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Investigating the combined effect of Hydrogen Cyanamide and Abscisic Acid on Improving Berry Color in 'Flame Seedless' Grapevines (*Vitis vinifera* L.)

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ABSTRACT: The grapevine (*Vitis vinifera* L.) holds significant importance as a horticultural crop in tropical and subtropical regions. In terms of seedless cultivars, the grape variety "Flame Seedless" is considered to be the most widely grown table grape in the world. Flame Seedless vines exhibit vigorous growth and bright red fruit clusters having medium to large-sized seedless berries. These berries possess a crisp skin, juicy pulp, and a noticeably muscat-like flavor. The current investigation helps to understand the role of the combined impact of abscisic acid (ABA) and hydrogen cyanamide (HCN) in the improvement of the berry color of the "Flame Seedless" grapevine (*Vitis vinifera* L.). Immediately after pruning, the canes of the 14-year-old vines were subjected to a spray of 2% HCN. At the time of bunch emergence, 75–80 bunches per vine were retained in all the treatments except control, where 110–120 bunches per vine were retained. The treatment T_6 (2% HCN + 100 ppm ABA + 400 ppm ethephon) had the lower lightness of berry peel color (l = 15.83), which was statistically similar with treatments T_3, T_4 and T_5. Whereas, the highest value of a* (from green to red) was also obtained from treatment T_6 (5.80), which was statistically at par with treatment T_5. The treatment T_2 (HCN 2% + 75 ppm of ABA) had the highest blueness of peel color b* value (4.91), which was statistically similar to T_3 and T8. However, T_6 (5.8) and T_3 (5.5) had the highest values of the color index of red grapes (CIRG) in comparison to control (4.34). The lowest percentage of uneven color of berries/bunch was observed with the application of 2% HCN + 100 ppm ABA + 400 ppm ethephon (16.78%). The current study will help mitigate the challenge of climate change effects due to elevated temperatures, which cause poor anthocyanin accumulation in berries. The findings of the current study revealed that application of 2% HCN (applied at the end of December) and 100 ppm ABA (applied at veraison stage) was effective in advancing berry ripening and improving berry peel color with minimal postharvest loss in fruit quality during marketing.

Keywords: Abscisic acid, Berry colour, Ethephon, Flame Seedless, Hydrogen cyanamide.

INTRODUCTION

The grape (*Vitis vinifera* L.) holds the distinction of being among the oldest fruits to be domesticated and cultivated. Although its origins lie in temperate zone, it has proven capable of flourishing in diverse climates across the globe, including tropical, subtropical, and temperate zones. It possesses a significant position in both acreage and economic returns in the world's fruit industry. It stands as one of the most delicious, refreshing, and nutritious fruits. Depending on the variety, the sugar content of the fresh fruit ranges between 12% and 24%. The dried grape berry contain up to 72% sugar and are good sources of minerals, including phosphorus, iron, calcium, vitamins B_1 and B_2. Furthermore, grapes serve as a valuable source of sugars like glucose, fructose, and sucrose, in addition to containing the acids malic and tartaric. In the realm of commerce, grapes are processed into products such as raisins, wines, juices, jellies, and syrups.

Following citrus and bananas, it ranks as the third most extensively grown fruit across the world. China holds the position of the world's largest grape producer, followed by Italy, Spain, and France. India is ranked 14th in terms of production and 7th in total area among the world's leading grape-producing countries (FAOSTAT, 2020-21). India accounts for 3.31% of the world's grape production. Currently, grapes are grown on 140 thousand hectares, producing 3125 thousand metric tons of grapes annually (NHB, 2019-20). It is an important and profitable crop for farmers among the horticulture crops growing in India.

Approximately 90% of grape cultivation in India is concentrated in Peninsular India. The major grape growing regions comprise Maharashtra, Karnataka, Andhra Pradesh, and Tamil Nadu. Moreover, grapes are also cultivated in limited scale in north-western states such as Punjab, Haryana, Uttar Pradesh, Rajasthan, and Madhya Pradesh. Despite being the leading producer, Maharashtra contributes 75% of total domestic grape production.

