The results are compared with those obtained when abscisic acid (ABA), a plant growth regulator involved in several physiological processes, was sprayed in the same vineyard. The results obtained indicated that, although the application of ABA increased the content of skin anthocyanins and tannins, these positive effects were not reflected in the wines made from these grapes. BTH+MeJ-treated grapes also presented higher anthocyanin and flavonol contents, and in this case, their wines presented better chromatic characteristics that the wine made from control grapes.

KEYWORDS: *grape, wine, phenolic compounds, elicitors, benzothiadiazole, methyl jasmonate, abscisic acid, tannins, anthocyanins*

INTRODUCTION

In winegrapes, the technological importance of phenolic compounds, especially flavonoids, is well-known. They are responsible for the color of wines, especially anthocyanins (colored pigments responsible for the chromatic characteristics of red wines), proanthocyanidins (compounds responsible for the long-term stability of red wine color), and flavonols (compounds that may influence wine color through copigmentation). They are also responsible for some other wine organoleptic properties such as astringency, bitterness, and body. Another important aspect that has been widely studied in recent years is the role that grape and wine phenolic compounds can play in the human diet and health.[1−3]

Grape phenolic compounds also impart benefits to the plant itself, since they protect it from biotic and abiotic stress factors; indeed, some of these phenolic compounds are induced when a stress factor is present.[4]

A variety of chemical compounds have been tested for their use to increase the level of plant phenolic compounds. One of these compounds is abscisic acid (ABA), a plant growth regulator involved in various physiological processes, including seed maturation and germination and signaling when a plant is under stress as a result of high salinity, cold, and/or microbial infections, etc.[5] ABA also participates in the initiation of ripening,[6−8] and some results indicate that it may play a significant role in triggering the flavonoid biosynthetic pathway.[9,10] Berli et al.[8] proposed that ABA is involved in the protective responses of grape plant tissues to some abiotic stresses by enhancing both the enzymatic and nonenzymatic response systems.

Other compounds used to increase phenolic compound levels in plants belong to the group of so-called elicitors. In plants, phenolic compounds are part of the plant-inducible defense mechanisms, which, upon recognition of the attacker, are activated at the site of infection as well as in uninfected distant tissues, using signaling molecules and processes for the activation.[11] Among these, the resistance process mediated by the accumulation of endogenous salicylic acid (SA), called systemic acquired resistance (SAR), involves the induction of secondary metabolic pathways and the increased synthesis of products from this metabolism, phenolic compounds among them, as a response to pathogen attack.[12] However, defense signaling pathways that are independent of SA have also been described. For example, jasmonic acid (JA) and its derivative methyl jasmonate (MeJ) are also signaling molecules that can orchestrate a large set of defense responses,[11] including the synthesis of new phenolic compounds.

Chemical elicitors are agrochemicals that lack antimicrobial activity themselves but trigger inducible defense mechanisms. They were primarily designed to improve plant resistance to pathogens, but their ability to increase phenolic compounds also received much attention. Some of these agrochemicals may be the signaling molecules themselves (jasmonic acid and its derivate, methyl jasmonate, and salicylic acid), although other compounds can mimic these molecules (such as benzothiadiazole, a functional analog of salicylic acid) or simulate the attack

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of a pathogen (such as chitosan or harpin). The application of different elicitors to plants has proved to be useful to improve their phenolic content.[13−15] In this way, in grapes, the studies of Iriti et al.[16,17] demonstrated that the anthocyanin and proanthocyanidin contents increased after the application of benzothiadiazole (BTH), and this was accompanied by increased resistance to Botrytis attack. Other elicitiors such as MeJ[18,19] and chitosan[20] have also demonstrated their usefulness for increasing resistance in grapes while increasing the phenolic content. In 2009 and 2010, our research group studied the effect of the application of BTH and MeJ to grapes on their phenolic composition and that of the resulting wines and found a positive response, especially in the case of BTH.[21]

As mentioned previously, jasmonic acid and salicylic acid each trigger an array of biochemical response and products, some of which overlap, although many are distinct.[11] For this reason, some authors have tried the simultaneous application of both BTH and MeJ. Several lines of evidence suggest that there may be cross-talk between the jasmonate and the salicylate response pathways, with most of the reports indicating that such a cross-talk is negative,[22] although synergistic interactions have been also described.[23]

Given our interest in increasing the phenolic content of grapes and wines and the positive results previously reported for these two elicitors, we studied the effect of applying BTH and MeJ together to preharvest grapes. Also, the application ABA by itself on the phenolic composition of grapes and wines from Monastrell variety is discussed.

