

Effects of brassinosteroid (24-epibrassinolide) on yield and quality of grape (*Vitis vinifera* L.) 'Thompson Seedless'

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Summary

Berry growth ripening process and yield are directly related with nutrition status and phytohormones during fruit growth and development stages. Brassinosteroid (24-epibrassinolide) is one of the plant growth substances that influence different physiological processes including fruit growth and development. In this study, the effect of brassinosteroid (Br) application on the yield and berry quality of 'Thompson Seedless' was investigated in a commercial vineyard, Malayer, Hamedan, Iran. Br solution concentrations of 0, 0.2, 0.4, 0.6 mg·L⁻¹ at five stages (budbreak (S₁), prebloom (S₂), middle of bloom (S₃), post bloom (S₄) and veraison (S₅)) were sprayed. The results showed that the Br application has significant positive effects on bunch morphology and berry quantity and quality. Among levels of Br, the highest yields were related to the application of Br at the rate of 0.6 mg·L⁻¹ in the post bloom and veraison stages, and the lowest was found in the bud break stage. Spraying Br in the post bloom and veraison stages had the greatest effect on berry quality. Also, the highest content of total phenol and antioxidant capacity were obtained with 0.6 mg·L⁻¹ of Br application at veraison stage. In this study, we provided evidence to show that using steroidal plant hormones (Br) may play an essential role in improving the yield and quality of 'Thompson Seedless'.

Key words: brassinosteroid; grape; growth; content of total phenol; antioxidant capacity.

Introduction

Grapevine is one of the most important horticultural crops produced in Iran in terms of total grown acreage and economic value. According to the Food and Agriculture Organization (FAO) in 2012, Iran in terms of acreage and grape production ranked eighth in the world, but in terms of yield per hectare ranked thirty-first. One of the reasons for the yield deficit is the lack of nutrition and hormones. The quality of grapes (*Vitis vinifera* L.) depends on concentrations of total soluble solids (TSS), total acid

(TA), total phenol, and antioxidant capacity in the berry. The increase in yield and quality of grapes by various plant growth regulators will be done (CREASY and CREASY 2009).

Brassinosteroids (Br) are a new class of phytohormone regulating many aspects of plant growth and development (BISHOP and YOKOTA 2001, CLOUSE 2002). Br are essential for normal plant development and regulate a range of diverse cellular responses, such as stem elongation and pollen tube growth, induction of ethylene biosynthesis and fruit ripening, xylem differentiation, yield and reproductive development (SYMONS *et al.* 2006, BROSA 1999, SASSE 2003, CLOUSE 2002).

Recent studies have shown the significant increase in yields of various crops after Br are applied. Meanwhile, the application of Br has increased the acceleration of the ripening process and yields in tomato (VARDHINI and RAO 2002). Similarly, the application of Br to yellow passion fruit also elevated yield, corresponding to 20 t·ha⁻² compared to the control yield of 12 t·ha⁻² (GOMES *et al.* 2006).

ZAHARAH *et al.* (2012) reported that the exogenous application of Epi-BL (45 and 60 ng·g⁻¹ FW) promoted mango fruit ripening but endogenous Br may not play a significant role in the climacteric ripening of mango fruit. In contrast to climacteric fruits, where ethylene is pivotal, the hormonal control of ripening in non-climacteric fruits, such as grape (*Vitis vinifera* L.), is poorly understood. In addition, Br also has involved in the increase of early fruit development in strawberry and fruit ripening quality (CHAI *et al.* 2013). The hormonal control of berry ripening in grapevine appears to be relatively complicated. Application of Br to grape berries significantly enhances ripening, while the exogenous application of brassinazole (an inhibitor of Br biosynthesis) significantly delays fruit ripening (SYMONS *et al.* 2006). Furthermore, Br are involved in the ripening of berry, leaf and stem elongation, flowering, anther development, and fruit set in grapes (SYMONS *et al.* 2006, SASSE 2003).