The major commercial varieties cultivated in this region consist of Thomson Seedless and its clones like Tas-e-Ganesh, Manik-Chaman, and Sharad Seedless. Grape cultivation is limited to the north-western plains of India, specifically in Punjab, Haryana, and certain areas of Rajasthan and Uttar Pradesh. The grape sector in this area mainly relies on a single variety, "Parlette". In Punjab, grape cultivation covers an area of 290 hectares, with an annual production of 8.2 thousand metric tons. Punjab has a higher productivity of 28.4 tons of grapes, which is almost three times higher as compared to other states (Anonymous, 2018–19). "Perlette" occupied approximately 95% of the area among various recommended varieties for commercial cultivation in the state. This prolific-bearing variety has several desirable traits, like early ripening, seedlessness, and bearing on basal buds. However, this variety has the disadvantages of a high percentage of shot berries, compact clusters, and small berry size. In Punjab and the neighbouring states, monoculture of this type causes gluts in the market within a short period of 15–20 days. Furthermore, as a result of concerns about pre-monsoon rains, there is a panic to harvest poor-quality grapes out of fear.

The PAU, Ludhiana, has recommended "Flame Seedless" (a colored variety) grapes be commercially grown in the region, aiming to diversify from the prevalent "Perlette" monoculture. J. Weinberger and F. Harmon developed this variety in 1989. This variety has several advantages over Perlette because of its higher yield, crimson red colour, seedless berries, high TSS:Acid ratio, medium bunch, and long shelf life. It matured a week after Perlette, effectively mitigating the market glut caused by Perlette. While the "Flame Seedless" grape variety offers several benefits compared to "Perlette", it exhibits a problem in sub-tropical climates: uneven color development because of high temperatures hindering the build-up of anthocyanin in the skin of grape berries (Spayed *et al*., 2002; Yamane and Shibayama 2006). In 'Benitaka' table grapes, abscisic acid (ABA) has significantly improved color development and anthocyanin accumulation (Shahab *et al*., 2019; Shahab *et al*., 2020).

Initiatives have also been undertaken to enhance the color development of "Flame Seedless" grapes using cultural methods such as managing crop load, which involves removing leaves, thinning shoots, and thinning clusters (Dokoozlian *et al*., 1995). Previously, various researchers improved the color of "Flame Seedless" by cluster thinning with foliar application of ethephon. However, the uniform ripening of "Flame Seedless" berries has not been achieved. Therefore, the present study focuses on the alone or combined use of HCN, ABA, and ethephon to improve the quality and color of berry in "Flame Seedless" variety of grapes.

MATERIALS AND METHODS

A. Plant material

The experiment of the present study was conducted on 14-year-old Flame Seedless grapevines planted at a spacing of 3 m × 3 m. Bower's system was used to train the grapevines. During the course of study, grapevines obtained uniform cultural practices. The various treatments consist of T_1 (Hydrogen Cyanamide 2% + 50 ppm ABA); T_2 (Hydrogen Cyanamide 2% + 75 ppm ABA); T_3 (Hydrogen Cyanamide 2% + 100 ppm ABA); T_4 (Hydrogen Cyanamide 2% + 50 ppm ABA + 400 ppm Ethephon); T_5 (Hydrogen Cyanamide 2% + 75 ppm ABA + 400 ppm Ethephon); T_6 (Hydrogen Cyanamide 2% + 100 ppm ABA + 400 ppm Ethephon); T_7 (Hydrogen Cyanamide 2% + 400 ppm Ethephon); T_8 (75% crop load + 400 ppm Ethephon (Local recommended practice) and T_9 Control (untreated).

At the end of December, vines were pruned to 4 basal buds, except for vines from treatments T_8 and T_9. Immediately following pruning, hydrogen cyanamide (HCN) at 2% a.i. was applied to the pruned vine cane. For treatments T_8 (recommended methodology) and T9 (control), the vines were pruned down to four bud level during the last week of January, aligning with the recommended pruning time for grapes in the northern Indian. Apart from untreated control (T_9), 75–80 bunches were retained in all the treatments during bunch emergence. At the color break stage, various ABA and ethephon concentrations were sprayed using different treatments. Tween-20 was added at 1 ml per liter during the spraying to increase the effectiveness of growth regulators.

B. Berry colour measurement

Visual assessment parameters. The visual assessment parameters (I) were examined using a Hunter Lab color difference meter (Colour Flex® EZ, USA), and the resulting variables from its equatorial section were acquired: L* (lightness), C* (chroma), and h° (hue). Lightness values range from 0 (representing black) to 100 (representing white). Chroma is derived from the a* and b* values of the CIELab scale and signifies color saturation, indicating the distance from gray (achromatic) to a pure color. Hue pertains to the color wheel and is quantified in angles; green, yellow, and red correspond to 180, 90, and 0 degrees, respectively (Lancaster, 1992). Using this information, the Color Index of Red Grapes (CIRG) was computed.

