YIELD AND QUALITY OF SULTANI GRAPES (*VITIS VINIFERA* L.) TREATED WITH 28-HOMOBRASSINOLIDE AND GIBBERELLIC ACID

IŞÇI, B.

Department of Horticulture, Faculty of Agriculture, Ege University, Bornova/İzmir, Turkey
(ORCID ID: 0000-0002-6542-0271)
e-mail: burcak.isci@ege.edu.tr

(Received 10th May 2019; accepted 16th Jul 2019)

Abstract. In this study, the effects of gibberellic acid (GA_3) and brassinosteroid (BRs) applications on the yield and berry quality of Sultani grapes were determined during the 2017-2018 period. 28-homobrassinolide (Hbl) solutions with concentrations 4 ppm (Hbl-4) and 8 ppm (Hbl-8) were spray-applied at veraison. Three GA_3 concentrations were implemented by spraying method. The applications times were as follows; Application 1: 10 ppm, clusters 5-10 cm in length at pre-bloom stage (first week of May), Application 2: 15 ppm at 45-50% flowering (mid-June), and Application 3: 20 ppm with 3-5 mm berry diameter stage. GA_3 and BRs on yield components, some quality characteristics relating to clusters and berries (weight, breadth and length, etc.), color of bunches, total phenol content and antioxidant activity were investigated. Results showed that the effects differed, and they were not consistent on all the variables. Effects of the HBl treatments on yield were not as high as GA_3 but there were some positive improvements. Hbl was found to be influential on increasing cluster breadth. Berries were harder with the hormone applications. Quality characteristics were more influenced by the seasons rather than the hormones. Color and total anthocyanin contents were better with the hormones. Fructose content indicated a significant increase with the Hbl-4 application. 28-homobrassinolide might carry some potentially useful effects on improving yield, not to the extent of GA_3, and also on color along with increasing sweetness during the production of ‘Sultani’ grapes.

Keywords: *Vitis vinifera, Sultani, berry, hormone, gibberellic acid* (GA_3)*, brassinosteroid*

Introduction

In Turkey, only about 50-60 grape cultivars are economically cultivated despite their vast variety (about 1200). Seedless table grape production constitutes about 37% of all production. Sultani (aka ‘Thompson Seedless’), originated from the Aegean region of Turkey, since it is the most valuable raisin and table grape variety in the world. Its berries are green-yellow in color, seedless and small, with ellipsoid shaped berries, and the bunches are very large in size with long cylindrical body with wings on both sides (İşçi and Altındişli, 2015).

Applications of growth regulators such as gibberellins, ethylene or auxin in grape growing aim to improve fruit yield and quality. Especially gibberellic acid is the most effective growth regulator in improving bunch compactness, berry characters as well as yield and quality of berries. Gibberellic acid is mostly commercially used in seedless varieties to increase yield of grapes depending on its concentration and the time of application (İşçi and Gökbayrak, 2015; Reynolds et al., 2016; Ghorbani et al., 2017).

Brassinosteroids promote a dual response of cell division and cell elongation resulting in both enhancement and acceleration of the overall growth of the plants (Bartwal et al., 2013; Ali, 2017; Chai, 2012). They show growth regulatory effects in plants similar to those of endogenous plant hormones, auxins and gibberellins. In carrot, application of 24-epibrassinolide resulted in longer plants with increased vegetation weight (Que et al., 2017). Chai et al. (2013) determined that keeping brassinosteroids

DOI: http://dx.doi.org/10.15666/aeer/1705_1244112450

from expression resulted in delayed color development in strawberries. It was shown that the application of brassinosteroid to grape berries evidently enhanced skin coloration and the final sugar level of the flesh, and significantly promoted ripening (Mitchell et al., 1970; Vardhini and Rao, 2002; Symons et al., 2006).

The objective of this study was to determine and compare the effects of gibberellic acid (GA_3) and 28-homobrassinolide applied at different phenological stages on quantity and quality of the 'Sultani' vines.