Moreover, the grapes treated with Br had higher enhanced phenolics contents and antioxidant capacity in the grape skin (XI *et al.* 2013). 0.4 mg·L⁻¹ EBr treatment induced the most highly significant increases in phenolics content and antioxidant capacity of 'Cabernet Sauvignon' and 'Yan73' grapes in relation to the control (Xu *et al.* 2015). However, very little is known about the possible

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role of Br involved in yield and its components of grapevines including bunch length and bunch width and berry length and berry diameter. Therefore, it is important to investigate whether Br can enhance the yield and quality of grape. We examined whether the exogenous application levels of Br, during growth and development of fruit stages influenced the yield and quality of grape (*Vitis vinifera* 'Thompson Seedless').

Material and Methods

Site and experimental conditions: The experiment was conducted in order to analyze qualitative and quantitative changes in *Vitis vinifera* 'Thompson Seedless' berries, under the influence of different concentrations of Br (epi-brassinolide, SIGMA-ALDRICH, USA). This research was carried out in a commercial vineyard, Malayer, Hamedan, Iran in 2014. Vines were uniformly pruned to 30 to 35 nodes at per vine. The experiment was conducted at the base of a factorial experiment in a block complete randomized design with 20 treatments and three grapevines per replications.

Treatment and sample collection: Br was applied in four levels (0, 0.2, 0.4 and 0.6 mg·L⁻¹) at five stages of growth and berry development including budbreak (S₁), prebloom (S₂), middle of bloom (S₃), post bloom (S₄) and veraison (S₅). Bunches for measurements were randomly harvested from treated vines when berries well ripened. To define the stage of harvest, 50 berries from control vines were randomly selected. The level of total soluble solids (°Brix) was measured in the selected fruit and harvested when the control vines reached 23 °Brix.

Determination yield and yield components of grapevine: In order to measure yield, bunches from each vine per treatment were collected. The bunches were immediately weighed using a digital balance. Four bunches from each vine per treatment were selected and in order to evaluate components of yield, length and width of bunch, length and diameter of berry were measured. Next, 100 grape berries were collected from the upper, middle and lower sections of the four bunches, and were weighed using a digital balance.

Analysis of TSS and titratable acidity: Juice samples were analyzed for pH with a conductivity meter. Titratable acidity was determined by titration with 0.1 M NaOH, with an endpoint of pH 8.2. The concentration of tartaric acid in each treatment was determined based on the procedure described by KAMPFENKEL *et al.* (1995).

Total soluble solids were quantified on a separate aliquot of supernatant with a hand-held refractometer (Reichert A2R200, Reichert GmbH, Seefeld Germany) and reported as °Brix.

Determination of total phenolic contents. Extraction: The fruit of each treatment were tap washed followed by being washed with distilled water and completely dried in the shade at room temperature. Berries of each plant (100 g) were separately extracted with methanol solvents (85 %) and distilled water at room

temperature then each extract was filtered using Whatman filter paper no.1 and concentrated by using a rotatory evaporator (Buchi, Switzerland) (LARRAURI *et al.* 1997).

Determination of total phenolic contents and antioxidant capacity: To calculate the content of total phenols, we used the method of Folin-Ciocalteu (SINGLETON and ROSSI 1965). For the colorimetric assay, 1 mL of Folin-Ciocalteu (1:3) reagent, 2 mL Na₂CO₃ at 20 % and 2.85 mL of distilled water were added to 150 µL of extract. After incubation in the dark for 30 min, absorbance was measured at 700 nm. Gallic acid (Acros Organics) was used as the standard, and results were expressed as gallic acid equivalents (GAE) mg·100 g⁻¹ FW (fresh weight).

To measure antioxidant capacity, extracts were prepared according to BRAND-WILLIAMS *et al.*'s method (1995) and determined as described by DPPH (ARNOUS *et al.* 2001). 500 µL extract (0.5 g sample were extracted with 3 mL methanol 85 %) was diluted with 500 µL distilled water and solutions were centrifuged with rate 10,000 rounds per min for 5 min. Then, the 2925 µL of DPPH methanol solution were added to 75 µL solution and vortexed for few seconds. The absorbance was read at 0 and 30 min. The optical absorption of the samples was read at the wavelength of 515 nm by the spectrophotometer (model Carry 100). The optical absorption of the samples was read again and calculated through the use of the following equation:

$$DPPH\% = \frac{ABS_{t0} - ABS_{t30}}{ABS_{t0}} \times 100$$

Where ABS_{t0} is the absorbance of the reaction at time 0 min and ABS_{t30} is the absorbance the sample at time 30 min.

