Erwerbs-Obstbau (2022) 64(Suppl 1) :S1–S10
https://doi.org/10.1007/s10341-022-00664-z

ORIGINAL ARTICLE / ORIGINALBEITRAG

Alterations in Chromatic Color Characteristics and Phenolic Compounds of 'Early Cardinal' Grape (*V. vinifera* L.) as affected by Various Concentrations of Foliar Abscisic Acid and Melatonin Treatments

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Received: 3 September 2021 / Accepted: 27 February 2022 / Published online: 22 March 2022

Abstract
There has recently been a revival interest in use of distinct foliar treatments for obtaining uniform skin coloration of some table grape varieties. Poor coloration of grape skin in early ripening red table grape growing is one of the major problems that grape growers may run across. In order to cope with these difficulties in uneven skin coloration in table grape varieties, different foliar treatments such as abscisic acid and melatonin may be utilized. Grape skin color is one of the major quality factors, providing remarkable contributions to market values of table grapes and is found out by assessing anthocyanin content or color characteristics. In present research, it was benefited from various concentrations of foliar abscisic acid (S-ABA) and melatonin (Mt), including control, S-ABA 300 mgL^{-1}, S-ABA 400 mgL^{-1}, S-ABA 500 mgL^{-1}, Mt 300 µM, Mt 400 µM and Mt 500 µM and CIELAB color system was used for quantifying skin color of berries. Study results showed that abscisic acid treatments were more effective than melatonin treatments on grape yield and quality characteristics and respectively, treatments of S-ABA 500 mgL^{-1}, S-ABA 400 mgL^{-1}, S-ABA 300 mgL^{-1}, Mt 500 µM, Mt 400 µM, Mt 300 µM resulted in darker red skin color and higher total phenolic compounds content in 'Early Cardinal' grape variety.

Keywords Early ripening table grape · Uneven coloration · Abscisic acid · Melatonin · Chromatic characteristics · Phenolic compounds

Beeinflussung der Farbeigenschaften und phenolischen Verbindungen von Früchten der frühen Tafeltraubensorte 'Early Cardinal' (*V. vinifera* L.) durch Blattapplikationen mit Abscisinsäure und Melatonin

Schlüsselwörter Früh reifende Tafeltraube · Ungleichmäßige Färbung · Abscisinsäure · Melatonin · Fruchtfarbe · Phenolische Verbindungen

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Introduction

'Early Cardinal' is a very early-season, colored and seeded table grape variety that can be grown in hot regions of Turkey. Its berries are firm, moderately juice, have no special aroma and skin color can alter from dark red to purple (Sabır 2008).

Nowadays, grape size, firmness, sugar and color from high quality characteristics have been demanded by global table grape markets and intensity and uniformity of skin color in both grapes and whole bunch have a crucial role on table grape quality (Arın and Akdemir 2004; Kok and Bal 2017; Kok 2018, Defilippi et al. 2019).

Commercial values of table grapes are immensely influenced by grape appearance and one of the difficulties that grape growers occasionally encounter is also lack of uniform skin color of grapes when the early table ripening grape varieties are grown (Kok and Bal 2018a).

Skin coloration of colored fruits is attributable to the presence of anthocyanin pigment (Willson and Whelan 1990). Anthocyanin biosynthesis and accumulation in grape are influenced by various factors such as grape variety (Kok and Bal 2017), environmental conditions (Mori et al. 2004), viticultural practices (Downey et al. 2006) as well as different exogenous foliar applications (Kok and Bal 2018b).

CIELAB color scale is widespread employed in food industry to describe color of food and fruits. It comprises of L*, a*, b* coordinates, where L* value represents lightness, a* represents redness or greenness and b* represents yellowness or blueness. Besides, hue angle (h°) is considered quality characteristic of color and is pertained to traditional color expressions, including reddish and yellowish (Doğan Cömert et al. 2020).

In recent years, there has been a growing interest in utilization of different foliar applications such as biostimulants, plant growth regulators and fertilizers for improving grape skin coloration and ripening process in some table grape varieties (Lurie et al. 2009; Ferrar and Brunetti 2008, 2010).

Growth regulators are the chemicals, analogous to plant hormones in cellular activity, and are derived from natural and artificial sources. Since exogenous treatments may act in a direct way on hormonal balances by regulating the physiological processes of plants, these chemicals have remarkable roles, contributing to help to obtain ripening faster and enhance the must quality by promoting processes of synthesis and accumulation of grape metabolites in grape growing (Szyjewicz et al. 1984; Cantin et al. 2007; Roberto et al. 2012; González et al. 2018).

Abscisic acid is a phytohormone, playing crucial roles in different physiological process such as induction of seed dormancy, adaptive responses to environmental stresses (Nambara et al. 1998) and enhancing of anthocyanin biosynthesis and accelerating grape ripening (Olivares et al. 2017; Li et al. 2019a). In order to achieve a homogeneous skin color in some table grape varieties, including Flame Seedless (Peppi et al. 2006), Redglobe (Peppi et al. 2007a) and Crimson Seedless (Peppi et al. 2007b; Lurie et al. 2009), exogenous treatments of abscisic acid can be used for increasing anthocyanin content of berry skin (Peppi and Fidelibus 2008).

