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Influence of new generation plant bio-regulators on physio-biochemical alterations in grapes cv. Beauty Seedless

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ABSTRACT

Influence of benzothiadiazole (BTH) and pro-hexadione calcium (Pro-Ca), abscisic acid (ABA) and ethephon on leaf gas exchange parameters such as net photosynthesis rate (*A*), stomatal conductivity (g_s), intercellular CO_2 concentration (*Ci*) and leaf transpiration rate (*E*), leaf stomatal density, leaf relative water content (RWC) and leaf biochemical parameters along with berry surface colour characteristics such as *L (lightness), *a** (greenness to redness), *b** (blueness to yellowness), chroma (*C**), hue angle (*h* º) and CIRD (Colour Index for Red Grapes) were examined on grapevine cv. 'Beauty Seedless' especially under hot subtropical conditions. The highest leaf *A* (12.20 µmol CO_2 m^{-2} s^{-1}) and g_s (0.15 mmol m^{-2} s^{-1}) were recorded with Pro-Ca 400 ppm treatment, while the lowest *A* (7.97 µmol CO_2 m^{-2} s^{-1}) and g_s (0.05 mmol m^{-2} s^{-1}) were recorded with ABA 400 ppm and ethephon 400 ppm, respectively. Among other physiological parameters, the highest value of *Ci* was measured with ABA 400 ppm (265.29 µmol m^{-2} s^{-1}) treated vines followed by ethephon 400 ppm (261.78 µmol m^{-2} s^{-1}). The vines sprayed with ethephon 400 ppm (3.18 mmol m^{-2} s^{-1}) were recorded had the lowest value of leaf *E*. Similar trends were also recorded for leaf RWC. Treatments ABA (400 ppm) and ethephon (400 ppm) decreased the stomatal density significantly. The highest chlorophyll '*a*' content was observed in case of ethephon 400 ppm (2.39 mg g^{-1}) treated leaves having similarity statistically with all the treatments except control, while the highest chlorophyll '*b*' and total chlorophyll contents were noted with ABA 400 ppm. Treatment ethephon 400 ppm (0.89 mg g^{-1}) improved the total carotenoids content, which proved similar statistically with ABA 400 ppm (0.88 mg g^{-1}). The least values of *L**, *C** and the highest CIRG index were recorded for ABA 400 ppm (19.63, 2.36 and 7.61, respectively) followed by ethephon 400 ppm (20.14, 2.67 and 7.43, respectively).**

Key words: *Vitis vinifera* L, carotenoids, leaf gas exchange, PBRs, phenolics, stomatal density

INTRODUCTION

Grape is one of the nourishing fruits having advantageous effects on human health. The European grape (*Vitis vinifera* L.) is one of the most prominent species of cultivated grape worldwide, while other important cultivated species are American grapes (*V. labrusca* and *V. rotundifolia*) and also some French hybrids. Grape has been used mostly for wine making, while on limited scale for table, raisins and juice making. In general, the plant hormones have the ability to regulate various growth and development phases in the plants, and the discovery of their roles have been considered crucial in horticulture production. Nowadays, plant bioregulators (PBRs) are widely used in viticulture for enhancing bunch production and berry quality improvement.

One of these bioregulators was paclobutrazol, which is another form of 2-oxaglutarate (Rademacher, 14) affecting accumulation of uncommon flavonoids in young grape leaves and also altering the flavonoid metabolism. Exogenous application of abscisic acid (ABA) at veraison stage, starting from one week prior to veraison is known to accelerate the transpiration rate in grapevine cv. 'Cabernet Sauvignon' (Balint and Reynolds, 3) as well as total carotenoids and total phenolics content (Berli *et al*., 6). Contrastingly, treatment of ABA to grapevine leaves results in the reduction in net transpiration rate and increases the leaf water potential (Fidelibus and Williams, 9). However, grapevines treated with ethephon after fruit-set efficiently reduces the leaf chlorophyll content, leaf area and net photosynthesis rate as compared to the untreated control, which can be compensated by enhanced growth of principle shoots (Gonzalez *et al*., 11).

There is very little information about the influence new generation PBRs on physiology of grapevines. These PBRs are expected to influence several leaf physico-chemical characteristics, thus indirectly impacting the berry yield and quality significantly. Hence, this study was conducted to examine the influence of new generation PBRs on the physiology of grapevine cv. 'Beauty Seedless' raised under hot subtropical conditions.