MATERIAL AND METHODS

Plant Material and Open Field Treatments. Treatments were carried out in an experimental vineyard at Bullas (Murcia, SE Spain) in 2011. The study was performed on 8-year-old *Vitis vinifera* L. Monastrell (syn. Mourvedre) red wine grapevines grafted onto R110 rootstock. A bilateral cordon training system trellised to a three-wire vertical system was used. Vine rows ran N−NW to S−SE, and planting density was 3 m between rows and 1.25 m between vines. Six two-bud spurs (12 nodes) per vine were retained at pruning. The vineyard was drip-irrigated.

All treatments were applied to three replicates and were arranged in a complete randomized block design, with 10 vines for each replication. Plants were sprayed at the beginning of véraison and 3 and 6 days after the first application, with a water suspension of a mixture of BTH [benzo(1,2,3)thiadiazole-7-carbothioic acid *S*-methyl ester] (Sigma Aldrich, St. Louis, MO) at a concentration of 0.3 mM and methyl jasmonate (Sigma Aldrich, St. Louis, MO) at a concentration of 10 mM or with ABA at a concentration of 400 ppm (Valent Biosciences, Libertyville, IL). Tween 80 (Sigma Aldrich, St. Louis, MO) was used as wetting agent. Control plants were sprayed with a water suspension of Tween 80 alone. Each plant received approximately 120 mL of suspension. When grapes reached optimum maturity, they were harvested and transported to the winery in 20 kg boxes. For chemical analysis of the polyphenolic compounds, five mature clusters per plant were randomly collected at harvest from treated and untreated grapevines. Clusters were immediately transported to the laboratory and frozen at −20 °C until analysis.

Vinifications. The grapes were crushed, destemmed, and sulfited (8 g of SO_2/100 kg of grapes). Total acidity was corrected to 5.5 g/L, and selected yeasts were added (Laffort, DSM, Servian, France, 10 g of dry yeast/100 kg of grapes). All the vinifications were conducted in triplicate, in 100 L tanks, at 25 ± 1 °C. Throughout the fermentative pomace contact period (10 days for all vinifications), the cap was punched down twice a day, and the temperature and must density were recorded. At the end of this period, the wines were pressed at 1.5 bar in a 75 L tank membrane press. Free-run and press wines were combined and stored at room temperature. One month later, the wines were racked and analyzed.

Physicochemical Determinations in Grapes. Grape analysis involved the traditional flesh measurements. Total soluble solids (°Brix) were measured using a digital refractometer (Atago RX-5000). Titratable acidity and pH were measured using an automatic titrator (Methrom, Herisau, Switzerland) with 0.1 N NaOH. Tartaric and malic acids were measured using enzymatic kits from Boehringer Mannheim GmbH (Mannhein, Germany). The methodology for carrying out these analyses is described in EEC regulation no. 2676/90.

Anthocyanins, Flavonols, and Proanthocyanidins in Grapes and Wines. Grapes were peeled with a scalpel and the skins and seeds were separated and stored at −20 °C until analysis. To isolate the anthocyanin and flavonol, grapes samples (2 g) were immersed in methanol (40 mL) in hermetically closed tubes and placed on a stirring plate at 150 rpm and 25 °C. After 4 h, the methanolic extracts were filtered through 0.45 μm nylon filters (OlimPeak, Tecknochroma, Barcelona, Spain) and analyzed by high-performance liquid chromatography (HPLC) and HPLC−MS. To evaluate the anthocyanins and flavonols in wines, samples of wines were filtered through the 0.45 μm nylon filters and directly analyzed by HPLC. The chromatographic conditions were previously described.[21] Anthocyanins were quantified at 520 nm as malvidin 3-*O*-glucoside, using malvidin 3-*O*-glucoside chloride as external standard (Extrasynthèse, Genay, France). Flavonols were quantified at 360 nm using quercetin (Sigma Aldrich, St. Louis, MO) as external standard.