Melatonin (N-acetyl-5-methoxytrptamine) is a novel bioactive plant hormone, synthesizing from tryptophan metabolism by means of serotonin (Wang et al. 2017; Yan et al. 2021). Melatonin is pointed out to function as a growth regulator (Arnao and Hernández-Ruiz 2015) and has crucial roles in controlling fruit ripening and senescence (El-Mogy et al. 2019; Li et al. 2019b). For instance, melatonin can enhance plant growth with substantial auxinic effect (Hernández-Ruiz et al. 2004), advance tomato ripening (Sun et al. 2015) and raise size and synchronicity of grapes (Meng et al. 2015).

The purpose of the current study is to determine the influences of various concentrations of foliar abscisic acid and melatonin treatments on characteristics of skin color and phenolic compounds of 'Early Cardinal' grape variety, thus increasing consumer preference.

Material and Methods

Vineyard Conditions and Plant Materials

This research was conducted in an experimental vineyard of Tekirdağ Viticulture Institute, Turkey (40°58′24.03″N; 27°28′03.49″E; 39 m a.s.l.) in the course of 2019 growing season.

Table 1 Meteorological data of Tekirdağ province for 2019 year

Climatic characteristics	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Mean temperature (°C)	5.6	5.8	9.3	11.6	17.9	24.1	23.9	25.3	21.6	17.5	15.5	9.2
Max temperature (°C)	8.1	9.1	13.1	14.9	21.2	28.2	27.7	29.6	25.8	21.1	18.9	12.5
Min temperature (°C)	3.2	3.1	5.7	8.2	14.4	20.1	19.6	21.2	17.6	14.0	12.4	6.4
Sunshine duration (h)	55.1	113.5	210.9	177.7	191.7	237.1	278.9	279.9	209.8	175.0	123.0	71.1
Precipitation (mm)	63.8	44.8	30.2	42.9	31.2	7.5	18.7	0.0	9.6	46.2	17.4	22.3
Relative humidity (%)	76.3	74.3	70.8	71.9	70.5	64.8	65.0	62.7	65.1	73.3	75.7	75.5

In the study, 13-year-old 'Early Cardinal' grapevines grafted onto 1103P rootstock (*V. berlandieri* × *V. repestris*) were used as plant material. The grapevines were trained to a bilateral guyot system, spacing of 3.0 m between rows and 1.5 m between grapevines within a row in the vineyard. All vineyard operations were carried out as stated by standard viticultural practices for grape cultivar and the region.

The research area is under the influence of the temperate climate zone and some meteorological data of Tekirdağ province, where the research was carried out, were given in Table 1.

Foliar Treatments of Abscisic Acid and Melatonin and Their Treatment Times

To prepare aqueous solutions, two distinct plant growth regulators, abscisic acid (S-ABA) and melatonin (Mt), were used in this study. ProTone SL® is a liquid commercial growth regulator with an active ingredient concentration of 100 g/L S-ABA (Valent BioSciences, Libertyville, IL, United States). The other growth regulator was powder melatonin (Sigma-Aldrich, >98%).

Before starting research, uniform grapevines were chosen for the foliar abscisic acid and melatonin treatments and it was benefited from various concentrations of foliar abscisic and melatonin treatments with various concentrations, including control, S-ABA 300 mgL^{-1}, S-ABA 400 mgL^{-1}, S-ABA 500 mgL^{-1}, Mt 300 µM, Mt 400 µM and Mt 500 µM. In the research, foliar abscisic acid and melatonin treatments were implemented three times with 10-day intervals, comprising 10-day before vérasion period (first treatment time), at vérasion period (second treatment time) and 10-day after vérasion period (third treatment time) by using a back pump. Before foliar treatments, abscisic acid and melatonin were initially dispersed in water with 0.01% (v/v) of Tween 20. Thereafter, grapevines were treated by foliar abscisic acid and melatonin with various concentrations till run-off.

Yield and Quality Characteristics

In the current study, following measurements were taken: berry length (mm), berry width (mm), berry weight (g), berry firmness (N), cluster length (cm), cluster width (cm) and cluster weight (g) as yield characteristics. Moreover, total soluble solids content (%), titratable acidity (g/L), must pH, *p*-value of must (µW), chromatic characteristics such as color values of a*, b*, L*, hue angle (h°) and total phenolic compounds content (mg GAE/kg fw) were determined as quality characteristics.

