Indian Journal of Agricultural Sciences **91** (6): 920–3, June 2021/Article
https://doi.org/10.56093/ijas.v91i6.114300

Effect of new generation bio-regulators on anthocyanins and berry quality of grape cv. Beauty Seedless

V B MHETRE[1], V B PATEL[1]*, S K SINGH[1], M K VERMA[1], G P MISHRA[1],
ANIL DAHUJA[1] and CHAVLESH KUMAR[1]

ICAR-Indian Agricultural Research Institute, New Delhi 110 012, India

Received: 29 December 2020; Accepted: 05 January 2021

ABSTRACT

Five-year-old grapevines of Beauty Seedless spaced at 3 m × 3 m distance and trained on the ‘Bower’ system were sprayed with different plant bioregulators, namely, abscisic acid (ABA: 200 and 400 ppm), ethephon (200 and 400 ppm), benzothiadiazole (BTH: 0.3 and 0.6 mM), prohexadione-calcium (Pro-Ca: 200 and 400 ppm) and control (water spray) at the veraison stage during 2019–20 with the aim of studying their influence on berry physical and biochemical characteristics. The experiment was conducted in the randomised block design with three replications for each treatment. Different berry physical characteristics like weight, length, diameter and firmness were positively influenced with the different treatments except for ethephon (400 ppm) and ABA (400 ppm) treatments, which reduced the berry firmness significantly. Berry biochemical parameters were positively improved by almost all the plant bioregulators, they were most pronounced with ethephon (400 ppm) and ABA (400 ppm). The most prominent effects of ABA and ethephon treatments were noted on the total anthocyanins biosynthesis in the berries, which is major bottleneck under the warm subtropical conditions in different coloured grape genotypes. Hence, application of new generation plant bioregulators such as BTH, Pro-Ca, ABA and ethephon resulted in improvement in berry quality parameters of cv. Beauty Seedless.

Key words: Anthocyanins, Antioxidants, Beauty Seedless, Berry quality, Bioregulators

Beauty Seedless, a prominent table and wine grape cultivar is a hybrid between Koenigin Der Weingaerten (Queen of Vineyards) × Black Kishmish evolved by the famous grape breeder Prof. H P Olmo in 1941 at University of California, USA (VIVC). However, under sub-tropical situations, the non-uniform colour development is one of the major problems in case of growing coloured grape genotypes (Roberto *et al*. 2012). In Beauty Seedless in spite of early maturing, there is non-uniform and poor colour development in berries. Such poor colour of berries is mainly attributed to lesser accumulation of anthocyanins in the peel caused by high temperature (Peppi *et al*. 2006). The visual appearance of the grape berries is the prime factor influencing their market pricing, besides poor consumer preference (Peppi *et al*. 2006).

The treatment of different plant bio-regulators at various phases of fruit development coupled with endogenous concentrations of phytohormones marked up their role for the proper fruit development and several quality attributes (Srivastava and Handa 2005). The hormone ethylene is involved in the ripening of the grape berry and other ripening related processes including accumulation of anthocyanins (Amiri *et al*. 2010). The exogenous treatments of abscisic acid significantly boosted the colour in Flame Seedless (Peppi *et al*. 2006) by enhancing anthocyanin levels in the berry peel. Of late, benzothiadiazole (BTH) - a functional analogue of salicylic acid, which has a positive role in triggering defence related genes inducing systemic acquired resistance as well as overall enhancement in the phenolics content (Ruiz-Garcia *et al*. 2012). Similarly, prohexadione calcium (Pro-Ca), a new peer group biosynthesis blocker of gibberellin with low toxicity and better retention in the crops (Mandemaker *et al*. 2005) which improves total anthocyanins and monoterpenes content in grape berries (Thomidis *et al*. 2018). The aim of this study was to examine the influence of exogenous application of established and some new plant bioregulators such as ethephon, abscisic acid, benzothiadiazole and prohexadione-calcium on berry physical characteristics, and quality parameters of grape cv. Beauty Seedless under warm sub-tropical conditions.