Statistical analysis: Statistical analysis was performed with statistical software SAS, followed by a Duncan multiple range test to determine significant differences with regard to all parameters. The values were significantly different at *P* < 0.05 probability level.

Results

Yield and its components: Results in Fig. 1 showed that the effect of Br sprays on yield was significant at 0.05 level of significance. Results indicated that application of Br at veraison and post bloom stages with plant growth leads to yield increase (Fig. 1). The highest value of yield per vine was obtained through the use of 0.6 Br at veraison and post bloom stages (10 and 9.4 kg per vine, respectively). The yield at veraison and post bloom stages with 0.6 Br treatments was increased due to an increase in the length and width of bunch and berry (Tab. 1). In the second-year, also application 0.6 Br at veraison resulted in higher yield (Fig. 2). The increase in grape yield and the positive impact on several parameters may be attributed to Br uptake from the leaf from the applied Br sprays. There was a significant difference in weight of hundred berries of the grape between Br treatment and control (Tab. 1). By the application of Br sprays, maximum and minimum hundred weight of berry was, respectively, obtained at the highest (0.4 and 0.6 mg·L⁻¹) and lowest (control level) levels of

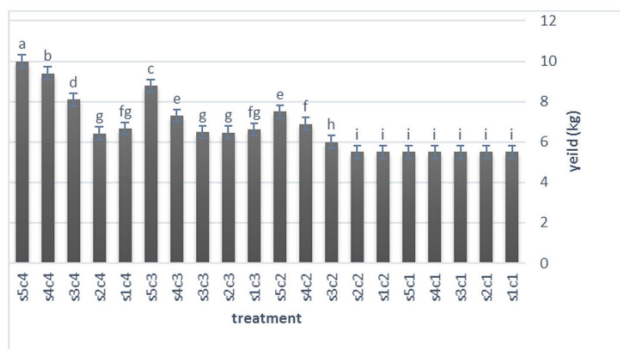


Fig. 1: Response of Br on yield (kg) of grapevine 'Thompson Seedless' in 2014 year; s₁c₁ (Bud break, 0), s₁c₂ (Bud break, 0.2 mg·L⁻¹), s₁c₃ (Bud break, 0.4 mg·L⁻¹), s₁c₄ (Bud break, 0.6 mg·L⁻¹), s₂c₁ (pre bloom, 0), s₂c₂ (pre bloom, 0.2 mg·L⁻¹), s₂c₃ (pre bloom, 0.4 mg·L⁻¹), s₂c₄ (pre bloom, 0.6 mg·L⁻¹), s₃c₁ (middle of bloom, 0), s₃c₂ (middle of bloom, 0.2 mg·L⁻¹), s₃c₃ (middle of bloom, 0.4 mg·L⁻¹), s₃c₄ (middle of bloom, 0.6 mg·L⁻¹), s₄c₁ (post bloom, 0), s₄c₂ (post bloom, 0.2 mg·L⁻¹), s₄c₃ (post bloom, 0.4 mg·L⁻¹), s₄c₄ (post bloom, 0.6 mg·L⁻¹), s₅c₁ (veraison, 0), s₅c₂ (veraison, 0.2 mg·L⁻¹), s₅c₃ (veraison, 0.4 mg·L⁻¹), s₅c₄ (veraison, 0.6 mg·L⁻¹). Values followed by different letters are significantly different between samples according to Duncan test at P < 0.01.