Berry Sampling and Storing Until Analysis

When the berries on control grapevines of 'Early Cardinal' variety attained nearly 16%, the grapes were harvested in the course of 2019 growing season. After grapes had been harvested, standard measurements and biochemical analyses were promptly performed for grapes. After grapes were picked at harvest time, samples of 250-grapes were collected from each foliar treatments and were finally used to determine total soluble solids content, titratable acidity, must pH, *p*-value of must. Later, 350-berry samples were also used for each foliar treatment to determine chromatic characteristics and total phenolic compounds content. All berry samples were stored at –25 °C until the analysis of total phenolic compounds. Prior to this analysis, berry samples were moved away from –25 °C, permitted to thaw overnight at 4 °C and were followingly homogenized in a commercial laboratory blender for 20 s.

Determination of Berry Firmness

Berry firmness was gauged with an analog penetrometer (FT 02, Wagner Instruments, Riverside, USA) and measurement values were read as g/mm. Thereafter, berry firmness values were converted from g/mm to N by using conversion factor and were expressed as Newton.

$$Berry\,firmness(Newton, N) = firmness\,value(gram - force, g - f) \times 0.009807$$

Calculation of *P*-value

P-value, containing redox potential (mV), pH, *p*-value (µW) and resistivity (′Ω) were formulated with an equation reported by Hoffmann (1991). In the study, *p*-values of berry must sample from various concentrations of foliar treatments were calculated and were explained as µW.

Analysis of Total Phenolic Compounds by Spectrophotometer

In the present study, spectrophotometric method clarified by Singleton et al. (1978) was used for determining total phenolic compounds content and analysis results were expressed as milligrams of gallic acid equivalent per kilogram of fresh weight (mg GAE/kg fw).

Measurements of Chromatic Characteristics

Skin colors of berries were analyzed by using CIELAB color system in which L*, a*, b* values, detected by reflectance spectrophotometry, defines a three-dimensional color space, where L* is the vertical axis and describes

Table 2 Effects of various concentration of foliar abscisic acid and melatonin treatments on yield characteristics

Treatments	Berry length (mm)	Berry width (mm)	Berry weight (g)	Berry firmness (N)	Cluster length (cm)	Cluster width (cm)	Cluster weight (g)
Control	21.42[c]	22.00[c]	6.41[c]	6.81	20.92	11.67	299.05
S-ABA 300 mgL^{-1}	22.74[ab]	23.56[a]	8.90[a]	7.81	23.97	13.25	365.72
S-ABA 400 mgL^{-1}	22.91[a]	23.68[a]	8.99[a]	7.92	24.07	13.54	371.00
S-ABA 500 mgL^{-1}	23.01[a]	23.77[a]	9.08[a]	8.07	24.52	13.72	387.93
Mt 300 µM	21.89[bc]	22.49[bc]	7.49[b]	7.51	22.86	12.76	312.33
Mt 400 µM	22.23[abc]	23.28[ab]	8.35[a]	7.56	23.02	12.95	334.63
Mt 500 µM	22.40[ab]	23.41[ab]	8.74[a]	7.78	23.47	13.06	347.17
$LSD_{5\%}$	0.96	1.00	0.79	N.S.	N.S.	N.S.	N.S.

Means in a column with the same letters are not significantly different at 5% level according to Least Significant Difference (LSD) multiple range test, *S-ABA 300 mgL^{-1}*: S-abscisic acid 300 mgL^{-1}, *S-ABA 400 mgL^{-1}*: S-abscisic acid 400 mgL^{-1}, *S-ABA 500 mgL^{-1}*: S-abscisic acid 500 mgL^{-1}, *Mt 300 µM*: Melatonin 300 µM, *Mt 400 µM*: Melatonin 400 µM, *Mt 500 µM*: Melatonin 500 µM, *N.S.* Non-significant

the lightness, from utterly opaque (0) to utterly transparent (100); a* and b* are the horizontal axes and define respectively redness (or-a* of greenness) and the yellowness (or-b* of blueness). Later, a* and b* values were transformed into hue angle value (h°, color shade) (Bakker et al. 1986; McGuire 1992). Hunter-Lab Colorimeter was used for assessing chromatic characteristics (Hunter Lab DP-9000 color, Virginia, USA).

Statistical Analysis

The research was performed by a completely randomized blocks design with four replicates. All data were subjected to analysis of variance (ANOVA) with the aid of TARIST statistical software program. Differences among the means were compared by Fisher's Least Significant Difference (LSD) multiple range test at 5% level.

Results and Discussions

Yield Characteristics

Table 2 represents that some of yield characteristics of 'Early Cardinal' grape variety, including berry length, berry width and berry weight are significantly influenced by foliar abscisic acid and melatonin treatments ($p < 0.05$).

As stated by Kasimatis et al. (1977) and Gougoulias and Masheva (2010), ecological factors, grape variety and cultural practices affect yield and quality characteristics in grape varieties. Berry size is one of the leading factors, determining table grape quality (Strydom 2014). In the present study, berry length is statistically influenced by foliar abscisic acid and melatonin treatments ($p < 0.05$) and S-ABA treated berries had higher berry length than melatonin treated berries and control berries. As indicated in Table 2, the highest berry length means were respectively 23.01 mm (S-ABA 500 mgL^{-1}) and 22.91 mm (S-ABA 400 mgL^{-1}) when compared to control treatment (21.42 mm).

