Timing and abscisic acid concentration enhancing color of seedless grapes

Renata Koyama[1], Wellington Fernando Borges[1], Luciane Gomes Barros[1], Ibrar Hussain[1], Lilian Yukari Yamamoto[1], Ronan Carlos Colombo[1], Reginaldo Teodoro de Souza[2] and Sérgio Ruffo Roberto[1].

[1]Londrina State University, Department of Agronomy, Celso Garcia Cid Road, km 380, P.O. Box 10.011. Londrina, PR Brazil. Email: emykoyama@hotmail.com
[2]Empresa Brasileira de Pesquisa Agropecuaria. CNPUV. Rua Livramento 515, P.O. Box 130. 95700-000 Bento Gonçalves, RS, Brazil.

Abstract. The objective of this study was to evaluate the effect of timing and concentrations of *S*-ABA, aiming to intensify the color of 'Clone 21' berries. The experiment was conducted in a vineyard grown in a trellis, located in Marialva PR, Brazil, The trial was conducted on two consecutive seasons 2013 and 2014. A randomized block design was used with four replications and five treatments: control; *S*-ABA 200 mg L^{-1} 7 days after veraison (DAV); *S*-ABA 400 mg L^{-1} at 7 DAV; *S*-ABA 200 mg L^{-1} at 7 DAV + *S*-ABA 200 mg L^{-1} at 15 days after the first application (DAFA); and *S*-ABA 400 mg L^{-1} at 7 DAV + 400 mg L^{-1} at 15 DAV. The variables studied were weight and diameter of berries, weight and length of bunches, and skin anthocyanins concentration. There was no significant difference between treatments regarding the weight and diameter of the berries, as well as the weight and width of bunches. However, exogenous application of *S*-ABA significantly increased the grape skin concentration of anthocyannins, independent of concentration and timing of application, and that treatment with two applications of 400 mg L^{-1} of *S*-ABA (7 DAV + 15 DAFA) resulted in higher average.

1 Introduction

Seedless grapes have certain characteristics that make them a high quality fruit and have better acceptance by consumer. Thus, Embrapa Wine and Grape through its program of Genetic Improvement of Table Grapes, has developed new seedless cultivars adapted to the diverse conditions of growing areas of Brazil, such as the 'Clone 21', which features by low color of the peel, lightly pink.

Grape color has great influence on product market value due to the visual aspect. So, commercial value of grapes is influenced by their appearance, including color. Therefore, poor coloration of red grapes, grown in warm regions is a frequent problem that decreases production efficiency [1].

The color of grapes is related to the anthocyanins, plant pigments responsible for the majority of blue, purple and all tones of red found in flowers, fruits and some leaves, stems and roots of plants [2]. The accumulation of anthocyanins, which starts from veraison (the onset of grape ripening), is responsible for the intensity of the color of berries and appears to be regulated, at least in part, by abscisic acid (ABA) [3]. Various studies have suggested that the exogenous application of this regulator increases the anthocyanin content of the skins of Table grape cultivars, without changing berry maturation [4, 5].

In view of this, the aim of this study was to assess the effect of different concentrations of *S*-ABA applied at different times to clusters of 'Clone 21' grapes for improving accumulation of anthocyanins in berry.

2 Materials and Methods

This study was conducted in a commercial vineyard of 'Clone 21' grapes grafted on IAC 766, from 4-year-old vines, located in Marialva, state of Paraná (PR), Brazil (23º29'52,8''S, 51º47'58''0 W, elevation 570 m), on two consecutive crop seasons, 2013 and 2014. According to the Köppen classification, the climate is type Cfa. The vines were trained on overhead trellis and spaced 2,5 x 2,5 m apart. Pruning was performed to leave two to three buds per spur. Subsequently, 5% hydrogen cyanamide was applied to the buds to induce and standardize sprouting. During the trials, the standard regional cultivation practices with regard to nutrition, weed control, and pest and disease management were used.

The effects of the plant growth regulator *S*-ABA applied at different concentrations and times on the grape clusters were evaluated. *S*-ABA, at an active concentration of 100 $g \cdot L^{-1}$, was supplied by Valent BioSciences Corporation® (Libertyville, IL).

Treatments and experimental design. The following treatments were tested: 1) control (no application); 2) *S*-ABA at 200 $mg \cdot L^{-1}$ at 7 DAV; 3) *S*-ABA at 400 $mg \cdot L^{-1}$ at 7 DAV; 4) *S*-ABA at 200 $mg \cdot L^{-1}$ at 7 DAV + 200 $mg \cdot L^{-1}$ at 15 DAFA (days after the first application); and 5) *S*-ABA at 400 $mg \cdot L^{-1}$ at 7 DAV + 400 $mg \cdot L^{-1}$ at 15 DAFA. The randomized blocks design was used as a statistical model with five treatments and four replicates with five vines per plot. Fifteen representative clusters in each plot were marked before the application of treatments for further evaluation.