C. Colour index of red grapes (CIRG)

A Hunter Lab color difference meter (Colour Flex® EZ, USA) was employed to measure the color of the berry peel. The measurements of L*, a*, and b* were documented, and from these values, the hue angle (h°) as arc tangent (b*/a*), along with the chroma (C*) $[(a^*)^2 + (b^*)^2]^{1/2}$, were computed. These collected data were then utilized to determine the red grape color index (CIRG), following the method outlined by Carreno *et al*. (1999).

$$\text{CIRG} = \frac{(180° - h°)}{C^* + L^*}$$

D. Uneven colour of berries/bunch (%)

The berry skin <50% area showing color development were considered as berries with uneven coloring. The percentage of unevenly colored berries was determined by counting the total count of such berries against the total number of berries per bunch.

E. Statistical Analysis

A randomized block design (RBD) was used to analyze the data recorded at the time of harvest for studies on quality improvement. Using SAS (V 9.3, SAS Institute Inc., USA), the data were analyzed for variance. The treatment means are subjected to mean separation by Least Significant Difference (LSD, p≤0.05).

RESULTS AND DISCUSSION

Berry peel colour (l, a, b)

A. Berry peel colour (l)

The findings pertaining to the impact of HCN, ABA, and ethephon on the brightness of the berry's peel color (l) are shown in Fig. 1. The maximum lightness of berry peel color was observed in T_9 control (l = 23.07) when compared to other treatments, whereas the lowest berry peel color lightness was observed in treatment T_6 (2% HCN + 100 ppm ABA + 400 ppm ethephon), which was statistically similar to T_3, T_4 and T_5. The CIRG (color index of red grapes) can be improved with these treatments since they have a lower lightness (L) value. The findings of the present study are similar to those of Setha's (2012), which indicated a positive association between alterations in ABA content and color change as well as pigment content. Likewise, Link (2000) also found that a positive linear correlation exists between color and crop load, with correlation coefficients ranging from 0.60 to 0.76 in apple cultivars like 'Elstar', 'Golden Delicious', and 'Jonagold'. According to Mcdonnell *et al*. (2008), Cabernet Sauvignon grapes consistently showed the lowest color density in the lowest crop load treatments.

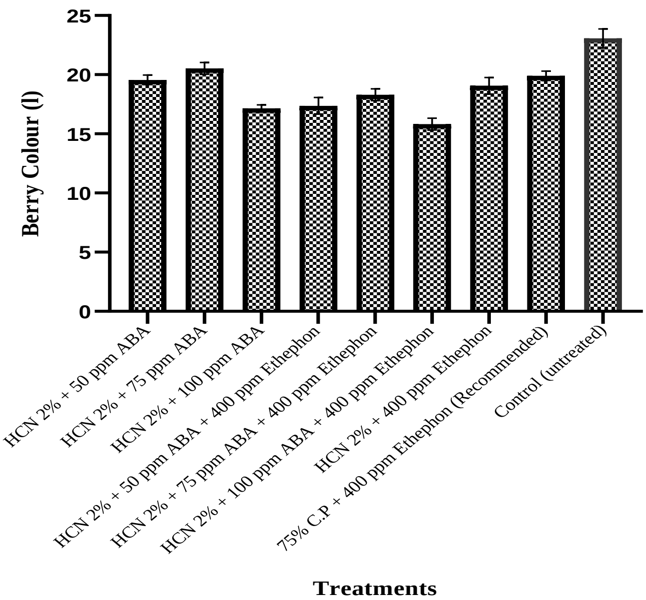

Fig. 1. Effect of pre-harvest application of hydrogen cyanamide, ABA and ethephon on berry colour (l*) L*=0: black, 100: white.