Concerning berry width, it is noticed in Table 2 that foliar abscisic acid and melatonin treatments significantly affect berry width ($p < 0.05$). The highest berry width means were successively recorded for treatments of S-ABA 500 mgL^{-1} (23.77 mm), S-ABA 400 mgL^{-1} (23.68 mm) and S-ABA 300 mgL^{-1} (23.56 mm), the lowest berry width was 22.00 mm for control treatment.

As far as berry weight are concerned in Table 2, there are significant differences among the foliar abscisic acid and melatonin treatments ($p < 0.05$) and the highest berry weight means were respectively obtained from treatments of S-ABA 500 mgL^{-1} (9.08 g), S-ABA 400 mgL^{-1} (8.99 g), S-ABA 300 mgL^{-1} (8.90 g), Mt 500 µM (8.74 g) and Mt 400 µM (8.35 g) in comparison with control treatment (6.41 g).

Berry texture is a determinant quality characteristic for table grape varieties and berry firmness of table grapes is one of the substantial factors, determining their eating quality (Carreño et al. 2015). Based on berry firmness depicted in Table 2, foliar abscisic acid and melatonin treatments have no significant effects on berry firmness ($p < 0.05$) and berry firmness means varied from 6.81 N (control) to 8.07 N (S-ABA 500 mgL^{-1}).

Table 2 reveals that cluster length is significantly unaffected by foliar abscisic acid and melatonin treatments ($p < 0.05$). In the study, S-ABA 500 mgL^{-1} treatment resulted in the highest cluster length (24.52 cm) whereas the lowest cluster length was recorded in control treatment (20.92 cm).

As can be observed in Table 2, there are no significant differences among the foliar abscisic acid and melatonin

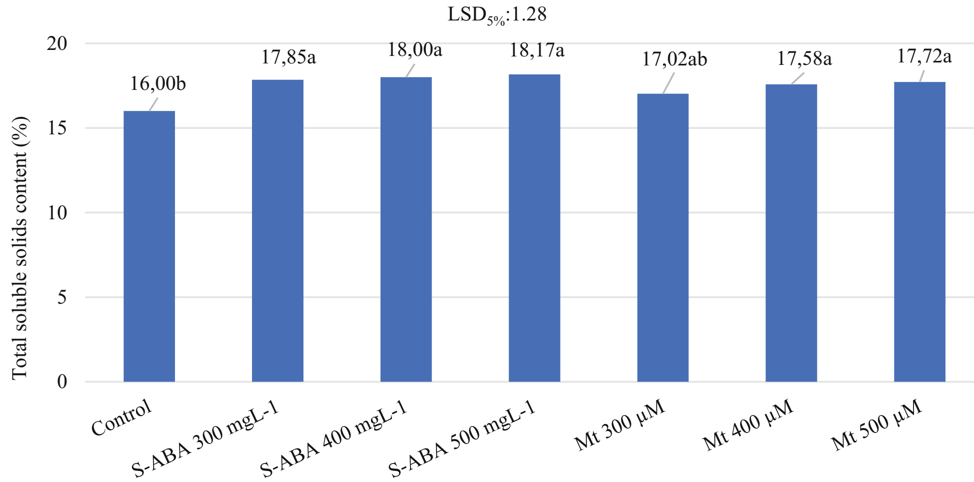

Fig. 1 Effects of various concentration of foliar abscisic acid and melatonin treatments on total soluble solids content

treatments in terms of cluster width ($p<0.05$) and increasing means of cluster width were successively obtained from treatments of S-ABA 500 mgL^{-1} (13.72 cm), S-ABA 400 mgL^{-1} (13.54 cm), S-ABA 300 mgL^{-1} (13.25 cm), Mt 500 µM (13.06 cm), Mt 400 µM (12.95 cm), Mt 300 µM (12.76 cm) and control (11.67 cm).

With respect to cluster weight in Table 2, it is viewed that foliar abscisic acid and melatonin treatments have no significant effects on cluster weight ($p<0.05$). The highest cluster weight mean was 387.93 g for S-ABA 500 mgL^{-1} treatment, the lowest cluster weight mean was recorded for control treatment (299.05 g).

Quality Characteristics

Figs. 1, 5, 6 and 7 and 8 reveal that foliar abscisic acid and melatonin treatments have substantial roles in total soluble solids content, values of a*, b*, L* hue angle (h°) and total phenolic compounds content.

The taste of table grapes is constantly influenced through sugar and organic acid constituents, measuring via total soluble solids and titratable acidity (Ferguson and Boyd 2002; Shiraishi et al. 2010). With respect to total soluble solids content displayed in Fig. 1, there are significant effects among the foliar abscisic acid and melatonin treatments ($p<0.05$) and means of total soluble solids content changed from 16.00% (control) to 18.17% (S-ABA 500 mgL^{-1}).