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MATERIALS AND METHODS

The experiment was conducted on 5-year-old fruiting Beauty Seedless grapevines planted at 3 m × 3 m distance, and trained on Bower system during 2019 at the Experimental Field of the Division of Fruits and Horticultural Technology, ICAR-IARI, New Delhi. 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 pH and clay loam texture. Total nine treatments with three replications for each treatment were arranged in Randomized Block Design (RBD). Different bioregulators namely, abscisic acid (ABA) (200 and 400 ppm), benzothiadiazole (BTH) (0.3 and 0.6 mM), ethephon (200 and 400 ppm) and pro-hexadione Calcium (Pro-Ca) (200 and 400 ppm), and water spray as control were sprayed at veraison stage (24^{th} May) on whole vine canopy using a handheld sprayer in the evening hours. The leaf gas exchange parameters, such as net photosynthesis (*A*), stomatal conductivity (g_s), intercellular CO_2 concentration (*Ci*), and leaf transpiration (*E*) were measured between 9 a.m. and 11 a.m. with the help of photosynthesis System (LCi-SD Ultra-Compact system, ADC BioScientific Ltd., UK) after harvest of berries. Three fully expanded leaves were marked for observations, from the cluster opposite to leaves of each experimental vine. The leaf stomatal density and relative water content (RWC) were determined by the methods described by D'Ambrogio (8) and Barrs and Weatherley (5), respectively. The Folin-Ciocalteu reagent was used to determine the leaf total phenols (Singleton *et al.*, 15). To determine chlorophyll '*a*', chlorophyll '*b*' and total chlorophyll content, dimethyl sulfoxide (DMSO), method was employed (Hiscox and Israelstam, 12), while total carotenoids content was estimated as per the method described in AOAC (1). The estimated yield was calculated by counting the number of bunches per vine and bunch weight. A colour meter (Color Tec PCM/PSM, USA) was used to determine the berry skin colour. The instrument was standardized using a white calibration reference before assessing the berry surface colour. L^* (lightness), a^* (greenness to redness) and b^* (blueness to yellowness) values were measured for all treated samples along with control. On the basis of these values, the chroma (C^*) and hue angle (h^o) were derived by using the formula, $C^* = [(a^*)^2 + (b^*)^2]^{½}$ and $h^o = \arctan(b^*/a^*)$, respectively. The CIRG (Colour Index of Red Grapes) values were calculated using, CIRG = $(180 – h^o/(C^* + L^*)$. Univariate ANOVA (Analysis of Variance) and correlation analysis were done with the help of SAS (Statistical Analysis System: Version 9.2) and the mean values for each treatment were calculated by using LSD (Least significant differences) at $p \leq 0.05$.

RESULTS AND DISCUSSION

The highest leaf *A* (12.20 µmol CO_2 m^{-2} s^{-1}) and g_s (0.15 mmol m^{-2} s^{-1}) were recorded with Pro-Ca 400 ppm treatment, while the lowest values of *A* (7.97 µmol CO_2 m^{-2} s^{-1}) and g_s (0.05 mmol m^{-2} s^{-1}) were recorded with ABA 400 ppm and ethephon 400 ppm, respectively. Of the other physiological parameters, the highest value of *Ci* (263.29 µmol m^{-2} s^{-1}) was noticed in ABA 400 ppm treated leaves followed by ethephon 400 ppm (261.78 µmol m^{-2} s^{-1}), ABA 200 ppm (253.09 µmol m^{-2} s^{-1}) and ethephon 200 ppm (252.36 µmol m^{-2} s^{-1}). The vines sprayed with ethephon 400 ppm showed the lowest value of

Table 1. Influence of PBRs on leaf gas exchange and stomatal parameters of 'Beauty Seedless' grapevine.