For the isolation of proanthocyanidins in grapes, the method of Hernández-Jiménez et al. [24] was followed. Briefly, whole seeds and skins, previously ground to a powder with liquid nitrogen, were extracted separately in covered Erlenmeyer flasks with 10 mL of 2:1 acetone/water at room temperature for 24 h on an orbital shaker at 200 rpm. Following extraction, the extract was concentrated under reduced pressure at 35 °C to remove acetone and then lyophilized to a dry powder. This powder was redissolved in 1 mL of methanol in a volumetric flask.

Skin and seed proanthocyanidins were determined using the phloroglucinolysis methods according to the methodology described by Kennedy and Jones[25] with the modifications described by Busse-Valverde et al.[26] Briefly, a solution of 0.2 N HCl in methanol, containing 100 g/L phloroglucinol and 20 g/L ascorbic acid, was prepared (phloroglucinolysis reagent). The methanolic extract was reacted with the phloroglucinolysis reagent (1:1) in a water bath for 20 min at 50 °C and then combined with 2 volumes of 200 mM aqueous sodium acetate to stop the reaction.

For wines, the samples (5 mL) were evaporated in a centrivap concentrator (Labconco), redissolved in 3 mL of water, and then passed through a C18-SPE column (1 g, Waters, Mildford, MA), previously activated with 10 mL of methanol followed by 20 mL of water. The cartridge was washed with 20 mL of water and the compounds of interest were eluted with 10 mL of methanol, evaporated, and then dissolved in 1 mL of methanol. Phloroglucinolysis was then carried out as described above. HPLC analysis followed the conditions described by Busse-Valverde et al.[26]

Color Determinations in Wines. Absorbance measurements were made in a Shidmazu UV-1603 spectrophotometer (Shimadzu Deutschland GmbH) with 0.2 cm path length glass cells. Color density (CI) was calculated as the sum of absorbance at 620, 520, and 420 nm, and tint was calculated as the ratio between absorbance at 420 and 520 nm. Total phenol content (TP_{wine}) and total anthocyanins were spectrophotometrically measured as described by Ribéreau Gayon et al.[27] The CIELab parameter L^* (lightness) was determined by measuring the transmittance of the wine every 10 nm from 380 to 770 nm, using the D65 illuminant and a 10° observer.

Total Antioxidant Capacity Determination. This assay is based on the decoloration that occurs when the radical cation $ABTS^{\bullet+}$ is reduced to ABTS (2,2′-azinobis-3-ethylbenzothiazoline-6-sulfonic acid). The radical was generated by reaction of a solution of ABTS in tampon phosphate salin (pH 7.4) with MnO_2. This solution was filtered with a 0.2 μm filter and it was kept at low temperature. The

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assay was conducted with 1000 μL of ABTS$^{\bullet+}$ solution and 100 μL of the sample and carried out in darkness at room temperature. Absorbance measurements at 734 nm were made after 2 min of reaction time. Results were compared with a standard curve prepared with different concentrations of Trolox, a water-soluble analogue of vitamin E. The results are expressed in millimolar of Trolox equivalents.

Statistical Data Treatment. Significant differences among wines and for each variable were assessed by analysis of variance (ANOVA) using Statgraphics 5.0 Plus. LSD test was used to separate the means ($p < 0.05$) when the ANOVA test was significant.

RESULTS AND DISCUSSION

Physicochemical Composition. Table 1 shows the physicochemical data of the grapes at the moment of harvest.

Table 1. Physicochemical Characteristics of the Grapes at the Moment of Harvest[a,b]

	wt of 100 berries	°Brix	total acidity (g/L)	pH	tartaric acid (g/L)	malic acid (g/L)
control	118.3 a	23.2 a	3.4 a	3.7 a	4.9 a	1.4 a
BTH+MeJ	118.1 a	23.5 a	3.3 a	3.8 a	5.0 a	1.5 a
ABA	133.8 a	23.4 a	3.4 a	3.7 a	5.0 a	1.3 a

[a]BTH, benzothiadiazole; MeJ, methyl jasmonate; ABA, abcisic acid. [b]Different letters in the same row indicate significant differences according to the LSD test ($p < 0.05$).