Materials and methods

Plant material

Vitis vinifera L. 'Sultani', a seedless grape, was grafted on 41B rootstock in 2005. Implementations were carried out at the experimental vineyard located in Bornova, İzmir. The vineyard is on sandy and silt soil 2.0 × 3.0 m spacing. Each vine carried approximately 20 grape clusters. Guyot-trained vines with four canes carrying 11-12 buds were drip irrigated. All viticulture practices were performed according to standard vineyard practices. During winters of 2017 and 2018, Bordeaux mix was applied following pruning, and wettable powder sulphur was used when the summer shoots were 10 cm long. Lateral shoots were accordingly removed in order to facilitate pest management.

Hormone treatments including the control were carried out freshly prepared solutions that contained 0.1% Tween 20 as the surfactant. Control vines were sprayed with distilled water. There were no other manipulations on the clusters. 28-homobrassinolide (Hbl, 0.1%; Repar, MD, USA) solutions with concentrations 4 ppm (Hbl-4) and 8 ppm (Hbl-8) were applied at veraison (last week of July) by spraying with a handgun sprayer at a rate of 10 ml/cluster, and the solution was made sure to cover the entire surface area of the berries in each cluster. Three GA_3 concentrations were implemented by spraying method. The applications times were as following; 1. application: 10 ppm, clusters 5-10 cm in length at pre-bloom stage (first week of May), 2. application: 15 ppm at 45-50% flowering (mid-June), and 3. application: 20 ppm with 3-5 mm berry diameter stage (last week of July).

The weather conditions in the two seasons (2017 and 2018, respectively) were as follows: average monthly temperature in year round was 18.2 °C and 19.2 °C. Mean temperature during the months of vegetation period (April-October) was 24.2 and 24.3 °C. Mean total precipitation was 1.78 mm and 1.65 mm, during which the vegetation period it was recorded as 0.64 and 0.93 mm. Average humidity was 60.6% and 64.3% throughout the seasons. The relative humidity during the vegetation period in 2018 was 4.2% higher compared to that in 2017.

The vines were monitored and harvested when the control vines reached 16°Brix. Yield components (yield per vine and per hectare) were recorded and some quality characteristics relating to clusters and berries (weight, breadth and length, etc.) were determined under lab conditions. Tensile strength of the pedicel (Newton, N) was measured with a penetrometer (Somyftec, France) on 50 berries taken from the clusters. The external berry color was measured at the equatorial area of each side of 50 berries using a colorimeter (CR-300, Minolta Co, Osaka, Japan), average scores were recorded in terms of CIE-L* a* b* values. The colorimeter had an 8 mm diameter viewing area and was calibrated with a white tile ($L^* = 97.26$, $a^* = +0.13$, $b^* = +1.71$). From these data, the Color Index of Red Grapes (CIRG) (Carreño et al., 1996) was calculated as:

CIRG = (180- h^o)/(C* + L*). Based on this index, the berries were classified into five categories: green-yellow (CIRG < 2); pink (2 < CIRG < 4); red (4 < CIRG < 5); dark red (5 < CIRG < 6); and blue-black (CIRG > 6).

The total soluble solids (°Brix) content in the juice was determined with a digital refractometer (PR-1, Atago, Tokyo, Japan) and expressed as percentage. Titratable acidity was measured by titration with 0.1 N NaOH to pH 8.1, and the results were expressed as g tartaric acid·100 mL-1 fruit juice. Sugar composition of grape (fructose and glucose) was determined with HPLC (Thermo Dionex Ultimate 3000) according Camara et al. (1996) only in year 2017. Total phenol content and extraction for antioxidant activity was measured by Thaiponga et al. (2006).

Statistical analysis

The trial was completely randomized with three replicates in each treatment. Each replicate had four vines. A total of 12 vines was used for each application. The mean data collected relating to different parameters of 'Sultani' grapes were statistically analyzed by Minitab® 16.1.1 statistical software. The differences between the significant means were determined with the Tukey's test ($p \leq 0.05$).