Treatment	*A* (µmol CO_2 m^{-2} s^{-1})	g_s (mmol m^{-2} s^{-1})	*Ci* (µmol m^{-2} s^{-1})	*E* (mmol m^{-2} s^{-1})	Stomatal density (mm^{-2})	RWC (%)
ABA 200 ppm	8.22^d	0.06^b	253.09^b	3.34^{de}	425.16^c	73.32^{cd}
ABA 400 ppm	7.97^d	0.06^b	265.29^a	3.19^e	357.14^e	72.95^{cd}
BTH 0.3 mM	11.10^b	0.11^a	228.58^{cd}	5.29^b	476.18^{ab}	74.82^{bc}
BTH 0.6 mM	11.39^b	0.11^a	225.26^{cd}	5.15^{bc}	459.18^b	75.46^b
Ethephon 200 ppm	9.77^c	0.05^b	252.36^b	3.60^d	425.17^c	72.84^{cd}
Ethephon 400 ppm	9.50^c	0.0^5b	261.78^a	3.18^e	374.14^{de}	77.75^a
Pro-Ca 200 ppm	11.47^{ab}	0.12^a	227.06^{cd}	5.14^{bc}	425.17^c	74.48^{bc}
Pro-Ca 400 ppm	12.20^a	0.15^a	231.69^c	4.84^c	391.15^d	74.60^{bc}
Control	10.81^b	0.12^a	222.37^d	5.80^a	493.19^a	71.55^d
General Mean	10.27	0.09	240.83	4.39	425.16	74.2
LSD (p < *0.05*).	0.73	0.05	8.47	0.36	22.63	2.08

Values within a column with same letter(s) are not significantly different by LSD (p < *0.05*).

leaf *E* (3.18 mmol m^{-2} s^{-1}). The similar trends were also noticed for leaf RWC (Table 1). It is known that Pro-Ca is basically a new generation gibberellin biosynthesis inhibitor that decreases the endogenous levels of active gibberellins in plant by acting on 3ß hydroxylation, and thereby improving the other physiological response in higher plants (Rademacher, 14). Medjdoub *et al*. (13) from his investigation using Pro-Ca in apple found increase in the *A* and leaf chlorophyll content. Similar results in grapevines with regard to *A* at mid-maturity stage might be because of positive action of Pro-Ca. The drop in *A* might be due to inhibitory effect of both ABA and ethephon on the vines. Similar findings were also reported by Gonzalez *et al*. (11) due to ethephon after fruit-set, which efficiently reduced the lateral bud sprouting and *A* as compared with the control.

Stomatal behaviour is one of the important factors in CO_2 assimilation in the plants and net assimilate production. Stomatal pores help in diffusion of CO_2 into the leaves and allow water vapour to escape in the environment. Stomatal opening is governed by several factors. During the stress conditions, stomata opening are altered or leaves develop fewer stomata similar to elevated CO_2 exposure. In the present study, the least stomatal density (357.14 mm^{-2}) was recorded for leaves treated with ABA 400 ppm solution followed by ethephon 400 ppm (374.14 mm^{-2}) (Table 1; Fig. 1). There was decrease in the g_s in the same treatment with enhancement in *Ci* due to application of ABA and ethephon 400 ppm, which could be the reason for low *A* in the vines. The use of different exogenous bio-regulators might have been helpful in optimizing the marginal water cost of carbon gain as proposed by the Franks *et al.* (10) where relative gradient for diffusion of CO_2 inside the leaves is relatively constant and specified by compatible feedback mechanism, hereby resulting in improved berry quality parameters.

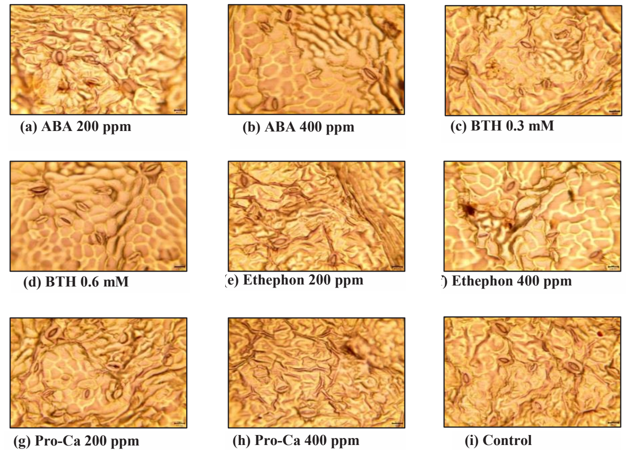

Fig. 1. Influence of plant bioregulators on stomatal density of cv. 'Beauty Seedless'

Phenolic compounds are stored within the cell wall of the plant and provide an important first line protection mechanism against the different ailments (Cartea *et al*., 7). Although no significant differences were observed in total phenols content of the leaves treated with different bioregulators, yet slight variations were noted in our investigation. Among the treatments, maximum total phenols content was estimated in the leaves treated with ABA 400 ppm (259.33 mg 100 g^{-1}) (Table 2). Berli *et al*. (6) also reported that weekly applications of ABA on grapevine leaves from bud-break to harvest improved the accumulation of phenolics and UV-absorbing compounds. In the

Table 2. Influence of PBRs on leaf biochemical parameters of 'Beauty Seedless' grapevine.