No changes in physicochemical parameters or berry weight were observed compared with the control grapes. Our previous studies[21] showed that the application of BTH or MeJ had no effect on berry weight and only a very slight effect on berry composition. Similarly, Fumagalli et al.[28] found no adverse conditions when BTH was applied to grapes. With regard to the application of ABA, Gu et al.[29] applied it to Cabernet Sauvignon grapes at different moments and doses and observed that berry weight was not affected and that total soluble solids, pH, and acidity were only marginally influenced. Sandhu et al.[30] made similar observations for muscadine grape. In addition, Omran[31] found that ABA did not affect vine yield. In contrast, some authors observed that ABA enhanced ripening, when applied to grapevine, and in this way, Garibaldi et al.[32] reported that ABA acts through the over- or underexpression of the same proteins that are involved in the ripening process. However, they stated that these effects were mostly observed when berries were treated before véraison and not at later stages, probably due to the fact that in these stages the endogenous ABA content was already high. The fact that our treatments started at the moment of véraison might explain the lack of an effect of the ABA treatment on the physicochemical parameters.

Grape Anthocyanins and Flavonols. The results are shown in Tables 2 and 3. The application of BTH+MeJ doubled the quantities of grape anthocyanins, expressed as both μg/g skin or mg/kg of grapes. The treatment with ABA also significantly increased the concentration of anthocyanins.

It may be thought that the uptake of these compounds through the waxy cuticle is likely to be an inefficient process; however, in the case of ABA, Berli et al.[8] showed that the ABA levels increased in berry skin as a result of exogenous application to clusters, whereas when applied to leaves, no effect was observed.[29]

The effect of the application of BTH or MeJ in grapes have been previously proved to be an interesting option for increasing grape phenolic compounds.[16,21,28] However, less information is available on the join effect of BTH+MeJ. As stated before, it seems clear that both SA and JA act by defending plants against pathogens but through distinct signaling processes. In nature, it seems that, depending on the type of attacker, the plant activates different signaling pathways to synthesize an optimal mixture of defensive

Table 2. Concentration of Anthocyanins in Berries Treated with Benzothiadiazole and Methyl Jasmonate, and Abscisic Acid[a,b]

anthocyanins (μg/g skin)	control	BTH+MeJ	ABA
Del	420.7 a	1348.0 b	622.0 a
Cyan	322.0 a	1535.5 c	682.0 b
Pet	720.3 a	1256.7 b	671.6 a
Pn	497.0 a	1149.6 b	622.1 a
Malv	2401.9 a	4188.5 b	2943.5 a
total nonacylated	4361.9 a	9478.3 b	5541.1 a
Del Ac	50.6 a	106.2 b	78.2 ab
Cyan Ac	36.6 a	69.6 c	51.7 b
Pet Ac	77.0 a	120.8 b	91.8 ab
Pn Ac	45.4 a	76.0 b	66.7 b
Malv Ac	283.7 a	377.1 a	356.0 a
total Ac	493.4 a	749.7 b	644.3 ab
Del Coum	179.5 a	240.8 a	205.7 a
Mal Caf	139.7 b	82.0 a	95.7 ab
Cyan Coum	139.3 a	237.5 b	175.9 a
Pet Coum	259.6 a	306.0 a	288.5 a
Pn Coum	154.3 a	232.8 b	200.3 ab
Malv Coum cis	58.2 a	70.1 a	77.5 a
Malv Coum trans	940.7 a	1092.1 a	1129.9 a
total Coum	1871.3 a	2261.3 a	2173.6 a
total acylated	2364.7 a	3011.0 a	2817.9 a
total 3′-substituted	1194.7 a	3301.0 c	1798.7 b
total 3′,5′-substituted	5531.9 a	9188.3 b	6560.3 a
total anthocyanins (μg/g skin)	6726.6 a	12489.3 c	8359.0 b
total anthocyanins (mg/kg grapes)	656.7 a	1343.8 c	905.3 b

[a]Abbreviations: Del, delphinidin 3-*O*-glucoside; Cyan, cyanidin 3-*O*-glucoside; Pet, petunidin 3-*O*-glucoside; Pn, peonidin 3-*O*-glucoside; Malv, malvidin 3-*O*-glucoside; Ac, acetylglucosides; Cum, coumarylglucosides; Caf, caffeate glucoside; BTH, benzothiadiazole; MeJ, methyl jasmonate; ABA, abscisic acid. [b]Different letters in the same row show and for each year indicate significant differences according to the LSD test ($p < 0.05$).

