Scientific Papers. Series B, Horticulture. Vol. LXII, 2018
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

# INCREASE OF ANTHOCYANINS ACCUMULATION BY PRE-HARVEST 24-EPIBRASSINOLID (24-EBL) APPLICATIONS IN 'HOROZ KARASI' GRAPE CULTIVAR

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***Abstract***

*In this study, the effect of pre-harvest 24-eBL (a BR analogue) applications on the accumulation of anthocyanin of 'Horoz Karasi' grape cultivar was examined. 24-eBL was applied to vines with 13 combinations including different application times (veraison, 7 days after berry set + veraison, 7 days after berry set + veraison + 30 days after veraison) and 4 different concentrations of 24-eBL (0.2, 0.4, 0.6 and 0.8 mg/L) and control. As a result of the study, the highest anthocyanin content was obtained from the vines applied with 0.2 mg/L of 24-eBL at 7 days after fruit set and at veraison stages in both years (respectively, 47.98 and 71.16 mg/100 g FW).*

***Key words:*** *anthocyanin, brassinosteroid, Grape 24-eBL, pre-harvest application.*

## INTRODUCTION

Grapes are one of the most consumed fruits, and due to its rich phenolic compounds, such as antiradical and antioxidant properties, interest in grapes and grape products (especially food additives, pharmaceutical industry and natural cosmetic products) is increasing (Bourgaud et al., 2001; Ghafoor et al., 2009).
Anthocyanins in grapes have well-known many beneficial effects for human health including the reduction in the incidence of coronary heart disease, enhancement of visual acuity, maintenance of normal vascular activity, as well as pharmacological properties and strong biological functions such as antiinflammatory and antioxidant activities (Hohnova et al., 2008). Also, anthocyanins in grapes play an important role in the quality of colour and they are successfully used as food colourants and nutraceuticals (Espín et al., 2007). Therefore, the determination of anthocyanins in red grapes has acquired of increasing interest during last decade. Many different applications can be made in cultivation in order to enrich the nutrient and antioxidant contents of grapes. The most important treatments in grape growing for this purpose are the usage of plant growth regulators.
Brassinosteroids (BRs), known as the sixth group of hormones, have highly beneficial effects on plant growth, development and physiological aspects in plants such as seed germination, rhizogenesis, flowering, rooting, senescence, abscission, cell expansion and elongation and they are considered as plant hormones with having pleiotropic effects (Clouse and Sasse, 1998; Vardhini and Rao, 2002; Luan et al., 2013).
Moreover exogenous applications of BRs increase the synthesis of secondary metabolites in plants (Biesaga-Koscielniak et al., 2014; Ghorbani et al., 2017).
Symons et al. (2006) and Luan et al. (2013) showed that the exogenous application of BR to grape berries evidently enhanced skin coloration and anthocyanin accumulation. Although there are some studies showing that BRs influence the expression of anthocyanin biosynthesis genes in grapes (Luan et al., 2013; Xi et al., 2013), little is known about the possible roles of BRs involved in anthocyanin accumulation. In this study we investigated the effect of BRs on anthocyanin accumulation in grapes.
Thus, the objective of this study was to determine the effect of pre-harvest 24-eBL application at different doses and application

times on anthocyanin accumulation in 'Horoz Karasi' grape cultivars.

## MATERIALS AND METHODS

The experimental vineyard was located in Senirkent-Isparta Province in the Mediterranean region of Turkey. Nine-year old *Vitis vinifera* L. cv. 'Horoz Karasi' grapevines grafted 41 B.M.G. were used. The vines were planted at 2 x 3 m spacing trained on a bilateral cordon system. 24-eBL was sprayed during two years at concentrations of 0, 0.2, 0.4, 0.6 and 0.8 mg/L and prepared by dissolving in DMSO. Tween 20 at the rate of 0.1% (v/v), as a surfactant was added to 24-eBL solution. The prepared solutions were sprayed directly onto the vine (1L per vine) at veraison (approximately 10% of the berries of 50% of the clusters become soft and at colour break), at 7 days after fruit set (3-5 mm diameter berry size) + at veraison and 7 days after fruit set and at veraison + 30 days after veraison by a hand pump sprayer until run-off early in the morning. Grapes were harvested at commercial maturity and bunches were packed in plastic crates and transported to the laboratory where they were frozen in liquid nitrogen and stored at -80°C until the analyses were performed.

Total anthocyanin content determination was based on a pH differential method and expressed as malvidin 3-glucoside equivalents (Wrostad, 1976). For this purpose, aliquots of the extracts were adjusted to pH 1.0 and 4.5 with buffers. The absorbance of each solution was measured at wavelength of 520 and 700 nm.

The experiment was conducted in randomized complete block design with 3 (application time; at veraison, at 7 days after fruit set + at veraison and 7 days after fruit set + at veraison + 30 days after veraison) × 5 (24-eBL concentration; 0, 0.2, 0.4, 0.6 and 0.8 mg/L) × 2 (years; first and second) factorial arrangements. Each treatment had three replications of eight vines.

Statistical analyses were performed with LSD multiple range test.