For treatment applications, clusters were sprayed in the morning using a knapsack sprayer at a pressure of 568.93 psi (39.22 bar) with JA1 hollow cone nozzle tips at a volume of 800 $L \cdot ha^{-1}$ to provide complete and uniform coverage. In addition, 0.3 $mL \cdot L^{-1}$ of Break-Thru® (Evonik Industries, Germany), a non-ionic surfactant, was added to all treatments.

The physical characteristics of grapes were evaluated by determining the mass (g) and diameter (mm) of berries and the weight (g) and length (cm) of clusters using a scale and a digital caliper.

The clusters of each plot were manually harvested when soluble solids reached around 16 °Brix. For each plot, 30 berries were collected for anthocyanin analysis with two berries taken from the upper, middle, and bottom regions of each marked cluster. The concentrations of total anthocyanins of berries of all treatments were carried out according to Peppi et al. [1].

3 Results and Discussion

There were no significant differences among treatments in terms of berry mass and diameter, nor in terms of cluster mass and length (Table 1). Similar results were obtained by Roberto et al. [6], who assessed the effect of *S*-ABA on 'Benitaka' grapes and observed no difference in terms of berry mass and diameter, and cluster mass and length. It shows that S-ABA has no effect on these characteristics of 'Clone 21' grape, i.e., the exogenous application of this plant growth regulator does not modify the vineyard yield.

In regard to the total anthocyanins, of 'Clone 21' berries (Table 1), significant differences were detected with the highest averages observed for the treatments S-ABA 400 mg L^{-1} applied twice (at 7 days after veraison and 15 days after the first application). In general, *S*-ABA application resulted in increased anthocyanin concentrations in berries compared with the control. The increase in ABA levels at around the time of veraison is consistent with it having a role in the control of berry ripening [7], resulting from increased expression of the key anthocyanin pathway enzyme UDP-glucose:flavonoid 3-Oglucosyltransferase [8]. Thus, the application of exogenous ABA enhances the accumulation of anthocyanins [9].

The application of *S*-ABA also increased the anthocyanin concentration in other cultivars, especially on table grapes such as 'Flame Seedless' (Peppi and Fidelibus, 2008) [5] and 'Hongisul' [11] (Shin and Park, 2012).

4. Conclusion

Based on these results, it was observed that the use of *S*-ABA has an increasing effect on anthocyanins concentration. A double application of *S*-ABA at 400 $mg \cdot L^{-1}$ at 7 DAV + 15 DAFA, provides a significant increase of anthocyanin concentration in berry of 'Clone 21' grape.

References

1. M.C. Peppi, M.W. Fidelibus, and N. Dokoozlian. HortScience, **41,** 1440–1445 (2006).
2. P. Markakis. Stability of anthocyanins in foods. In: Anthocyanins as food colors. New York, NY (1982).
3. S.J. Owen, M.D. Lafond, P. Bowen, C. Bogdanoff, K. Usher, and S.R. Abrams. Amer. J. Enol. Viticult. **60**, 277–284 (2009).
4. M.C. Cantín, M.W. Fidelibus, and C.H. Crisosto. Post. Biol. Tech. **46**, 237–241 (2007).
5. M.C. Peppi, M.A. Walker, and M.W. Fidelibus. Vitis. **47**, 11–14 (2008).
6. S. R. Roberto, A. M. Assis, L. Y. Yamamoto, L. C. V. Miotto, A. J. Sato, R. Koyama and W. Genta. Scientia Horticulturae. **142**, 44-48, (2012).
7. S. Wheeler, B. Loveys, C. Ford, and C. Davies. Aust. J. Grape Wine Res. **15**, 195–204 (2009).
8. F. He, L. Mu, G.L. Yan, N.N. Liang, Q.H. Pan, J. Wang, M.J. Reeves, and C.Q. Duan. Molecules **15**, 9057–9091 (2010).
9. S.T. Jeong, N. Goto-Yamamoto, S. Kobayashi, and M. Esaka. Plant. Sci. **167**, 247–252 (2004).
10. K.H. Shin, And H.S. Park. Kor. J. Hort. Sci. Technol. **3,** 256–260 (2012).

Table 1 Physical characteristics and total anthocyanins of 'Clone 21' berries and clusters subjected to various treatments with *S*-ABA during two consecutive seasons, 2013 and 2014.

Treatment (concentration in mg L^{-1})	**berry mass (g)**	**berry diameter (mm)**	**cluster mass (kg)**	**cluster length (cm)**	**Total anthocyanins (mg/g skin weight)**
Control	16,9 a	4,0 a	0,5 a	19,4 a	0,2 d
S-ABA 200 (7DAV)	17,0 a	4,1 a	0,5 a	19,3 a	0,6 c
S-ABA 400 (7DAV)	16,6 a	4,1 a	0,5 a	18,9 a	0,8 b
S-ABA 200 (7DAV) + 200 (15DAFA)	16,7 a	4,1 a	0,5 a	19,1 a	0,8 b
S-ABA 400 (7DAV) + 400 (15DAFA)	16,7 a	4,4 a	0,5 a	18,8 a	1,2 a

Averages followed by the same letter in the columns did not differ according to the Scott-Knott test ($P<0.05$). Note: 7DAV = 7 days after veraison, 15DAFA = 15 days after the first application.