Treatment	Chlorophyll '*a*' (mg g^{-1})	Chlorophyll '*b*' (mg g^{-1})	Total chlorophyll (mg g^{-1})	Total carotenoids (mg g^{-1})	Total phenols (mg 100 g^{-1})
ABA 200 ppm	2.37^{a}	0.48^{d}	2.47^{bc}	0.68^{c}	249.86^{a}
ABA 400 ppm	2.34^{a}	0.67^{a}	2.63^{a}	0.88^{a}	259.33^{a}
BTH 0.3 mM	2.33^{a}	0.36^{ef}	2.31^{d}	0.73^{bc}	252.39^{a}
BTH 0.6 mM	2.33^{a}	0.34^{f}	2.29^{d}	0.75^{bc}	250.93^{a}
Ethephon 200 ppm	2.35^{a}	0.58^{b}	2.54^{ab}	0.78^{b}	252.13^{a}
Ethephon 400 ppm	2.39^{a}	0.37^{e}	2.38^{cd}	0.89^{a}	257.06^{a}
Pro-Ca 200 ppm	2.26^{a}	0.51^{c}	2.40^{cd}	0.73^{bc}	247.60^{a}
Pro-Ca 400 ppm	2.27^{a}	0.65^{a}	2.55^{ab}	0.76^{b}	252.66^{a}
Control	2.11^{b}	0.65^{a}	2.58^{ab}	0.44^{d}	246.26^{a}
General Mean	2.31	0.51	2.46	0.74	252.02
LSD ($p < 0.05$)	0.13	0.02	0.13	0.08	NS

Values within a column with same letter(s) are not significantly different by LSD ($p < 0.05$).

present experiment, different photosynthetic pigments showed variable levels due to exogenous application of PBRs (Table 2). The highest chlorophyll '*a*' content (2.39 mg g^{-1}) was observed in the leaves treated with ethephon 400 ppm, while the highest chlorophyll '*b*' (0.67 mg g^{-1}) and total chlorophyll content (2.63 mg g^{-1}) were determined in the leaves treated with ABA 400 ppm. However, Gonzalez *et al*. (11) valuated the effects of ethephon treatments on yield, vigour and quality of 'Verdejo' grapevines and revealed that plants treated with ethephon after fruit-set efficiently reduced leaf chlorophyll content. In wheat, ABA enhances the carotenoid accumulation in leaves and the carbohydrate in grains (Travaglia *et al*., 16). Earlier, Barickman *et al*. (4) observed the increase in carotenoid and chlorophyll concentrations in ABA treated leaves and fruit of two tomato genotypes. In present study, ethephon 400 ppm (0.89 mg g^{-1}) improved the total carotenoids content, which was statistically at par with ABA 400 ppm (0.88 mg g^{-1}) (Table 2).

There were no significant changes were observed in the estimated yield as influenced by plant bioregulators (Fig. 2). The results were in agreement with Amiri *et al.* (2), they carried out an experiment to compare the effects of application of ABA and ethephon on the 'Beidaneh Ghermez' grape and observed that both the treatments non-significantly affected the berry yield in comparison with the untreated controls.

The analysis of berry surface colour characteristics showed significant variations for all berry colour characteristics analysed in this experiment (Table 3). The treatments with ABA 400 ppm and ethephon 400 ppm presented the lowest values of L^*, 19.63 and 20.14, respectively whereas berries from control were measured with highest L^* values (26.24). L^* is luminosity or lightness of berries which means lower the value L^*, darker the berries. The maximum values of a^* (greenness to redness) and b^* (blueness to yellowness) were measured for ethephon 200 ppm (2.84) and control (2.82), respectively while least values of a^* and b^* were recorded for control (1.88) and ABA 200 ppm (0.68). The C* values were decreased most significantly for the berries sprayed with both, ABA 400 ppm (2.36) and ethephon 400 ppm (2.67) while variable measurements of hue angles (h^o) were obtained for all the treatments. The maximum berry colour index is of utmost importance in evaluating the effect of PBRs on berry colour. The highest CIRG values were determined for ABA 400 ppm (7.61) followed by ethephon 400 ppm (7.43) treatments. On the similar lines, the significant changes in berry skin colour characteristics were reported in 'Rubi' grapevine (Roberto *et al*., 17).