Results and discussion

Yield components of the cultivar were not affected by the treatments (*Table 1*). However, the seasonal variations exhibited significant differences and always in the second year that decreases were observed. The weather data indicated that although mean values of temperature, humidity and precipitation all year round were not quite apart, the values during the vegetation period showed that the second period was hotter, more humid and had more rain. This might have caused the yield to be lower. Interestingly, in the first year of hormone applications, GA_3 had always the highest values, followed by mostly the control group. In second year of the experiment, on the other hand, it was the control group that had the highest cluster and vine yield. One stipulation on this observation could be that hormone treated vines might be more prone to the adverse effects of the weather conditions or that they might exert some negative effects on bud fruitfulness. Vergara et al. (2018) also stated that exogenous applications of brassinosteroids at veraison did not improve yield. Senthilkumar et al. (2018) investigated preharvest application of GA_3 and brassinosteroid together and found that they provided best vine yield and highest cluster weight. Champa et al. (2015) showed that 0.5 ppm brassinosteroid at pea stage and veraison significantly increased bunch weight in 'Flame Seedless'. Baneh et al. (2017) reported positive effects of GA_3 on increasing yield of Iranian seeded grape cultivars. In their study involving application of a GA_3 based growth regulator, Nicolaescu et al. (2015) indicated its promoting activity on cluster productivity in 'Beauty Seedless' cultivar. Reynolds et al. (2016) also reported effects of GA_3 on increasing yield as positive and negative dependent on the cultivars. Ghorbani et al. (2017) stated that effects of 24-epibrassinolide on yield of 'Thompson Seedless'vines were mainly dependent on the time of the applications, such that after bloom it tended to provide the highest yield, but it gave the lowest yield when applied at budbreak.

Cluster characteristics, except for the cluster length, did not change with the hormone applications (*Table 2*). The clusters were wider than they were longer, showing that 28-homobrassinolide was as effective as GA_3. Fresh and dry weight of the rachis were higher in the first year. Cluster length showed a significant increase in the second year. Although Haubrick and Assmann (2006) stated promoting effect of brassinosteroids on stem elongation, this effect was not observed in this study. This might be due to circumstances of the trial conducted. The increase was seen in all the clusters in the experiment. Champa et al. (2015) stated in their study with double application of brassinosteroid that cluster size significantly increased in ‘Flame Seedless’.

The hormone treatments generally caused no significant differences in the characteristics of berries (*Table 3*). Berry breadth, berry weight and tensile strength of the pedicel differed between the seasons, being higher in the first season. Berry firmness was affected by the hormone application. Clear difference was observed between GA_3 and the Hbl-4/control groups. It seems that H8 was also capable of increasing berry firmness as GA_3 did. This might be the result of brassinosteroids, keeping the pectins in the cell walls from solubilization and/or depolymerization which lead to pulp softening (Villareal et al., 2008). Suehiro et al. (2018) reported enlarging effect of GA_3 in ‘Shine Muscat’ grapes. There are many reports on the effects of GA and CK on berry enlargement throughout the developmental stage (Peppi and Fidelibus, 2008; Zoffoli et al., 2009; Acheampong et al., 2015). In Flame Seedless (Champa et al., 2015) berry weight and berry size showed an increase after brassinosteroid applications at pea stage and veraison. GA_3 treated vines of ‘Thompson Seedless’ produced heavier berries with greater size compared to the control vines (Koukourikou et al., 2015). ‘Skookum Seedless’ berries increased their firmness following application of GA_3 (Reynolds et al., 2016).

Quality of the ‘Sultani’ berries were not affected by the treatment (*Table 4*), but they showed a significant increase in the second season compared to the first season. Soluble solids content between GA_3 and Hbl-4 treatment was comparably high in 2016, and a balance was observed in all the applications in 2017. Total acidity exhibited a slight increase in the second season. Maturity index was under the influence of the hormones. Hbl-4 resulted in the highest index value in both seasons. The others showed a minor decrease in the second season. Our findings were in consistent with those of Vergara et al. (2018) in ‘Red Globe’ and in contrast with those of Ashgari and Rezaei-Rad (2018) in ‘Thompson Seedless”. Reynolds et al. (2016) also showed that response of berry quality characteristics was cultivar specific. İşçi and Gökbayrak (2015) indicated no effects of homobrassinolide applied at anthesis on Brix and pH of ‘Alphonse Lavallee’, but they showed that seasonal effects were clear on total acidity.