MATERIALS AND METHODS

Plant materials and treatments: The present study was carried out at the experimental farm of the Division of Fruits and Horticultural Technology, ICAR-Indian Agricultural Research Institute (IARI), New Delhi during 2019 on five-year-old grapevines of Beauty Seedless spaced at 3 m × 3 m

---

Present address: ICAR-Indian Agricultural Research Institute, New Delhi. *Corresponding author e-mail: patelvb7@gmail.com.

distance and trained on the bower system. The experimental vineyard is located at an altitude of 228 m above MSL with a latitude of 28º 40' N and longitudinal geographical coordinates of 77º 13' E. The climate is typically sub-tropical and the soil is alluvial having alkaline *p*H and clay loam texture. The present study was carried out for nine treatments with three replications for each treatment the concentration applied were (i) abscisic acid (ABA) 200 and (ii) 400 ppm, (iii) benzothiadiazole (BTH) 0.3 and (iv) 0.6 mM, (v) ethephon 200 and (vi) 400 ppm, (vii) pro-hexadione Calcium (Pro-Ca) 200 and (viii) 400 ppm, and (ix) control (water spray). All these plant bioregulators were sprayed at veraison stage of berry development on the entire vine (24th May, 2019) using a handheld sprayer until runoff during the late evening.

Sampling and estimation of berry physical characteristics: At the time of harvesting, three normal bunches from each replication were collected randomly throughout the canopy for the determination of different berry physical characteristics, namely, average of 100-berry weight (g), berry length and diameter (mm), berry shape index (berry length/diameter) and juice percentage. These physical parameters were measured following standard procedures, while berry firmness was estimated following the compression test using a texture analyser (model: TA+Di, Stable Micro Systems, UK) (Yaman *et al.* 2002).

Sampling and estimation of bunch characteristics: Bunch weight, length and width were recorded by using standard procedures. There are five groups based on the adherence of the berries to the rachis, namely, very loose, loose, medium, dense and very dense bunches based on the descriptors of grapes provided by the International Plant Genetic Resources Institute (Bioversity International). The compactness of every bunch was assessed at the time of maturity and they were categorized accordingly.

Sampling and estimation of berry biochemical characteristics: After the harvest, a set of 100-berry samples were randomly collected in three replicates for each treatment and immediately stored at -20°C till further biochemical analysis. The juice TSS was measured with the help of digital refractometer and findings were presented as ºB as mentioned in AOAC. To test the titratable acidity of berry juice, the samples were titrated against with 0.1 N NaOH (Ranganna 1999) using the titration method, while ascorbic acid content were determined as described in AOAC. The total monomeric anthocyanins content was estimated by the pH-differential method as described by Wrolstad *et al.* (2005). Total phenolics as well as total flavonoids content were determined by the methods described by Singleton *et al.* (1999) and Zhishen *et al.* (1999), respectively. The total antioxidant activities were determined using modified DPPH method proposed by Sanchez-Moreno *et al.* (1998).

The experiment was laid out in Randomized Block Design and the data were analysed following univariate ANOVA (Analysis of variances) with the help of SAS (Statistical Analysis System) where mean values were compared using LSD at $P \leq 0.05$.

Table 1 Influence of plant bioregulators on bunch and berry physical characteristics of cv. Beauty Seedless