Correlation analysis more clearly predicted the relation among the different berry quality characteristics such as total soluble solids (TSS), titratable acidity (TA), ascorbic acid content (ASA), total phenolics content (TPC), total flavonoids content (TFC), total anthocyanins content (TAC), total antioxidant activity (TAA), luminosity (L^*), chroma (C^*), hue angle (h^o) and Colour Index of Red Grapes (CIRG) (Fig. 3). The TSS showed negligible correlation with TA (r =

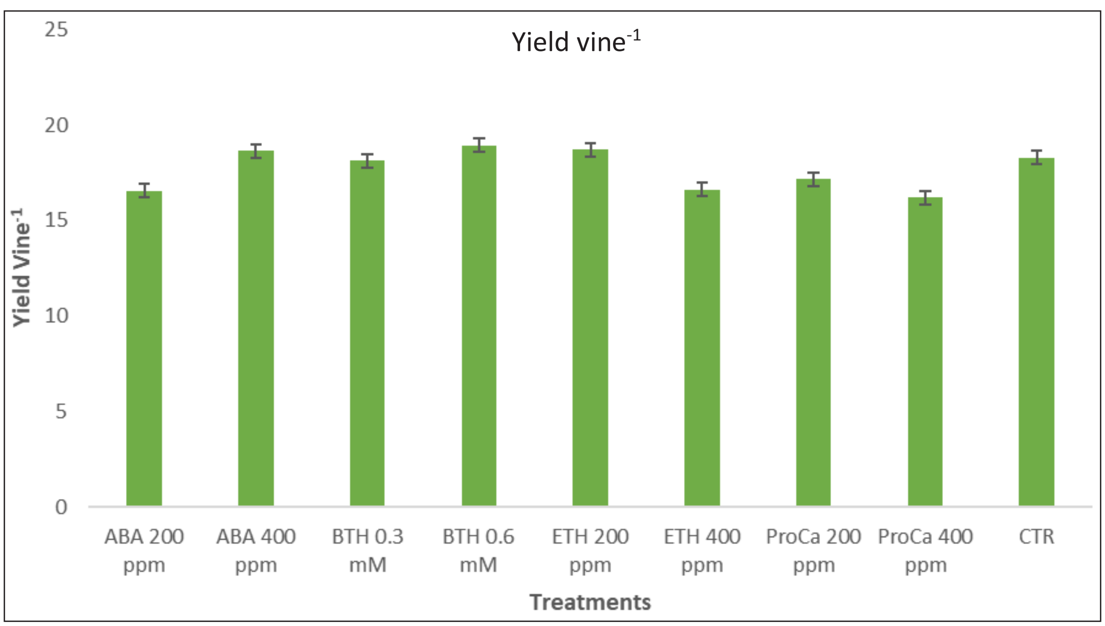

Fig. 2. Influence of plant bioregulators on berry yield

Table 3. Influence of PBRs on berry peel colour characteristics of 'Beauty Seedless' grapevine.

Treatment	*L**	*a**	*b**	*C**	*h °*	CIRG
ABA 200 ppm	21.70^{d}	2.73^{a}	0.68^{e}	3.96^{c}	14.01^{e}	6.47^{bc}
ABA 400 ppm	19.63^{e}	2.12^{d}	0.48^{g}	2.36^{g}	12.78^{f}	7.61^{a}
BTH 0.3 mM	24.00^{b}	2.06^{d}	1.72^{b}	3.61^{d}	39.95^{b}	5.07^{f}
BTH 0.6 mM	22.50^{cd}	2.38^{bc}	0.92^{d}	3.26^{e}	21.11^{d}	6.17^{cd}
Ethephon 200 ppm	21.57^{d}	2.84^{a}	0.59^{f}	4.21^{b}	11.80^{g}	6.52^{b}
Ethephon 400 ppm	20.14^{e}	2.27^{c}	0.42^{g}	2.67^{f}	10.39^{h}	7.43^{a}
Pro-Ca 200 ppm	23.04^{bc}	2.48^{b}	1.48^{c}	4.17^{b}	30.86^{c}	5.48^{e}
Pro-Ca 400 ppm	22.88^{c}	2.46^{b}	0.96^{d}	3.49^{d}	21.39^{d}	6.02^{d}
Control	26.24^{a}	1.88^{e}	2.82^{a}	5.75^{a}	56.46^{a}	3.86^{g}
General Mean	22.41	2.36	1.12	3.72	24.31	6.07
LSD ($p < 0.05$)	1.05	0.13	0.07	0.19	0.80	0.31