Color of the berries were interactively affected by the treatments and the season (*Table 5*). Brightness (L*) was higher and generally the same in the second season. Yellowness (b*) was generally similar between the seasons, except for the Hbl-8 treatment. As was with the brightness, b* was higher in the treated berries compared to the control berries. Redness (a*) stayed at similar levels among the hormone applications, but significantly increased between the seasons. CIRG value (Carreño et al., 1996) of the grapes showed that the ‘Sultani’ berries fall within the class of green-yellow. It did not change with the applications, but seasonal differences were found significant even though the class they belonged to be the same. In agreement with our findings, Vergara et al. (2018) stated that brassinosteroid analogs applied to Red Globe berries at veraison resulted in better coloration. However, the response here was also dependent on the seasonal effects.

Biochemical aspects of the berries treated with various hormones indicated that hormones resulted with significant contributions to the contents of total anthocyanin and total phenolics (*Table 6*). TAC increased with the hormones. TPC on the other hand responded differently to the type of the hormones. GA_3 treated berries had the highest TPC, but 28-homobrassinolid comparably lowered it. Ma et al. (2012), in agreement, showed that 24-epibrassinolide increased phenolic contents in grapes. Since the responses of TAC and TPC were similar to the treatments, it can be assumed that the phenolics contents in the 'Sultani' berries might be mainly related to anthocyanin accumulation as also stated by (Xi et al., 2013). Fructose content of the berries showed a significant increase with Hbl-4 treatment. Glucose accumulated more with the hormones than the control group. In a similar study Asghari and Rezaei-Rad (2018) indicated that three times applied 24-epibrassinolide made a significant contribution to the total anthocyanin contents of 'Thompson Seedless' table grapes. Vergara et al. (2018) also showed that brassinosteroid analogs improved the anthocyanin content of 'Red Globe' grapes. Application of 0.6 ppm at veraison resulted in highest total phenolics and total anthocyanin contents in 'Thompson Seedless' berries (Ghorbani et al., 2017).

Table 1. *Yield components of 'Sultani' vines at harvest for growing seasons of 2017 and 2018 (mean ± SD)*

	Cluster weight (g)		**Yield per vine (kg)**		**Yield per ha (ton)**	
Hormones	2017	2018	2017	2018	2017	2018
GA_3 (10-15-20 ppm)	762.58±181.2	383.93±57.0	15.23±3.62	7.68±1.14	25.41±6.03	12.79±1.90
Hbl 4 ppm	608.72±63.2	326.99±155.6	12.17±1.26	6.54±3.11	20.29±2.11	10.90±5.18
Hbl 8 ppm	548.33±72.1	352.77±124,2	10.97±1.44	7.05±2.48	18.27±2.40	11.75±4.14
Control	601.14±8.7	490.92±46.9	12.02±0.97	9.82±0.94	20.03±3.30	16.35±1.57
Mean	630.19±128A*	388.65±111.5B	12.60±2.56A	7.77±2.23B	21.00±4.26A	12.95±3.72B
p value	0.000		0.000		0.000	

*Uppercase letters in the rows indicate the significant differences between the seasons

Table 2. *Effects of hormone treatments on cluster traits of 'Sultani' grapes (mean ± SD)*