B. Berry peel colour (a)

The results of the present study concerning the impact of pre-harvest application of hydrogen cyanamide and abscisic acid on the color a* (green to red) are illustrated in Fig. 2. Application of HCN and ABA significantly alters the berry peel color a* values. The maximum value of berry peel color a* (5.80) were observed in treatment T_6 (HCN 2% + 75 ppm ABA + 400 ppm ABA), which was statistically similar with treatment T_5 (5.05) whereas the lowest a* value (2.75) were recorded in grapevines with 100% crop load as compared to other treatments. During the maturation process of Flame Seedless grapes, their color shifts from predominantly green to yellow, and eventually progressing to red. The outcomes of the present study are in conformity with those of Kliewer and Weaver

(1971). These researchers found that a variations in fruit color within vines that were subjected to thinning. The red coloration is attributed to the presence of anthocyanin pigment, with cyanidin-3-glucoside being the dominant pigment in "Flame Seedless”. These pigments are primarily confined to the skin (Fernanadez *et al*., 1998). Likewise, Peppi and Dokoozlian (2006) noted that the color of Flame Seedless grape berries improved when treated with abscisic acid (ABA). Applying ethephon treatment along with 200 or 300 mg/L ABA during veraison resulted in noticeably deeper skin color, decreased lightness (L*), and heightened b* values in "Crimson Seedless" grapes (Leao, 2012).

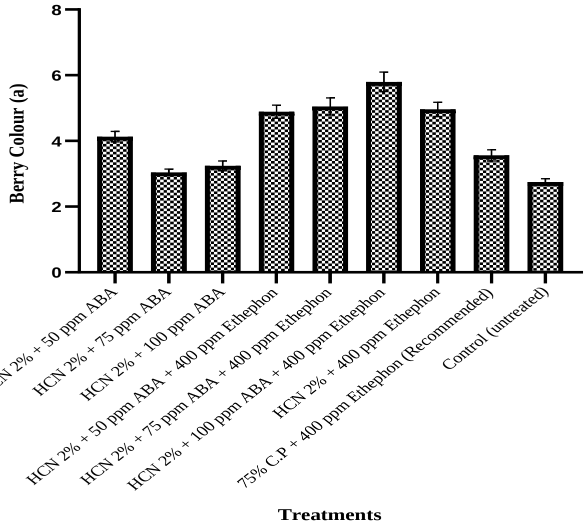

Fig. 2. Effect of pre-harvest application of hydrogen cyanamide, ABA and ethephon on berry colour (a*) a*= (-): greenness, (+): redness.

C. Berry peel colour (b)

The information regarding the berry's peel color (b) is presented in Fig. 3. The vines treated with HCN 2% + 75 ppm of ABA showed the highest b* value (4.91), at par with T_3 (HCN 2% + 100 ppm ABA) and T_8 (75% crop load + 400 ppm ethephon), while the lowest value of b* (3.71) was exhibited by Untreated vines with 100% crop load per vine. The findings of the present study align with Mcdonnell *et al*. (2008), which consistently identified the lowest color density within the lowest crop load treatments in 'Cabernet Sauvignon' grapes. Similarly, Leao (2012) also found that the combined application of ethephon with 200 or 300 mg/L ABA during veraison led to berries with notably darker skin color, reduced lightness (L*), and increased b* value in 'Crimson Seedless' grapes. Likewise, Leao (2012), suggested that the concurrent use of ethephon and ABA at concentrations of 200 or 300 mg/L during veraison led to significant changes in 'Crimson Seedless' grapes. These changes encompassed increased skin darkness, reduced lightness (L*), and a higher b* value. Canon *et al*. (2014) also found that the combination of short summer pruning and cluster thinning treatments positively influenced the color intensity of the 'Pinot Noir' grape variety.

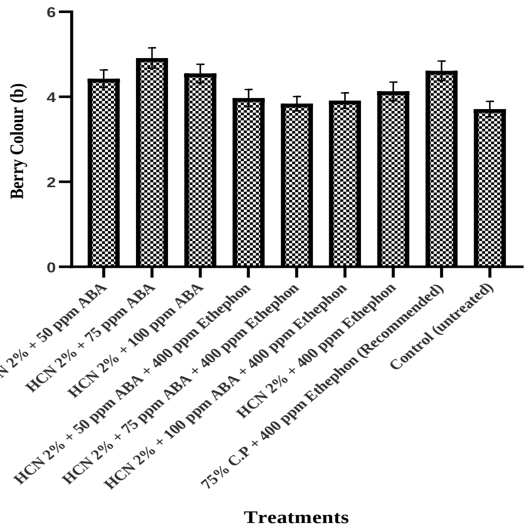

Fig. 3. Effect of pre-harvest application of hydrogen cyanamide, ABA and ethephon on berry colour (b*) b*= (-) blueness, (+): yellowness.