In view of titratable acidity elucidated in Fig. 2, foliar abscisic acid and melatonin treatments do not cause significant changes in titratable acidity ($p<0.05$). In the study, titratable acidity means were lower in abscisic acid treated berries than melatonin treated berries and the lowest titratable acidity means was respectively recorded in treatments of S-ABA 500 mgL^{-1} (4.92 g/L), S-ABA 400 mgL^{-1} (5.25 g/L), S-ABA 300 mgL^{-1} (5.48 g/L), Mt 500 µM (5.72 g/L), Mt 400 µM (5.81 g/L), Mt 300 µM (6.00 g/L) and control (7.17 g/L).

The pH values of grape must commonly may vary from 3.0 to 3.5 (Keller 2015). It is observed in Fig. 3 that must pH is significantly uninfluenced by foliar abscisic acid and melatonin treatments ($p<0.05$) and pH values of must were respectively higher in abscisic acid and melatonin treated

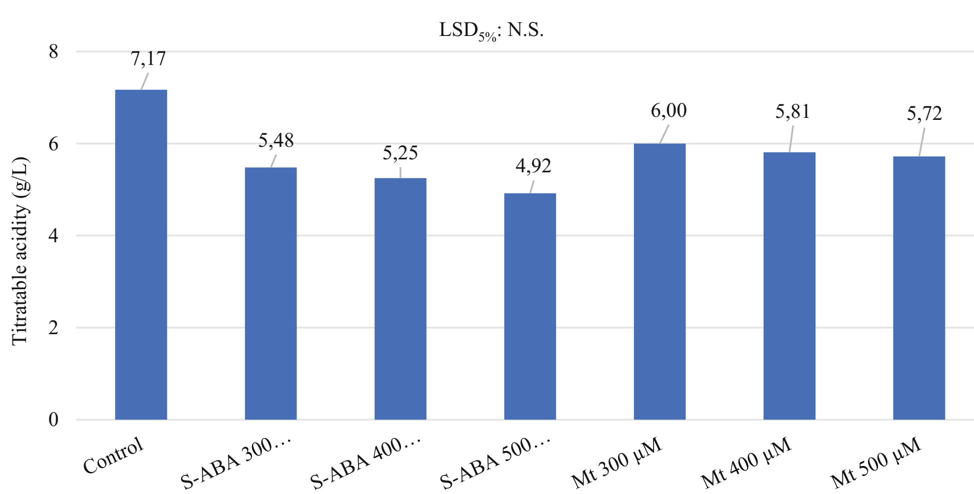

Fig. 2 Effects of various concentration of foliar abscisic acid and melatonin treatments on titratable acidity

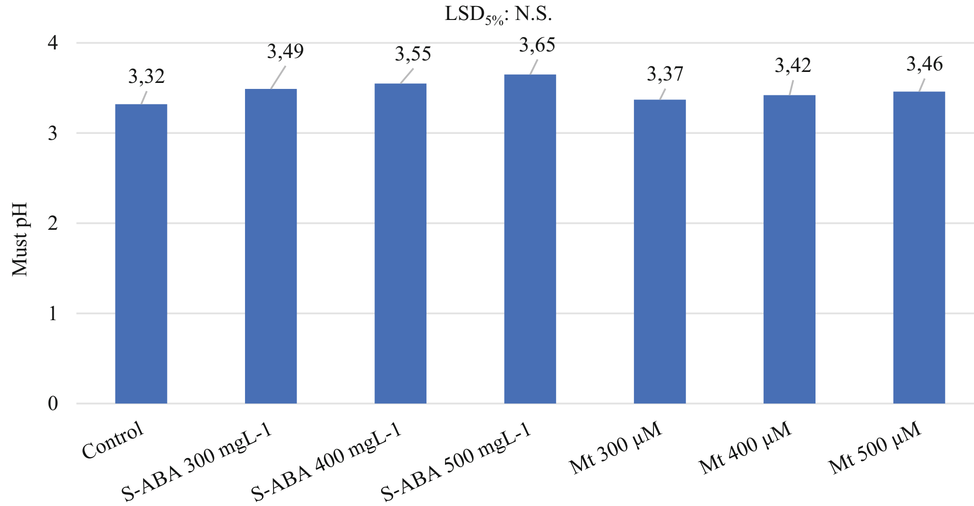

Fig. 3 Effects of various concentration of foliar abscisic acid and melatonin treatments on must pH

berries when the compared to control treatments. In the study, the highest means of must pH were consecutively obtained from treatments of S-ABA 500 mgL^{-1} (3.65), S-ABA 400 mgL^{-1} (3.55), S-ABA 300 mgL^{-1} (3.49), Mt 500 µM (3.46), Mt 400 µM (3.42), Mt 300 µM (3.37) and control (3.32).