Treatment	Bunch wt. (g)	Bunch length (cm)	Bunch width (cm)	Bunch compact ness	Berry weight (g)	Berry length (mm)	Berry diameter (mm)	Berry shape index	Berry firmness (N^{1*})	Berry elasticity (mm)	Juice content (%)
ABA 200 ppm	342.30	21.07^{ab}	12.97^{ab}	Dense	1.36^{bc}	13.11^{a}	12.40^{abc}	0.97^{abc}	3.25^{bc}	4.14^{a}	67.33^{a}
ABA 400 ppm	365.73	17.73^{d}	13.10^{ab}	Dense	1.56^{ab}	12.32^{ab}	12.67^{ab}	1.06^{a}	3.08^{bc}	3.77^{a}	58.33^{b}
BTH 0.3 mM	357.80	18.83^{cd}	13.03^{ab}	Dense	1.54^{ab}	12.61^{a}	12.66^{ab}	1.02^{b}	3.66^{b}	3.66^{a}	70.67^{a}
BTH 0.6 mM	368.93	21.13^{ab}	13.63^{ab}	Dense	1.66^{a}	13.34^{a}	13.05^{a}	1.07^{a}	3.40^{b}	2.14^{c}	66.67^{a}
Ethephon 200 ppm	368.80	19.67^{bc}	12.53^{bc}	Dense	1.65^{a}	12.83^{a}	12.04^{bc}	0.94^{bc}	3.12^{bc}	3.58^{a}	67.67^{a}
Ethephon 400 ppm	341.57	21.67^{a}	13.90^{a}	Dense	1.31^{bc}	13.46^{a}	13.13^{a}	0.94^{bc}	2.55^{c}	2.29^{bc}	68.00^{a}
Pro-Ca 200 ppm	343.43	17.13^{d}	11.47^{cd}	Dense	1.37^{bc}	11.16^{c}	11.93^{bc}	1.02^{ab}	3.48^{b}	3.44^{a}	59.67^{b}
Pro-Ca 400 ppm	337.30	18.33^{cd}	11.23^{d}	Dense	1.22^{c}	10.94^{c}	11.65^{c}	1.00^{abc}	3.10^{bc}	3.31^{ab}	68.33^{a}
Control	358.43	17.50^{d}	12.50^{bc}	Dense	1.54^{ab}	11.40^{bc}	12.45^{abc}	0.91^{c}	4.67^{a}	3.69^{a}	68.33^{a}
General Mean	353.81	19.23	12.71	-	1.47	12.35	12.44	0.99	3.37	3.34	65
LSD ($P \leq 0.05$)	NS^{2*}	1.85	1.25	-	0.28	1.16	0.91	0.10	0.77	1.09	7.51

Values within a column with same letter(s) are not significantly different (P< 0.05).

Table 2 Influence of plant bioregulators on berry biochemical characteristics of cv. Beauty Seedless

Treatment	TSS (°Brix)	Titratable acidity (%)	Ascorbic acid (mg/ 100 ml)	Total monomeric anthocyanins (C$_3$GE^{3*} mg/l)	Total phenols (GAE^{4**} mg/100 g)	Total flavonoids (QE^{5***} mg/100 g)	Total antioxidant activity (DPPH^{6****}) (TE^{7*****} µmol/g)
ABA 200 ppm	19.00^{ab}	0.36^{bcd}	17.61^{ab}	513.71^{b}	137.80^{b}	57.93^{bc}	6.99^{bc}
ABA 400 ppm	17.47^{bc}	0.41^{bc}	22.01^{ab}	562.10^{a}	150.99^{a}	61.77^{a}	7.98^{a}
BTH 0.3 mM	16.47^{c}	0.34^{bcd}	15.85^{bc}	468.65^{cd}	116.46^{f}	54.80^{de}	6.56^{cde}
BTH 0.6 mM	17.07^{c}	0.36^{bcd}	17.61^{ab}	481.45^{c}	128.59^{cd}	55.27^{cd}	6.28^{e}
Ethephon 200 ppm	16.53^{c}	0.29^{d}	16.73^{ab}	508.15^{b}	123.66^{de}	60.70^{ab}	7.22^{b}
Ethephon 400 ppm	20.03^{a}	0.32^{cd}	22.89^{a}	579.62^{a}	153.60^{a}	62.73^{a}	8.30^{a}
Pro-Ca 200 ppm	16.47^{c}	0.56^{a}	18.67^{ab}	451.13^{d}	122.79^{e}	54.90^{de}	6.41^{de}
Pro-Ca 400 ppm	16.17^{c}	0.32^{cd}	21.66^{ab}	518.99^{b}	129.34^{c}	55.97^{cd}	6.84^{bcd}
Control	16.03^{c}	0.43^{b}	9.68^{c}	446.40^{d}	104.93^{g}	52.17^{e}	6.26^{e}
General Mean	17.25	0.38	18.08	503.60	129.80	57.36	6.98
LSD at 5%	1.77	0.09	6.77	22.90	5.35	2.90	0.47