D. Colour index of red grapes (CIRG)

The effect of abscisic acid and ethephon treatments had a significant impact on the grape berry color quality, as indicated in the CIE lab parameters depicted in Figures 4 and 6. The highest color index of red grapes 5.80 (CIRG) was recorded in T_6 (HCN 2% + 100 ppm ABA + 400 ppm ethephon), which was at par with T_3. The current study's outcomes align with those of Vergara *et al*. (2018), where they recognized a statistically significant correlation between CIRG and the overall content and concentration of anthocyanins.

Similarly, Shahab *et al*. (2019) recorded that the treatment with ABA significantly improved both color development and anthocyanin accumulation in 'Benitaka' table grapes. Peppi and Dokoozlian (2006) suggested that application of abscisic acid (ABA) to "Flame Seedless" grapes might induce undesirable fruit softening, yet it also contributes to increased anthocyanin content and enhancement of berry color. Leao (2012) observed that when ethephon was applied together with either 200 or 300 mg/L ABA during veraison (97 days after pruning), it resulted in the development of grapes with significantly deeper skin color. Similarly, Ferrara *et al*. (2013) indicated that ABA could potentially help improve color.

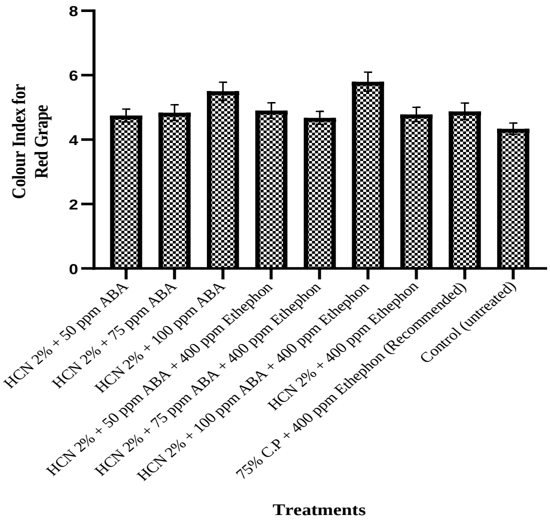

Fig. 4. Effect of pre-harvest application of hydrogen cyanamide, ABA and ethephon on colour index for red grapes (CIRG) Flame Seedless grapes.

E. Uneven colour of berries/bunch (%)

The observations related to the effect of HCN, abscisic acid, and ethephon on the uneven color of the berries per bunch are depicted in Fig. 5. The lowest uneven berries/bunch (16.78%) were observed with the application of HCN 2% + 100 ppm of ABA + 400 ppm ethephon, which was statistically at par with T_3 (17.97%) and T_7 (18.54%), respectively. The untreated vines with a 100 percent crop load exhibited the highest amount of uneven berries per bunch (42.52%).