Table 3. Concentration of Flavonols in Grape Berries Treated with Benzothiadiazole and Methyl Jasmonate, and Abscisic Acid[a,b]

flavonols (μg/g skin)	control	BTH+MeJ	ABA
M-3-glc	32.8 a	26.5 a	35.8 a
Q-3-glc	63.5 a	53.9 a	57.8 a
K-3-gal	2.06 a	1.84 a	2.18 a
K-3-glc + S-3-glc	12.8 a	11.6 a	12.6 a
I-3-glc	1.5 a	1.4 a	1.1 a
Q-3-glcU	15.8 a	15.4 a	14.8 a
total flavonols (μg/g skin)	131.5 a	110.7 a	121.2 a
total flavonols (mg/kg grapes fresh weight)	13.1 a	12.4 a	14.1 a

[a]Abbreviations: M, myricetin; Q, quercetin; K, kaempferol; I, isorhamnetin; glc, *O*-glucoside; gal, *O*-galactoside; glcU, *O*-glucuronide; BTH, benzothiadiazole; MeJ, methyl jasmonate; ABA, abscisic acid. [b]Different letters in the same row and for each year indicate significant differences according to the LSD test ($p < 0.05$).

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compounds.[11] However, when applied exogenously, both synergistic and antagonistic interactions between these molecules have been reported.[22,33] For example, Thaler et al.[22] reported that the application of jasmonic acid and BTH resulted in an attenuated expression of biochemical responses compared with plants elicited with only JA, and also SA responsive enzymes were reduced when JA+BTH were applied together, compared with BTH alone. This negative interaction resulted in a reduced resistance to herbivores. Kloek et al.[34] stated that the JA signal can be a potent inhibitor of SA dependent signaling but also pointed to the fact that cross-talk between SA and JA may be regulated differently depending on the plant species. This would explain the different results that were observed by O'Donnel et al.,[23] who described a cooperative interaction among SA, JA, and ethylene.

However, all the studies of cross-talk and interactions between MeJ (or JA) and BTH (or SA) are based on pathogen resistance or molecular studies. We could not find any other research on the effect of jointly applying MeJ and BTH on the phenolic content of plants, in general, or grapes, in particular. Only Considine et al.[35] applied SA and MeJ in combination to harvested table grapes and measured their antioxidant capacity several days after treatment. They found that, after an initial increase, the antioxidant capacity of MeJ-treated grapes decreased significantly but increased in SA+MeJ treated grapes, a result similar to that observed in grapes treated with SA alone, indicating that SA may override the effect of MeJ. Similarly, with a simultaneous SA and JA treatment in *Arabidopsis*, SA strongly suppressed JA-responsive gene expression.[36] In our previous study,[21] BTH increased the anthocyanin concentration by 14−23% and MeJ by 16%. The greater increases observed in this study when BTH and MeJ were applied together indicated that there was no negative interaction. However, a positive interaction cannot be totally ruled out, since, in this study, BTH and MeJ were not applied separately.

As regards the effect of ABA, in previous studies with grapes, its application always resulted in an increase in anthocyanins.[10,30,37] When ABA was applied at a concentration of 300 mg/L to table grapes that in warm climates fail to develop full color, the concentration of anthocyanins increased by 48%.[31] Also, the application of ABA to clusters at 300−600 mg/L significantly enhanced anthocyanin content of Cabernet Sauvignon grapes by 20−85% (depending on the year) but had no effect when applied to the leaves.[29]

All of these results are coincident with ours, although the positive action of ABA on anthocyanin synthesis does not apply to all grape varieties. For example, small grapes with a larger skin surface seem to be more susceptible to ABA treatment, since they might absorb ABA more efficiently.[30]

The increase was more marked in the case of 3′-substituted anthocyanins than 3′,5′-substituted anthocyanins (up to 176% and 50% of increase for BTH+MeJ- and ABA-treated grapes compared to an increase of 66% and 18% in 3′,5′-substituted anthocyanins, respectively) and in the case of nonacylated anthocyanins than in acylated ones (Table 2). Berli et al.[10] also found that flavonoid-3′-hydroxylase was more activated than flavonoid-3′,5′-hydroxylase when ABA was applied, a shift also observed in sun-exposed fruits.