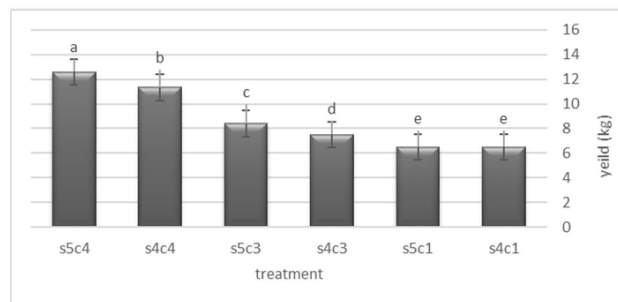


Fig. 2: Response of Br on yield (kg) of grapevine 'Thompson Seedless' in 2015 year; s₄c₁ (post bloom, 0), s₄c₃ (post bloom, 0.4 mg·L⁻¹), s₄c₄ (post bloom, 0.6 mg·L⁻¹), s₅c₁ (veraison, 0), s₅c₃ (veraison, 0.4 mg·L⁻¹), s₅c₄ (veraison, 0.6 mg·L⁻¹). Values followed by different letters are significantly different between samples according to Duncan test at P < 0.01.

Br. The length to width ratio of the bunch also increased (P < 0.01) with Br sprays at all growth stages (Tab. 1), the highest values are related to 0.6 Br at post bloom and veraison (4.91 and 5.3 cm respectively). The highest amount of length to width ratio of berry is related to 0.6 and 0.4 Br treatment at post bloom and veraison (4.69 and 5.11 cm respectively). This was also observed in the second-year (Tab. 2) sprayed with brassinolide (0.6 mg·L⁻¹) at veraison and post bloom.

TSS and titratable acidity: The means of comparison of this variable based on Duncan's test showed that different concentrations Br treatment enhanced the total soluble solids and decreased the titratable acids content

in the juices. The highest amounts of TSS/TA ratio exist in the vines treated with 0.6 Br at post bloom and veraison (Tab. 1). The result indicated that suitable level of Br treatment could promote grape berry ripening (TSS/TA). Our data in the second-year also corroborates the observations made in the first-year (Tab. 1).

Total phenol content and antioxidant capacity: Analysis of the variance for total phenol and antioxidant capacity indicated that the main effect and interaction effects of experimental factors were significant at 5 % probability levels. Mean comparison of interaction effect of time × Br showed that maximum content of total phenols was obtained at the highest level of Br, at veraison and post bloom (Tab. 1). The contents of total phenols from the four samples ranged from 3.96 to 5.65 mg·g⁻¹. According to data in Tab. 1, the highest amount of total phenol (5.65 mg·g⁻¹) was obtained from application of 0.6 Br at the final growth stage of grapevine 'Thompson Seedless'. The study of antioxidant capacity from berries showed that application of exogenous Br at three concentrations

Table 1

Effect of Br treatment at the different stage on the component yield, total phenol content and antioxidant capacity of treated grapevines in 2014 year