Calculation of *p*-value has recently been acknowledged as a remarkable method for appraising quality of food products (Keppel 2001; Velimirov 2004) and lower *p*-values indicate better product quality (Hoffmann 1991). In present study, it is sighted in Fig. 4 that foliar abscisic acid and melatonin treatments have no significant effects on *p*-value ($p < 0.05$) and *p*-values of must from foliar abscisic acid and melatonin treatments varied from 93.54 µM (S-ABA 500 mgL^{-1}) to 125.00 µM (control).

Anthocyanin composition of grape skin may be altered in colored grapes (Liang et al. 2008) and in order to assess color characteristics in grape varieties, it can be utilized from chromatic parameters, including a*, b* L* color values and hue angle value (h°), which are the indicators of grape quality (Bakker et al. 1986; McGuire 1992). The dark red skin color is an important quality characteristic, making the grapes attractive to consumer. As far as a* color value is concerned, higher positive a* color values indicate a reddish color in accordance with CIELAB color system (Al-Hooti et al. 1997; Ayala-Silva et al. 2005). As indicated in Fig. 5, a* color values of foliar abscisic acid and melatonin treated berries are significantly affected by foliar abscisic acid and melatonin treatments ($p < 0.05$) and a* color values changed from 3.03 (control) to 7.22 (S-ABA 500 mgL^{-1}).

In CIELAB color system, lower positive b* values represent blueness (Al-Hooti et al. 1997; Ayala-Silva et al. 2005). Concerning b* color values displayed in Fig. 6, foliar abscisic acid and melatonin treatments significantly affect b* color values ($p < 0.05$) and b* color values changed from 1.86 (S-ABA 500 mgL^{-1}) to 2.76 (control).

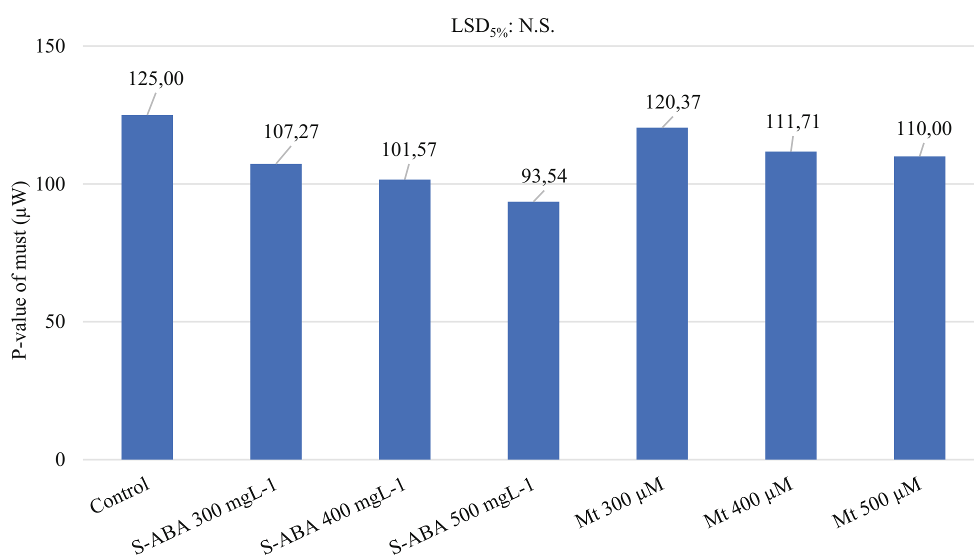

Fig. 4 Effects of various concentration of foliar abscisic acid and melatonin treatments on *p*-value of must

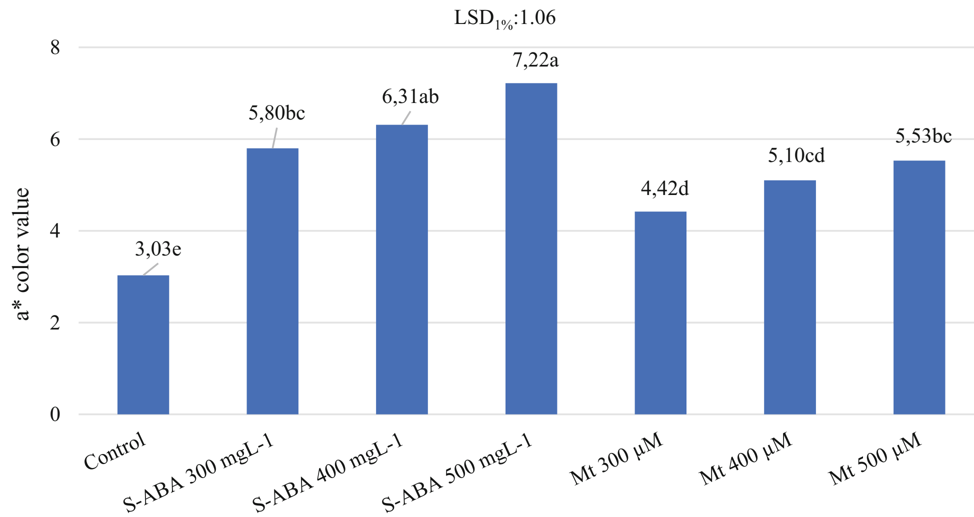

Fig. 5 Effects of various concentration of foliar abscisic acid and melatonin treatments on a* color value