	Cluster breadth (cm)		**Cluster length (cm)**		**Rachis fresh weight (g)**		**Rachis dry weight (g)**	
Hormones	2017	2018	2017	2018	2017	2018	2017	2018
GA_3 (10-15-20 ppm)	26.50±2.18	27.22±3.13	11.67±1.26	14.92±1.81	20.56±2.6	13.43±3.92	8.99±0.46	6.08±0.38
Hbl 4 ppm	28.35±3.43	26.53±3.33	10.33±2.57	11.78±1.36	20.42±1.42	12.67±1.96	8.42±1.37	6.32±0.55
Hbl 8 ppm	25.17±3.01	23.90±2.50	9.50±3.50	12.20±0.00	16.58±5.22	14.90±7.21	7.08±1.87	7.35±1.80
Control	27.50±4.3	26.47±1.70	9.33±1.04	12.25±2.21	22.03±6.43	23.48±9.72	9.16±2.59	9.62±3.16
Mean	26.88±3.11	26.03±2.68	10.26±2.20B	12.78±2.27A	19.90±4.27	16.12±7.12	8.41±1.72	7.34±2.15
p value	>0.05		0.013		>0.05		>0.05	

*Uppercase letters in the rows indicate the significant differences between the seasons

Table 3. *Effects of hormone treatments on berry traits of 'Sultani' grapes (mean ± SD)*

	Berry breadth (mm)		**Berry length (mm)**		**100 berry weight (g)**		**Berry firmness (N)**			**Tensile strength (N)**	
Hormones	2017	2018	2017	2018	2017	2018	2017	2018	Mean	2017	2018
GA_3 (10-15-20 ppm)	16.2±2.44	12.72±0.08	17.40±0.72	16.12±0.73	243.22±59.2	174.63±11.90	8.51±0.72	7.25±0.73	7.88±0.95 a	293.83±48.66	217.40±25.63
Hbl 4 ppm	14.26±0.77	12.25±0.60	16.51±0.84	15.33±0.40	218.41±29.66	148.63±33.48	6.42±0.84	5.60±0.40	6.01±0.74 b	246.83±9.02	211.00±19.94
Hbl 8 ppm	13.90±1.07	12.57±0.35	16.24±0.96	16.84±0.18	201.17±6.2	186.35±0.95	6.80±0.95	7.43±0.18	7.11±0.70 ab	243.17±22.83	231.10±24.30
Control	13.75±0.16	12.92±0.77	16.45±0.25	18.19±0.86	203.93±11.90	213.80±49.97	6.19±0.25	6.18±0.86	6.19±0.57 b	293.00±63.72	232.00±10.46
Mean	14.53±1.57A*	12.62±0.51B	16.72±1.14	16.63±0.95	216.68±39.15A	180.85±35.80B	6.98±1.14	6.62±0.94		269.21±43.81A	222.88±20.17 B
p value	0.000		>0.05		0.028		0.001			0.003	

*Lowercase letters within a column indicate significant differences among the treatments. Uppercase letters in the rows indicate the significant differences between the seasons

Table 4. *Quality parameters evaluated at harvest after hormone treatments in 'Sultani' grapes (mean ± SD)*

	Soluble solids (Brix)		**Total acidity g (100 g FW)**		**pH**		**Maturity index (SS/TA)**		
Hormones	2017	2018	2017	2018	2017	2018	2017	2018	Mean
GA_3 (10-15-20 ppm)	18.63±0.55	22.32±0.68	6.64±0.28	8.20±0.23	4.02±0.02	3.94±0.02	28.31±1.42	27.26±1.32	27.69±1.31 ab
Hbl 4 ppm	20.20±0.92	21.20±1.30	5.81±,0.47	7.13±0.05	4.11±0.07	3.98±0.01	35.02±4.27	29.71±1.64	32.37±4.10 a
Hbl 8 ppm	18.98±0.21	21.03±1.72	6.88±0.79	7.80±0.27	3.93±0.09	3.97±0.04	27.83±2.94	26.97±2.37	27.40±2.44 ab
Control	17.90±1.72	20.13±0.58	7.07±1.24	8.12±1.34	4.02±0.13	4.02±0.03	26.12±6.52	25.24±4.21	25.68±4.93 b
Mean	18.94±1.22B*	21.18±1.29A	6.60±0.84B	7.82±0.74A	4.02±0.10	3.98±0.04	29.87±5.06	27.30±2.80	
p value	0.000		0.001		>0.05		0.027		