Values within a column with same letter(s) are not significantly different ($P<0.05$). * Cyanidin-3-glucoside equivalent; ** Gallic acid equivalent; *** Quercetin equivalent; **** 2,2-diphenyl-1-picrylhydrazyl; ***** Trolox equivalent.

RESULTS AND DISCUSSION

Berry physical characteristics: The berry weight was found to be increased with the application of BTH 0.6 mM (1.66 g), which was on par with ethephon 200 ppm but application with Pro-Ca 400 ppm (1.22 g) led to slight reduction in the berry weight (Table 1). However, Amiri *et al*. (2010) reported the enhancement in the berry weight after exogenous application with ethephon on grapevine Beidaneh Ghermez. The treatment with ethephon 400 ppm (13.46 mm) led to increase in berry length, however, berries sprayed with Pro-Ca 400 ppm (10.94 mm) resulted in reduced berry size. The highest berry diameter was measured for ethephon 400 ppm (13.13 mm), which was on par with BTH 0.6 mM while the smallest berries were noted for treatment Pro-Ca 400 ppm (11.65 mm) (Table 1). Furthermore, exogenous application with Pro-Ca 400 ppm significantly reduced the berry weight as compared to control. On the similar lines, Thomidis *et al*. (2018) reported that Pro-Ca decreased the berry size and weight when applied at post bloom stage in seeded grape cultivars. The maximum value of berry shape index was noted for the treatments BTH 0.6 mM (1.07) and ABA 400 ppm (1.06) treatments. The berries sprayed with ethephon 400 ppm were found with least firmness (2.55 N) compared to control while ABA 400 ppm (3.08 N) also reduced the berry firmness (Table 1). Similarly, Marzouk and Kassem (2011) investigated that field treatments of ethephon can decrease berry firmness and encourage their abscission.

Bunch characteristics: Exogenous application of bioregulators resulted in changes in bunch length and width where the longest bunch length (21.67 cm) and width (13.90 cm) was recorded with ethephon 400 ppm. Regarding the bunch compactness, all the treated bunches in Beauty Seedless cultivar were of dense category as per the IPGRI grape descriptors (Table 1). However, in contrast there were no differences in weight, length and width of bunches of cv Rubi (Lerin *et al*. 2013) treated with ABA.