The concentration of flavonols did not increase following the treatments (Table 3). In contrast, Ruiz-Garcia et al.[21] found an increase in flavonols of up to 81% when plants were sprayed with MeJ alone. This different response may also corroborate the results of Considine et al.,[35] who indicated that SA may override the effect of MeJ. With regard to previous studies on the effect of ABA on flavonols, and contrary to our results, other authors found positive effects; for example, Sandhu et al.[30] found an increase in flavonols when ABA was applied to muscadine grapes, and Berli et al.[10] also found that ABA increased flavonol concentrations, especially quercetin and kampherol in Malbec grapes.

Tannins. The application of ABA increased grape skin tannin levels (Table 4), although only when expressed as mg/ kg of berries, and no difference was found between the control and BTH+MeJ-treated grapes. Also, no difference was observed in the mean degree of polymerization (mDP), while the composition only slightly varied between treatments. With regard to seed tannins (Table 5), no quantitative or qualitative differences were found between control and treated grapes. These results do not agree with our previous findings, since, when both elicitors were applied separately, an increase in skin tannins was observed,[21] especially when MeJ was applied. Perhaps the fact that BTH may override the effect of MeJ, as stated by many authors,[22,35,36] would partially explain the lack of positive effect on skin tannins when both elicitors were applied at the same time.

Table 4. Concentration and Composition of Skin Proanthocyanidins in the Grape Berries Treated with Benzothiadiazole and Methyl Jasmonate, and Abscisic Acid[a,b]

total tannins	C	BTH+MeJ	ABA
μg/g of skin	5492.6 a	5035.8 a	6411.1 b
μg/berry	691.9 a	737.8 a	726.1 a
mg/kg[c]	471.7 a	465.3 a	593.4 b
mDP	15.4 a	16.5 a	15.9 a
%G	1.9 b	1.6 a	2.0 b
%tCat	4.6 b	4.0 a	4.4 b
%tECat	1.9 a	2.1 a	1.9 a
%tECatG	0	0	0
%extCat	1.5 a	1.6 a	1.6 a
%extECat	63.7 a	65.8 a	63.4 a
%extECatG	1.9 b	1.6 a	2.0 b
%extEgCat	26.3 a	24.9 a	26.8 a

[a]mDP, mean degree of polymerization; %G, percentage of galloylation; %tCat, percentage of terminal (+)-catechin; %tECat, percentage of terminal (−)-epicatechin; %tECatG, percentage of terminal (−)-epicatechin gallate; %extCat, percentage of extension (+)-catechin; %extECat, percentage of extension (−)-epicatechin; % extEgCat, percentage of extension epigallocatechin; %extECatG, percentage of extension (−)-epicatechin gallate; C, control; BTH: benzothiadiazole; MeJ: methyl jasmonate. [b]Different letters in the same line indicate significant differences according to the LSD test ($p < 0.05$). [c]Milligrams of skin proanthocyanidins per kilogram of grapes (fresh weight).

The effect of ABA application to grapes on tannin biosynthesis has been less studied than the effect on other phenolic compounds, especially, anthocyanins. Only Lacampagne et al.[9] studied the effect of ABA on the proanthocyanin biosynthesis pathway, reporting that ABA had a positive impact on tannin biosynthesis during véraison and suggesting that anthocyanin reductase and leucoanthocyanin reductase were coregulated by ABA.

Wine Phenolic and Chromatic Composition. The analysis of the corresponding wines at the end of alcoholic fermentation (Table 6) showed no significant increase in

Table 5. Concentration and Composition of Seed Proanthocyanidins in the Grape Berries Treated with Benzothiadiazole and Methyl Jasmonate, and Abscisic Acid[a,b]

total tannins	C	BTH+MeJ	ABA
μg/g of seed	40 186.1 a	42 062.8 a	42 414.8 a
μg/berry	2771.4 a	2950.7 a	2902.9 a
mg/kg[c]	1891.4 ab	1860.0 a	2377.5 b
mDP	6.0 a	5.8 a	6.0 a
%G	15.6 a	15.0 a	15.2 a
%tCat	5.7 a	5.7 a	5.4 a
%tECat	7.4 a	7.7 a	7.7 a
%tECatG	3.7 a	3.8 a	3.7 a
%extCat	7.1 a	7.2 a	6.9 a
%extECat	64.3 a	64.5 a	64.8 a
%extECatG	11.9 a	11.2 a	11.5 a