*Lowercase letters within a column indicate significant differences among the treatments. Uppercase letters in the rows indicate the significant differences between the seasons

Table 5. *Chromatic parameters of the 'Sultani' berries treated with hormones (mean ± SD)*

	L*		***a****		***b****		**CIRG (scale)**	
Hormones	2017	2018	2017	2018	2017	2018	2017	2018
GA_3 (10-15-20 ppm)	42.55±3.88Bab*	48.87±0.49Aa	-7.29±0.33	-8.67±1.12	20.62±1.68Aab	20.91±0.53 Aa	1.10±0.07	0.94±0.04
Hbl 4 ppm	44.03±3.4Ba	48.94±0.61Aa	-7.51±0.52	-9.16±0.73	20.76±1.21Aa	21.48±0.48 Aa	1.06±0.06	0.93±0.03
Hbl 8 ppm	42.08±2.32Bab	50.98±1.92Aa	-7.21±0.69	-8.87±0.36	20.37±1.16Bab	25.12±3.31 Aa	1.11±0.05	0.91±0.04
Control	34.59±7.55Bb	51.12±2.19Aa	-7.22±0.98	-8.26±0.73	16.32±0.76Ab	24.04±2.53 Aa	1.29±0.22	0.93±0.03
Mean	40.81±5.56	49.98±1.71	-7.31±0.59 B	-8.74±0.75 A	19.52±2.21	22.89±2.56	1.14±0.14 A	0.93±0.03 B
p value	0.049		0.000		0.005		0.000	

*Lowercase letters within a column indicate significant differences among the treatments. Uppercase letters in the rows indicate the significant differences between the seasons

Table 6. *Total anthocyanin, total phenolics, fructose and glucose contents of 'Sultani' berries treated with hormones (mean ± SD)*

	TAC (mg/100 g FW)			**Total phenolics (mg GAE/100 g FW)**			**Fructose (%)**	**Glucose (%)**
Hormones	2017	2018	Mean	2017	2018	Mean	2017	2018
GA_3 (10-15-20 ppm)	3.23±0.83	6.08±0.93	4.66±1.74 a*	40.38±4.90	76.51±6.0	58.45±20.39 a*	5.69±2.60 b	5.06±0.13 a
Hbl 4 ppm	2.32±0.37	5.86±0.92	4.09±2.03 ab	34.64±10.11	63.67±3.76	49.16±17.30 b	7.93±0.85 a	5.58±0.39 a
Hbl 8 ppm	2.37±0.22	5.43±1.69	3.90±1.69 ab	38.78±4.03	61.89±1.21	50.34±13.99 b	6.60±0.33 ab	5.01±0.17 a
Control	1.99±0.65	4.46±0.57	3.23±1.43 b	28.23±5.00	63.51±0.21	55.87±19.60 ab	5.38±0.48 b	4.33±0.03 b
Mean	2.48±0.66B	5.46±0.89A		35.51±7.38B	66.40±7.77A		6.40±1.14	4.92±0.51
p value	0.000 (years) 0.010 (treatments)			0.000 (years) 0.015 (treatments)			0.000	0.000

*Lowercase letters within a column indicate significant differences among the treatments. Uppercase letters in the rows indicate the significant differences between the seasons

Conclusion

No matter the information gathered from this study and from all previous conducted valuable studies indicate that hormones do not greatly influence fruit growth and quality, understanding the role of plant growth regulators in composing grape quality and improving yield still requires some researches. Despite its relatively new-found place among plant hormones, brassinosteroids do have some promising effects on quality and quantity, obviously depending on its type, concentration and the time of application. Due to their degradation and limited transport, it might be useful to apply at the closest time or phenological stage to the intended outcome and therefore, repeated applications may be more effective. Improvement shown by brassinosteroid applications in berry firmness, color development and anthocyanin increases validate these hormones to be the subject of further research.

Acknowledgment. The author thanks Dr. Bhushan Mandava and Prof. Dr. Zeliha Gökbayrak for kindly providing the 28-homobrassinolide.

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