Berry biochemical characteristics: The TSS of the berries was significantly enhanced with application of ethephon 400 ppm (20.03°Brix) and ABA 200 ppm (19°Brix) in comparison with the control (16.03°Brix) (Table 2). Few other workers have also reported improvement in TSS by the use of these bioregulators such as ethephon (De Souza *et al*. 2015; Costa *et al*. 2016), ABA (Balint and Reynolds 2013 and Fidelibus and Williams 2017), which supported the present findings. The least titratable acidity was recorded with ethephon 200 ppm (0.29%); however, ethephon 400 ppm (22.89 mg/100 ml) and ABA 400 ppm (22.01 mg/100 ml) treatments resulted in significant improvement in ascorbic acid content (Table 2). On the similar lines, Gonzalez *et al*. (2018) investigated that field treatments of ethephon can reduce acidity in grape berries. Moreover, the exogenous application of the plant bioregulators significantly enhanced the accumulation of total monomeric anthocyanins in the berries for which ethephon 400 ppm (579.62 C$_3$GE mg/l) and ABA 400 ppm (562.10 C$_3$GE mg/l) showed the most promising results (Table 2). The results obtained are in agreement with those of Roberto *et al*. (2013) for ethephon treatment. Peppi *et al*. (2006) found an upsurge in the anthocyanin content with the treatment of ABA in Flame Seedless grapes. The highest total phenolics content (153.60 GAE mg/100 g) and the highest total flavonoids (62.73 QE mg/100 g) were determined in the berries treated with ethephon 400 ppm (Table 2). Earlier, Yamamoto *et al*. (2015) recorded increment in flavonoids content due to application of ABA treatments and such treatments with ethephon and ABA might be responsible for enhancing the expression of genes coding for certain enzymes promoting phenolic biosynthesis in the early stages of berry development following the spray treatment. The maximum total antioxidant activity (8.30 TE µmol/g) was recorded from berries treated with ethephon 400 ppm, which was on par with ABA 400 ppm (7.98 TE µmol/g) (Table 2). Similar findings were also noted by Sandhu *et al.* (2011) with exogenous application of abscisic acid in *Vitis rotundifolia*.

Therefore, it can be concluded that the exogenous application of plant bioregulators such as abscisic acid, ethephon, pro-hexadione calcium and benzothiadiazole at veraison stage of grape berry development had a remarkable effect on several berry quality characteristics. However, amongst the bio-regulators, ethephon (400 ppm) and abscisic acid (400 ppm) had the most promising results in improving berry qualities in grape cv. Beauty Seedless under the sub-tropical conditions compared to other plant bioregulators.

ACKNOWLEDGEMENTS

The author acknowledges the support provided through the Junior Research Fellowship of the Indian Council of Agricultural Research, New Delhi and Director, IARI, New Delhi for the facilities.

REFERENCES

Amiri M E, Fallah E and Parseh S H. 2010. Application of ethephon and ABA at 40% veraison advanced maturity and quality of ‘Beidaneh Ghermez’ grape. *Acta Horticulturae* **884**: 371–77.

Balint G and Reynolds A G. 2013. Impact of exogenous abscisic acid on vine physiology and grape composition of Cabernet Sauvignon. *American Journal of Enology and Viticulture* **64**(1): 74–87.

Costa T V D, Facanha R V and Scarpare Filho J A. 2016. Ethephon sprays as a defoliant in ‘Niagara Rosada’ vine subjected to two crop productions per year. *Revista Brasileira de Fruticultura* **38**(2): 1–7.

De Souza Leao P C, Lima M A C, Costa J P D and da Trindade D C G. 2015. Abscisic acid and ethephon for improving red color and quality of crimson seedless grapes grown in a tropical region. *American Journal of Enology and Viticulture* **66**(1): 37–45.

Fidelibus M W and Williams L E 2017. Effects of exogenous abscisic acid (ABA) applications on water use and cropping of ‘Thompson Seedless’ grapevines. *Acta Horticulturae* **1206**: 57–62.

Gonzalez R, Gonzalez M R and Martin P. 2018. Abscisic acid and ethephon treatments applied to ‘Verdejo’ white grapes affect the quality of wine in different ways. *Scientia Agricola* **75**(5): 381–86.

Lerin S, Fagherazzi A F, Mario A E, Turmina A G, Gerber J, Boff C E and Kretzschmar A A. 2013. Physicochemical characteristics of cv. Rubi with ABA application. *Acta Horticulturae* **1157**: 55–58.

Mandemaker A J, Cutting J G M, Smith D B and Dixon J. 2005. Effect of prohexadione-Ca on shoot growth, fruit set and retention in ‘Hass’ avocado in New Zealand. *New Zealand Avocado Grower’s Association Annual Research Report* **5**: 35–42.