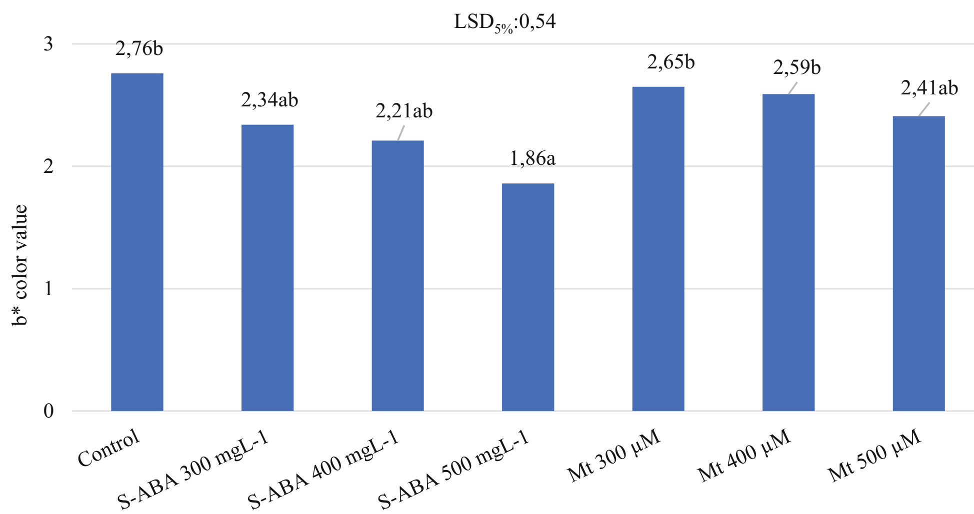

Fig. 6 Effects of various concentration of foliar abscisic acid and melatonin treatments on b* color value

Based on CIELAB color system, lower L* color values point out darkness in assessing color development of fruits (Al-Hooti et al. 1997; Ayala-Silva et al. 2005). As shown in Fig. 7, L* color values are significantly influenced by abscisic acid and melatonin treatments ($p<0.05$). In the present study, S-ABA 500 mgL^{-1} treated berries had the lowest L* color value (30.49), exhibiting darker berry color whereas control treatment had the highest L* color value (32.08), indicating brighter berry color.

Considering hue angle value (h°), lower hue angle values indicate dark shade color according to CIELAB color system (Al-Hooti et al. 1997; Ayala-Silva et al. 2005). As observed in Fig. 8, foliar abscisic acid and melatonin treatments have significant effects on hue angle values ($p<0.05$). In the present study, hue angle values decreased with rising concentrations of abscisic acid and melatonin treatments and S-ABA 500 mgL^{-1} treated berries had the lowest hue angle value (24.94), indicating the darkest shade berry color compared with treatments of S-ABA 400 mgL^{-1} (27.39), S-ABA 300 mgL^{-1} (27.70), Mt 500 µM (28.10), Mt 400 µM (28.64), Mt 300 µM (29.03) and control (31.25).

Grape is rich in phenolic compounds, contributing to characteristics of color, flavor, aroma, texture, astringency as well as antioxidant in table and wine grapes (Bucchetti et al. 2011; Teixeira et al. 2013; Luzio et al. 2021). As can be seen in Fig. 9, foliar abscisic acid and melatonin treatments have significant roles in total phenolic compounds content ($p<0.05$) and the highest total phenolic compounds contents were recorded for treatments of S-ABA 500 mgL^{-1} (2862.95 mg GAE/kg fw) and S-ABA 400 mgL^{-1} (2797.27 mg GAE/kg fw) when compared to control treatment (1897.09 mg GAE/kg fw).

Conclusion

While growing early ripening table grapes, grape growers may encounter the difficulties in lack of ideal and uniform