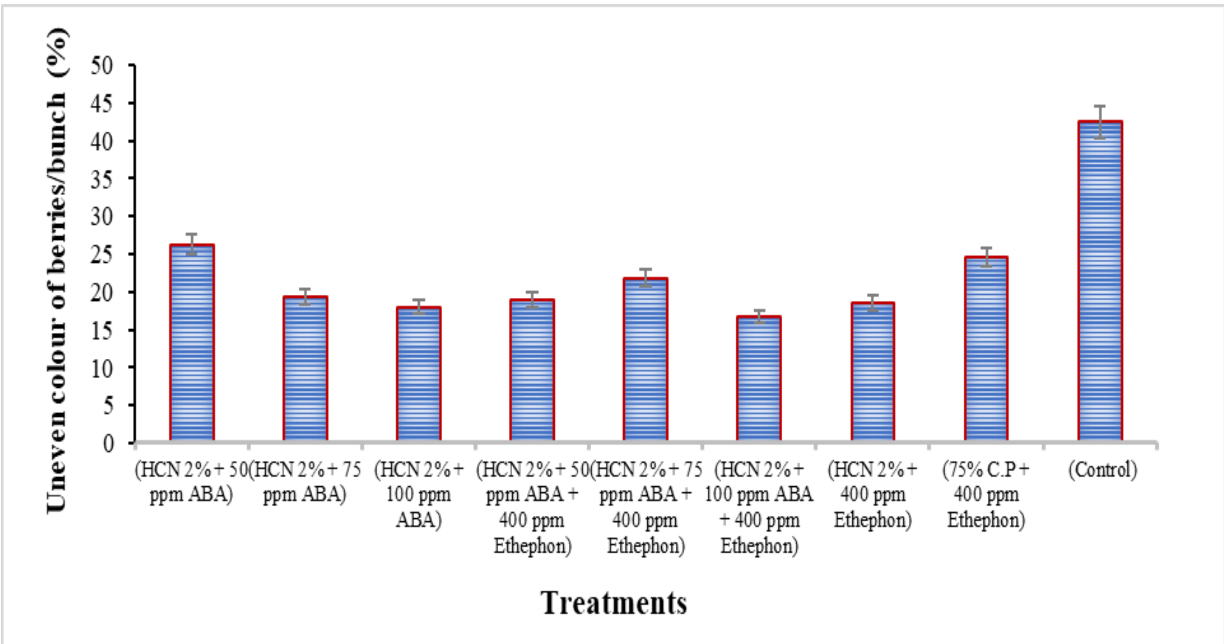

Fig. 5. Effect of pre-harvest application of hydrogen cyanamide, ABA and ethephon on uneven colour of barriers/bunches of Flame Seedless grapes.

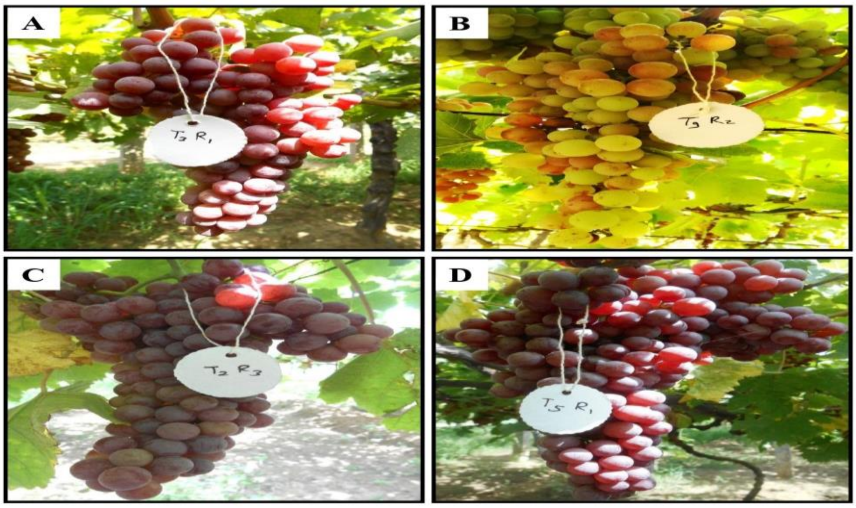

Fig. 6. Effect of pre-harvest application of hydrogen cyanamide, ABA and ethephon on Flame Seedless grape (A) 2% HCN + 100 ppm ABA (B) Control (100% crop load) (C) HCN 2% + 75 ppm ABA (D) HCN 2% + 75 ppm ABA + 400 ppm ethephon.

The alteration of anthocyanin content in berries due to increased sunlight exposure in clusters, leading to appropriate grape coloration, could be attributed to the efficacy of a decreased crop load. Likewise, there is a correlation between alterations in grape color and ABA content. The outcomes of the present study are similar to Mehta and Chundawat's (1979) findings, where they found that applying 500 ppm of ethephon during the veraison phase resulted in uniform color development in 'Beauty Seedless' and 'Pinot Noir' grapes. Kitamura *et al*. (2005) similarly found that managing the crop load enables achieving appropriate color development in 'Aki Queen' fruits by regulating anthocyanin concentration. Likewise, Panwar *et al*. (1994) noted that applying ethephon during the veraison phase resulted in a reduction of unevenly ripened berries in 'Beauty Seedless' grapes.

CONCLUSIONS

Conclusively, the current study demonstrated that application of HCN at 2%, applied at the end of December in combination with ABA (at the varaison stage), could significantly improve the color and quality of Flame Seedless grapevines while causing minimal deterioration in fruit quality during marketing.

FUTURE SCOPE

Further studies are needed to better understand the mechanisms involved in the physiological effects of HCN and ABA on anthocyanin accumulation and color development of grape berry parameters, as well as the factors involved in these physiological processes, which could help in better understanding the physiology of the grape ripening process.

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Conflict of Interest. None.

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