Values within a column with same letter(s) are not significantly different by LSD ($p < 0.05$)

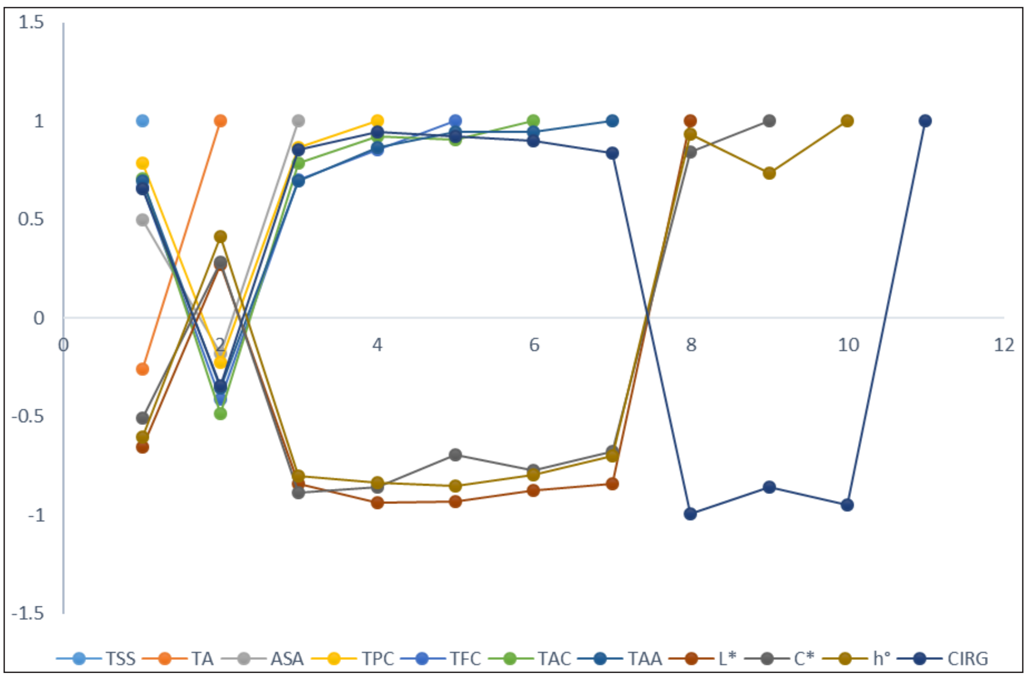

Fig. 3. Graph showing correlation between total soluble solids (TSS), titratable acidity (TA), ascorbic acid content (ASA), total phenolics content (TPC), total flavonoids content (TFC), total anthocyanins content (TAC), total antioxidant activity (TAA), luminosity (*L**), chroma (*C**), hue angle (*h °*) and Colour Index of Red Grapes (CIRG).

-0257), high positive correlation with TPC (r = 0.786), TAC (r = 0.708); moderate positive correlation with TFC (r = 0.663), TAA (r = 0.697); moderate negative correlation with *L** (r = - 0.656), *C** (r = - 0.508), *h °* (r = - 0.600) while moderate positive correlation with CIRG (r = 0.659). The correlation of TA with other parameters presented negligible (r = - 0.179) to low negative correlation (r = - 0.354). The ASA showed high positive correlation with TPC (r = 0.864), TFC (r = 0.703), TAC (r = 0.787) and high negative correlation with *L** (r = - 0.841), *C** (r = - 0.888), *h °* (r = - 0.801). TPC had very high positive correlation with TAC (r = 0.923), CIRG (r = 0.944); high positive correlation with TFC (r = 0.854), TAA (r = 0.863); very high negative correlation with *L** (r = - 0.937) and high negative correlation with *C** (r = - 0.857), *h °* (r = - 0.837). Further, TFC presented very high positive correlation with TAC (r = 0.908), TAA (r = 0.944), CIRG (r = 0.922); moderate negative correlation with *C** (r = - 0.693); high negative correlation with *h °* (r