[a]mDP, mean degree of polymerization; %G, percentage of galloilation; %tCat, percentage of terminal (+)-catechin; %tECat, percentage of terminal (−)-epicatechin; %tECatG, percentage of terminal (−)-epicatechin gallate; %extCat, percentage of extension (+)-catechin; %extECat, percentage of extension (−)-epicatechin; %extECatG, percentage of extension (−)-epicatechin gallate; C, control; BTH, benzothiadiazole; MeJ, methyl jasmonate. [b]Different letters in the same line indicate significant differences according to the LSD test ($p < 0.05$). [c]Milligrams of seed proanthocyanidins per kilogram of grapes (fresh weight).

Table 6. Concentration and Composition of Wine Flavonoids (As Determined by HPLC Analysis) Made with Grape Berries Treated with Benzothiadiazole and Methyl Jasmonate, and Abscisic Acid[a,b]

	C	BTH+MeJ	ABA
total tannins (mg/L)	539.7 a	548.8 a	519.1 a
mDP	5.4 b	5.5b	5.0a
total anthocyanins (mg/L)	405.2 b	422.3 b	366.9 a
total flavonols	59.7 b	48.9 a	50.8 a

[a]mDP, mean degree of polymerization; C, control; BTH, benzothiadiazole; MeJ, methyl jasmonate. [b]Different letters in the same line indicate significant differences according to the LSD test ($p < 0.05$).

HPLC-detected anthocyanins or flavonols compared with control wines or any difference in total tannins. These results were not expected, given the positive effect of both ABA and BTH+MeJ treatments had on grape phenolic composition, although, similarly, Fumagalli et al.[28] also reported that the increase in grape anthocyanin content that they observed with the use of elicitors was not reflected in the corresponding wines.

However, the wines elaborated with BTH+MeJ-treated grapes presented a higher spectrophotometrically measured total phenol content and color intensity than wines elaborated with control or ABA-treated grapes (Table 7). These findings confirm that our HPLC methods may provide only limited information on wine phenolic composition since, in the case of anthocyanins, only monomeric anthocyanins were analyzed, and in the case of tannins, it has been reported that part of wine tannins may not be depolymerized by the phloroglucinolysis analysis and therefore will not be measured in a HPLC analysis. An improvement of the analytical method for including the determination of new forms of anthocyanin-derived compounds could give more information on the wine phenolic composition. However, the spectrophotometric analysis clearly indicated that a higher presence of polyphenols was observed in BTH+MeJ wines, which resulted in a higher color intensity and total phenol content. Related to this higher phenol content, the BTH+MeJ wines also presented a higher total antioxidant capacity compared with wines from control and ABA-treated grapes.

Table 7. Wine Chromatic Characteristics and Antioxidant Capacity[a,b]

	L^*	TP_{wine}	CI	tint	TEAC
control	13.7 a	44.9 a	14.7 a	0.4 a	10.1 a
BTH+MeJ	13.3 a	48.1 b	15.6 b	0.4 a	11.1 b
ABA	14.3 a	44.2 a	14.2 a	0.5 a	9.4 a

[a]BTH, benzothiadiazole; MeJ, methyl jasmonate; TP, total phenols (measured as optical density at 280 nm); CI, wine color intensity; TEAC, Trolox equivalent antioxidant capacity. [b]Different letters in the same row indicate significant differences according to LSD test ($p < 0.05$).

In conclusion, although the preharvest exogenous application of ABA to grapes increased the skin content of anthocyanins and tannins, these positive effects were not reflected in the wines elaborated from these grapes. BTH+MeJ-treated grapes also presented higher anthocyanin content, and moreover, in this case, their wines presented better chromatic characteristics than the wine made from control grapes. However, these results did not improve on those observed in our previous study, which involved the separated application of BTH and MeJ, especially, as regards the BTH-treated grapes and wines.[21] These actual results (and given the fact that we did not use any advanced molecular tools) do not prove the existence of a negative cross-talk between BTH and MeJ when they were applied jointly to preharvest grapes, but they clearly indicate that the response was not improved compared with the results obtained with the separate application of these compounds.

AUTHOR INFORMATION

Corresponding Author

*Tel. +34868887323. Fax +34868884147. E-mail: encarna.gomez@um.es.

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Notes

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