Treatments (mg·L ⁻¹)	Length/width bunch				Length/width berry				Weight of 100 berry			
	0	0.2	0.4	0.6	0	0.2	0.4	0.6	0	0.2	0.4	0.6
Bud break	2.25 ^c	2.5 ^c	3.61 ^d	4.06 ^{cd}	2 ^g	2.4 ^{efg}	2.3 ^{fg}	2.5 ^{efg}	84.87 ^g	101.1 ^{ef}	96.4 ^f	101.56 ^{ef}
Pre bloom	2.25 ^c	2.4 ^c	2.3 ^c	4.0 ^{cd}	2 ^g	2.3 ^{fg}	2.4 ^{efg}	3.4 ^{cd}	84.87 ^g	114.02 ^{ef}	127.08 ^c	122.86 ^c
Middle of bloom	2.25 ^c	2.46 ^c	3.9 ^d	4.19 ^{cd}	2 ^g	2.9 ^{de}	2.8 ^{ef}	2.5 ^{efg}	84.87 ^g	104.04 ^{ef}	122.86 ^c	109.17 ^{de}
Post bloom	2.25 ^c	2.63 ^c	4.2 ^{cd}	4.91 ^{ab}	2 ^g	2.3 ^{fg}	3.8 ^{bc}	4.7 ^a	84.87 ^g	141.31 ^b	139.91 ^b	157.7 ^a
Veraison	2.25 ^c	2.71 ^c	4.6 ^{bc}	5.3 ^a	2 ^g	2.3 ^{fg}	4.1 ^b	5.1 ^a	84.87 ^g	143.05 ^b	156.7 ^a	142.9 ^b
Treatments (mg·L ⁻¹)	Phenol (mg·g ⁻¹)				Antioxidant capacity (%)				TSS/TA			
	0	0.2	0.4	0.6	0	0.2	0.4	0.6	0	0.2	0.4	0.6
Bud break	3.9 ^{bcd}	3.3 ^d	3.8 ^d	4.1 ^{bcd}	31.8 ^{hi}	32.2 ^{hi}	34 ^{efgh}	33 ^{fghi}	54 ^j	55 ^{ij}	55.4 ^{ij}	58 ^{efg}
Pre bloom	3.9 ^{bcd}	3.9 ^{bcd}	4 ^{bcd}	3.9 ^{cd}	31.8 ^{hi}	31.6 ⁱ	32.7 ^{hi}	35.1 ^{def}	54 ^j	55 ^{ij}	56 ^{hi}	60.1 ^{cd}
Middle of bloom	3.9 ^{bcd}	3.91 ^{bcd}	3.7 ^d	4 ^{bcd}	31.8 ^{hi}	34.6 ^{efg}	35.6 ^{ede}	37 ^{bcd}	54 ^j	55.2 ^{ij}	58.9 ^{def}	61.4 ^{bc}
Post bloom	3.9 ^{bcd}	4.9 ^{ab}	4.9 ^{abc}	5.5 ^a	31.8 ^{hi}	34.8 ^{efg}	37.2 ^{bc}	38.5 ^b	54 ^j	57.6 ^{fg}	59.4 ^{de}	62.1 ^{ab}
Veraison	3.9 ^{bcd}	5.4 ^a	5.4 ^a	5.6 ^a	31.8 ^{hi}	35 ^{def}	38.0 ^b	42.7 ^a	54 ^j	57.3 ^{gh}	59.6 ^d	63.5 ^a

Different letters within columns represent a significant difference at 1% level of probability.

Table 2

Effect of Br treatment at the different stage on the component yield, total phenol content and antioxidant capacity of treated grapevines in 2015 year

Treatments (mg·L ⁻¹)	Length/width bunch			Length/width berry			Weight of 100 berries		
	0	0.4	0.6	0	0.4	0.6	0	0.4	0.6
Post bloom	2.1 ^c	3.8 ^b	4.7 ^a	2.5 ^c	3.1 ^c	5.3 ^b	91 ^c	140.7 ^d	160.1 ^b
Veraison	2.1 ^c	5.1 ^a	4.9 ^a	2.5 ^c	3.9 ^c	5.9 ^a	91 ^c	152.6 ^c	161.2 ^a

Treatments (mg·L ⁻¹)	Phenol (mg·g ⁻¹)			Antioxidant capacity (%)			TSS/TA		
	0	0.4	0.6	0	0.4	0.6	0	0.4	0.6
Post bloom	3.6 ^c	5.1 ^b	6.1 ^a	35.6 ^d	39.1 ^c	40.4 ^b	56.1 ^c	60.8 ^c	62.6 ^b
Veraison	3.6 ^c	5.3 ^b	5.1 ^b	35.6 ^d	38.6 ^c	43.9 ^a	56.1 ^c	60.8 ^c	65.4 ^a

Different letters within columns represent a significant difference at 1% level of probability.

obviously increased antioxidant capacity (Tab. 1). There was no significant difference between 0.4 Br and 0.6 Br treatments at post bloom. However, there was a significant difference in the antioxidant capacity from berries between 0.6 Br and other treatments. Overall, the highest mean in the second and first year were recorded (43.9 and 42.7 % for 0.6 Br).