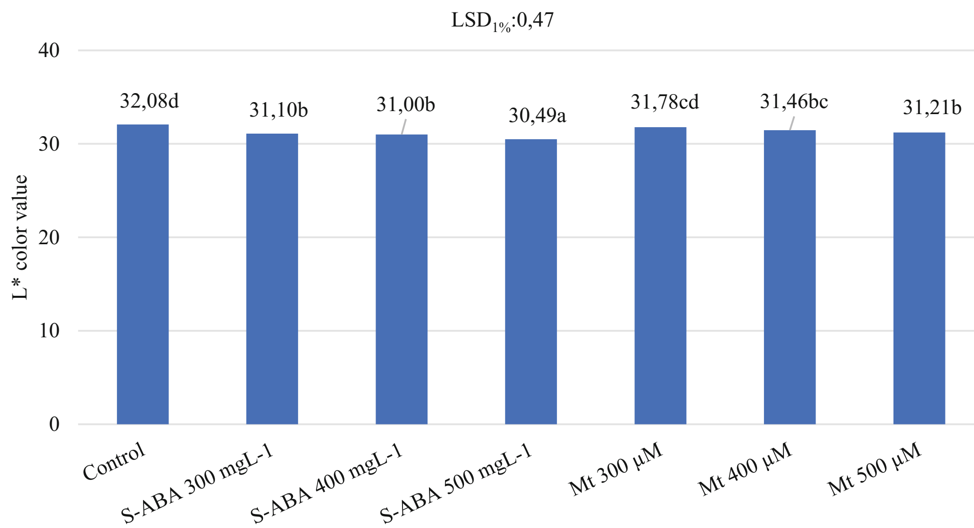

Fig. 7 Effects of various concentration of foliar abscisic acid and melatonin treatments on L* color value

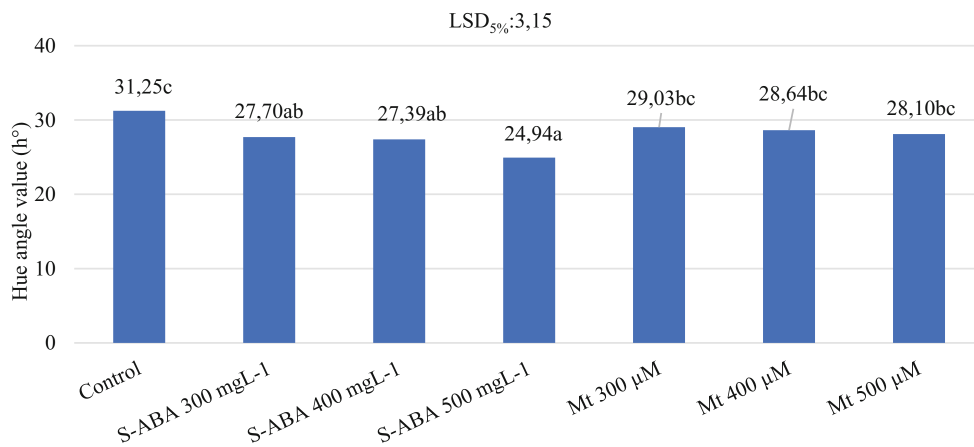

Fig. 8 Effects of various concentration of foliar abscisic acid and melatonin treatments on hue angle value

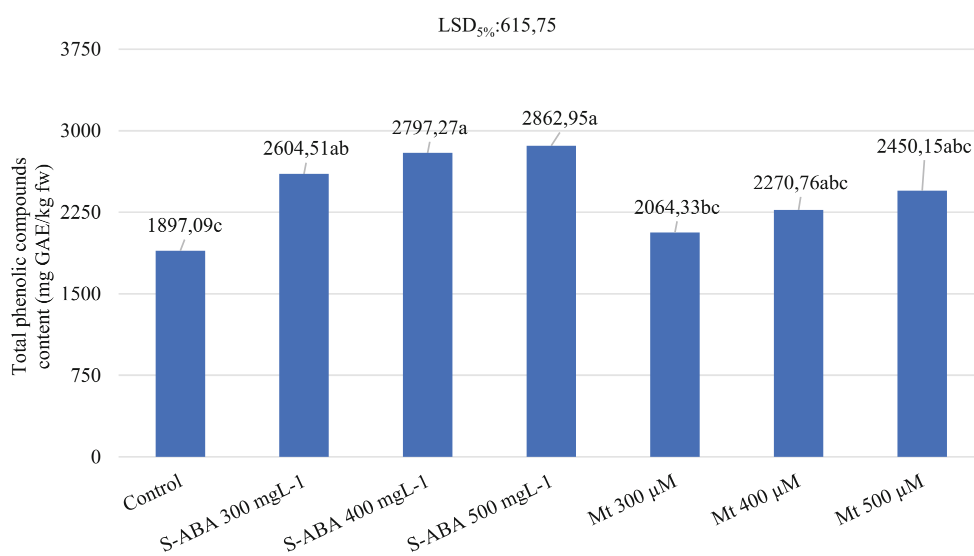

Fig. 9 Effects of various concentration of foliar abscisic acid and melatonin treatments on total phenolic compounds content

skin coloration of grapes. In the present study, different concentrations of foliar abscisic acid and melatonin treatments were utilized for enhancing poor skin color characteristics in 'Early Cardinal' grape variety. The findings revealed that abscisic acid treatments were found to be more effective than melatonin treatments and concentrations of abscisic acid and melatonin treatments had remarkable effects on the yield and quality characteristics of related grape variety. As a result, the best quality characteristics of 'Early Cardinal' grape variety, including color characteristics and total phenolic compounds content were especially obtained from treatments of S-ABA 500 mgL^{-1} and S-ABA 400 mgL^{-1}.

Conflict of interest D. Kok declares that he has no competing interests.

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