= - 0.854); very high negative correlation with L^* (r = - 0.930). TAC showed moderately positive correlation with TSS (r = 0.708); high positive correlation with ASA (r = 0.787), TAA (r = 0.974), TPC (r = 0.922) and TFC (r = 0.908) and CIRG (r = 0.899), whereas high negative correlation with L^* (r = - 0.873), C^* (r = - 0.771), $h°$ (r = - 0.854). This relation states that the berries with high anthocyanins content will be richer in phenolics, flavonoids content and antioxidant activity. The TAA presented high negative correlation with L^* (r = - 0.842); moderate to high negative correlation with C^* (r = - 0.676), $h°$ (r = - 0.698) while high positive correlation with CIRG (r = 0.837). Importantly, the TAA had nearly high positive correlation with TSS (r = 0.697) and ASA (r = 0.694); high positive correlation with TPC (r = 0.863) and very high positive correlation with TFC (r = 943) and TAC (r = 0.947) which clearly describes the relation between these berry quality characteristics. This indicates that coloured grape berries richer in anthocyanins content are the ones with high antioxidant properties. Among the berry surface colour characteristics, L^* showed very positive correlation with h^o (r = 0.935); high positive correlation with C^* (r = 0.841) and very high negative correlation with CIRG (r = 0.994). C^* presented high positive correlation with h^o (r = 0.734) and high negative correlation with CIRG (r = - 0.855) whereas h^o showed very high positive correlation with CIRG (r = 0.949).

Overall, TAC presented very high positive correlation with TPC (r = 0.923), TFC (r = 0.908) and TAA (r = 0.947); high positive correlation with TSS (r = 0.708); low to moderate negative correlation with TA (r = - 0.487); high positive correlation with ASA (r = 0.787) and nearly very high positive correlation with CIRG (r = 0.899), high negative correlation with L^* (r = - 0.873), C^* (r = - 0.771) and $h°$ (r = - 0.854). From this correlation data analysis, it can be concluded that berries of coloured grape cultivars such as 'Beauty Seedless' contain higher total anthocyanins content and present agreeable flavour. Also, the significant amount of positive correlation was found for the total antioxidant activity and other berry quality characteristics such as TSS, total anthocyanins content, ascorbic acid content and overall berry phenolics content indicating the role of plant bioregulators treatment in boosting the health promoting substances in grape berries. These results were in agreement with Neto *et al*. (18).

It can be concluded that the exogenous treatments with PBRs namely, abscisic acid, benzothiadiazole, ethephon and pro-hexadione calcium at the veraison stage had prominent influence on leaf gas exchange, stomatal as well as biochemical parameters. The lowest A (7.97 µmol CO_2 m^{-2} s^{-1}) and g_s (0.05 mmol m^{-2} s^{-1}) were recorded with ABA 400 ppm and ethephon 400 ppm, respectively, highest value of Ci was measured with ABA 400 ppm (265.29 µmol m^{-2} s^{-1}) followed by ethephon 400 ppm (261.78 µmol m^{-2} s^{-1}). The vines sprayed with ethephon 400 ppm (3.18 mmol m^{-2} s^{-1}) were recorded with lowest value of leaf E. Treatment ethephon 400 ppm (0.89 mg g^{-1}) improved the total carotenoids content, which proved similar statistically with ABA 400 ppm (0.88 mg g^{-1}), least values of L^*, C^* and the highest CIRG index were recorded for ABA 400 ppm (19.63, 2.36 and 7.61, respectively) followed by ethephon 400 ppm (20.14, 2.67 and 7.43, respectively). Among these plant growth regulators, ethephon and abscisic acid had the most remarkable results compared to other new generation plant bioregulators, though their effects were found to be superior to control.

AUTHORS' CONTRIBUTION

Conceptualization of research (VBP); Designing of the experiments (VBP, VBM, SKS, MKV, GPM, AD); Contribution of experimental materials (SKS); Execution of field/lab experiments and data collection (VBM, VBP, CK, GPM, AD, AK); Analysis of data and interpretation (VBM, VBP, SKS, AK); Preparation of the manuscript (VBM, VBP).

DECLARATION

The authors declare that they do not have any conflict of interest.

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