Discussion

Mean comparison of interaction effect of brassinosteroid × time sprays on grapevine yield showed that maximum yield was obtained at highest levels of brassinosteroid (0.6 mg·L⁻¹) and at timing of veraison (S₃) in both years. All the parameters used to measure the yield and ripening (TSS and TA) were increased by Br treatment. Results indicated that the application of brassinosteroids leads to increase in the ratio length to width of bunch and length to width of berry. The most significant effect of epi-Br on yield was detected at post bloom and veraison but there were no effects on yield and quality at budbreak, prebloom and middle of bloom. Having no effect on yield and quality of the treated grapevines at budbreak, prebloom and middle of bloom supported the findings of ISCI and GÖKBAYRAK (2015). It was reported that application of 10⁻³ and 10⁻⁴ mg·L⁻¹ brassinosteroid at anthesis to the vines had no clear effects of the compound on yield and quality, except for the cluster length. The profile of endogenous Br levels observed in grape berries showed that in grape tissues endogenous Br is low at veraison (SYMONS *et al.* 2006). Using epi-Br at post bloom and veraison increased yield, while the application of Br before budbreak, prebloom and middle of bloom stage did not have any effects.

The physiological mechanism by which Br-induced yield and components of yield may be due to stimulation of elongation, pollen tube growth and reproductive development. It was demonstrated that the elongation growth of plant tissue was promoted by exogenous application of Br (SASSE 2003). This phenomenon may be part of a mechanism whereby endogenous auxin is increased in the plant through growth stages (SASSE 1990). It is clear from other reports that Br is likely to be involved in cell expansion,

division, reproductive development, pollen tube formation and differentiation of plant tissues (BISHOP and KONCZ 2002, CLOUSE 2002, SASSE 2003). SERNA *et al.* (2013) also showed that in endives, treatment with brassinosteroids has caused an increase in yield, contents of total antioxidant activity and total phenols of field grown endives.

Furthermore, we show that the manipulation of Br levels *via* the application of exogenous Br can significantly promote berry ripening (TSS and TA) and increased quality of berry in 'Thompson Seedless' grape (Tab. 1). The results of this study were consistent with previous reports that Br could increase TSS in field trials of berries (SYMONS *et al.* 2006). To the best of our knowledge, this is the first report in which Br has been implicated for achieving high quality in grape fruit.

SYMONS *et al.* (2006) stated that Br also influence sugar accumulation in grape. Increases in endogenous Br levels coincided with an increase in the transcript levels of VvDWF1 and VvBRI1, between 8 and 10-week post flowering and led to an increase in yield and quality of grape. SYMONS *et al.* (2006) reported gene expression of Br synthesis and plant hormone levels of grape ('Cabernet Sauvignon') berry development which coincides with the process of berry ripening.

This is in accordance with previous studies showing significant maintenance of soluble-sugar content of cucumber (JIANG *et al.* 2012). Recently, some investigations have suggested that the application of Br could accelerate the ripening of tomato and jujube fruit by increasing ethylene production (VARDHINI and RAO 2002, ZHU *et al.* 2010).

In addition, the results showed that application of ep-Br increased contents of total phenol and antioxidant capacity of 'Thompson Seedless' (Tab. 1). The phenolics content in berries at 0.6 mg·L⁻¹ Br treatment was significantly higher than that of other treatments, especially control. Our results correspond with results of Xi *et al.* (2013), in which high total phenol and antioxidant capacity were found in *Vitis vinifera* 'Yan 73' and 'Cabernet Sauvignon' berries, when compared to control treatment. The secondary metabolism such as phenolics biosynthesis is regulated by EBr treatment in tomato (AHAMMED *et al.* 2013). However, the mechanism by which EBr effects on generation of phenolic compounds and grape ripening is not well under-

stood. There is a hypothesis that exogenous EBr acts as a signaling agent triggering the synthesis of endogenous Br (Xi *et al.* 2013). The ability of brassinosteroid to stimulate activation of antioxidants of endive (*Cichorium endivia* L.) has previously been reported (SERNA *et al.* 2013).

Conclusion

The present results clearly demonstrated that application of Br sprays to grapevine (*Vitis vinifera* 'Thompson Seedless') enhance yield and its components. It has been proposed that the increase in yield resulted from the utilization of the Br available by the sprays. On the other hand, it has been shown that Br enhances hundred berry weight, length and width berry and bunch, therefore, increases yield. Meanwhile, the exogenous Br could significantly enhance the total phenol and antioxidant capacity in berries.

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