Marzouk H A and Kassem H A. 2011. Improving yield, quality, and shelf life of Thompson seedless grapevine by preharvest foliar applications. *Scientia Horticulturae* **130**(2): 425–30.

Peppi M C, Fidelibus M W and Dokoozlian N. 2006. Abscisic acid application timing and concentration affect firmness, pigmentation, and colour of ‘Flame Seedless' grapes. *HortScience* **41**(6): 1440–45.

Ranganna S. 1999. *Handbook of Analysis and Quality Control for Fruits and Vegetable Products*, 2nd edn, p 105. New Delhi, India: Tata McGraw-Hill publishing, Co. Ltd.

Roberto S R, de Assis A M, Yamamoto L Y, Miotto L C V, Sato A J, Koyama R and Genta W. 2012. Application timing and concentration of abscisic acid improve colour of ‘Benitaka’ table grape. *Scientia Horticulturae* **142**: 44–48.

Roberto S R, Assis A M D, Yamamoto L Y, Miotto L C V, Koyama R, Sato A J and Borges R D S. 2013. Ethephon use and application timing of abscisic acid for improving colour of 'Rubi' table grape. *Pesquisa Agropecuária Brasileira* **48**(7): 797–800.

Ruiz-Garcia Y, Romero-Cascales I, Gil-Munoz R, Fernandez-Fernandez J I, Lopez-Roca J M and Gomez-Plaza E. 2012. Improving grape phenolic content and wine chromatic characteristics through the use of two different elicitors: Methyl jasmonate versus benzothiadiazole. *Journal of Agricultural and Food Chemistry* **60**(5): 1283–90.

Sanchez-Moreno C, Larrauri J A and Saura-Calixto F. 1998. A procedure to measure the antiradical efficiency of polyphenols. *Journal of the Science of Food and Agriculture* **76**: 270–76.

Sandhu A K, Gray D J, Lu J and Gu L. 2011. Effects of exogenous abscisic acid on antioxidant capacities, anthocyanins, and flavonol contents of muscadine grape (*Vitis rotundifolia*) skins. *Food Chemistry* **126**(3): 982–88.

Singleton V L, Orthofer R and Lamuela-Raventos R M. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in enzymology, Academic Press,* **299**: 152–78.

Srivastava A and Handa A K 2005. Hormonal regulation of tomato fruit development: a molecular perspective. *Journal of Plant Growth Regulation* **24**(2): 67–82.

Thomidis T, Zioziou E, Koundouras S, Navrozidis I and Nikolaou N. 2018. Effect of prohexadione-Ca on leaf chlorophyll content, gas exchange, berry size and composition, wine quality and disease susceptibility in *Vitis vinifera* L. cv. Xinomavro. *Scientia Horticulturae* **238**: 369–74.

VIVC (https://www.vivc.de/index.php?r=passport%2Fview&id=1065) Accessed on 28th November, 2020.

Wrolstad R E, Acree T E, Decker E A, Penner M H, Reid D S, Schwartz S J and Sporns P. 2005. *Handbook of Food Analytical Chemistry*, Vol 1: Water, proteins, enzymes, lipids, and carbohydrates. John Wiley and Sons.

Yamamoto L Y, de Assis A M, Roberto S R, Bovolenta Y R, Nixdorf S L, Garcia-Romero E and Hermosin-Gutierrez I. 2015. Application of abscisic acid (S-ABA) to cv. Isabel grapes (*Vitis vinifera* × *Vitis labrusca*) for colour improvement: Effects on colour, phenolic composition and antioxidant capacity of their grape juice. *Food Research International* **77**: 572–83.

Yaman M, Atalay V, Turker M, Gerek O and Cetin A. 2002. Texture segmentation by using adaptive poly phase sub and decomposition. (In) *Proceedings of the 17th international symposium on computer and information sciences,* Cicekli I, Cicekli N, Gelenbe E (Eds).

Zhishen J, Mengcheng T and Jianming W. 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry* **64**(4): 555–59.