Differences were considered statistically significant at the p≤0.05 levels. The software JMP 8 (SAS Institute, Inc., Cary, NC) was used for carrying out statistical analyses of the data.

## RESULTS AND DISCUSSIONS

Effects of 24-eBL concentrations and its application periods on anthocyanin contents were given in Table 1. 24-eBL applications generally increased the anthocyanin accumulation. In the both years, interaction effects of 24-eBL concentrations and application periods on the anthocyanin contents were statistically significant (p<0.05).

In the first year, the highest anthocyanin content was observed at 7 days after fruit set and at veraison periods with 0.2 mg/L 24-eBL (47.98 mg/100 g FW) and at 7 days after fruit set + at veraison periods with 0.6 mg/L 24-eBL (45.35 mg/100 g FW) and at 7 days after fruit set + at veraison + at 30 days after verasion periods with 0.6 mg/L 24-eBL (45.35 mg/100 g FW).

In the second year, the treated with 0.2 mg/L 24-eBL at 7 days after fruit set and at veraison periods grapes had the highest anthocyanin contents. The lowest anthocyanin contents were observed in control grapes for both years.

Table 1. Anthocyanin contents (mg/100 g FW) in 'Horoz Karasi' grapes as effected by 24-eBL concentrations and application periods

| Application period (AP) | Concentrations (C) (mg/L) | 1st year | 2nd year |
|---|---|---|---|
| Veraison (V) | 0 | 29.36 g | 39.73 e |
| | 0.2 | 33.38 f | 41.24 de |
| | 0.4 | 36.40 d | 57.14 abcd |
| | 0.6 | 35.22 e | 62.34 abc |
| | 0.8 | 22.73 j | 51.46 bcde |
| 7 days after fruit set + veraison (FS+V) | 0 | 29.38 g | 39.73 e |
| | 0.2 | 47.98 a | 71.16 a |
| | 0.4 | 21.19 l | 42.23 de |
| | 0.6 | 45.35 b | 65.35 ab |
| | 0.8 | 29.41 g | 47.33 cde |
| 7 days after fruit set + veraison + 30 days after veraison (FS+V+30V) | 0 | 29.38 g | 39.73 e |
| | 0.2 | 27.50 h | 42.78 de |
| | 0.4 | 22.40 k | 52.02 bcde |
| | 0.6 | 39.75 c | 41.08 de |
| | 0.8 | 23.86 l | 60.83 abc |
| **Main effect (Stages)** | | | |
| V | | 31.42 | 50.38 |
| FS+V | | 34.66 | 53.16 |
| FS+V+V30 | | 28.58 | 47.29 |
| **Main effect (Concentrations)** | | | |
| | 0 | 29.38 | 39.73 |
| | 0.2 | 36.29 | 51.73 |
| | 0.4 | 26.66 | 50.46 |
| | 0.6 | 40.11 | 56.26 |
| | 0.8 | 25.33 | 53.21 |
| ***p values*** | | | |
| AP | | <0.0001 | 0.2991 |
| C | | <0.0001 | 0.0197 |
| AP x C | | <0.0001 | 0.0035 |

Different letters indicate significant differences between groups (p<0.05).

Also, the main effect of 24-eBL application periods and concentrations on anthocyanin contents were shown in Table 1, too. In both years, when the main effects of 24-eBL application periods and concentrations examined, the application at 7 days after fruit set + at veraison periods and 0.6 mg/L 24-eBL concentration had the highest anthocyanin contents, respectively. Anthocyanins are synthesized by the biosynthetic way regulated by enzymes such as PAL, chalcone synthase (CHS), chalcone isomerase (CHI), F3H, DFR, leucoanthocyanidin dioxygenase (LDOX) (UFGT). The rise in these enzymes causes the synthesis and accumulation of anthocyanin. BRs can increase the amount of anthocyanin as a result of the enhancement in the enzymes on this synthetic route, and increase in the expressions of the genes encoding these enzymes. Indeed, it was determined that BRs can stimulate the expressions of the genes involved in the synthesis of anthocyanin, thus increasing the amount of anthocyanin in grapes (Xi et al., 2013). Peng et al. (2011) reported that BRs affect the accumulation of anthocyanin induced by jasmonates, through the regulation of late anthocyanin biosynthetic genes such as BRL, DFR, LDOX and UFGT. Similarly, previous studies showed that pre-harvest BRs applications increase the amount of total anthocyanin compared to control application in grapes, too (Luan et al., 2013; Xi et al., 2013; Champa et al., 2015).

## CONCLUSIONS

As a result of investigations, it was seen that all pre-harvest 24-eBL applications increased the amount of anthocyanin compared to the control application. Accordingly, it is revealed that among the 24-eBL applications, the better results were obtained from 0.2 mg/L 24-eBL applicated at 7 days after fruit set and at veraison periods. So, this application could be recommended for ‘Horoz Karasi’ cultivar.

## ACKNOWLEDGEMENTS

This research work was carried out with the support of TUBITAK (Scientific and Technological Research Council of Turkey) and also was financed from Project (Project number: TOVAG 112O517).

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