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THE INFLUENCE OF PRE-HARVEST BORIC ACID APPLICATIONS ON THE ACCUMULATION OF SOME ANTIOXIDANT COMPONENTS IN ALPHONSE LAVALLÉE GRAPE CULTIVAR

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Abstract

The aim of the research was carried out to determine the effects of boric acid (BA) applications on the accumulation of anthocyanins, β-carotene, ascorbic acid and total phenolics in 'Alphonse Lavallée' grape cultivar. BA was applied to vines with four different concentrations (250, 500, 750 and 1000 mg/L) and at two different periods (at blooming and at blooming+fruit set). At the end of the study, it was determined that the BA applications consist of 250mg/L and 750 mg/L of boric acid concentrations during both blooming and fruit set stages were the most effective applications providing the highest anthocyanins, β-carotene and total phenolics. On the other hand, boric acid applications had no important effects on the ascorbic acid contents in berries.

Key words: *'Alphonse Lavallée', antioxidant components, boric acid, pre-harvest applications.*

INTRODUCTION

Antioxidants are substances that, at relatively low concentrations, acting to scavenge and stabilize of free radicals. The benefits of antioxidants are very important to good health. Antioxidants can protect the human body free radicals that may cause some chronic diseases including cancer, cardiovascular diseases and cataract (Shahidi, 2000; Pham-Huy et al., 2008). Consumers are becoming increasingly prefer aware antioxidant substances rich in fruits and vegetables because of their beneficial effects on health. Grape is one of the most important fruit commodities as widely grown through the world. Also, it is a significant source of nutritional antioxidants, such as polyphenols, anthocyanins as well as biologically active dietary components (Orak, 2007). Therefore, 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). To enhance the production of antioxidant substances, strategies such as treating with elicitors (UV, salicylic acid, methyl jasmonate, ozone) and invoking biotic and abiotic stresses have been used in both plant cell culture and intact plants (Dong et al., 2010; Krzyzanowska et al., 2012). However, optimizing the agricultural practices also provide a rapid and efficient way to increase the antioxidant content. Mineral nutrients are essential for the growth, survival and reproductive success of plants. Among the essential elements for healthy plants, boron (B) is responsible for activation of dehydrogenase enzymes, sugar translocation, nucleic acids, plant hormones and effect on cell wall structure cell elongation, root growth and transfer of sugar and altered production of a wide range of phenol content and metabolism. In addition to it is helpful in growth and productivity, fruit setting and yield. B deficiency has visual symptom on root and leaves growth, flower, cluster and berry development in grapevine. Severely reduced in internodes and shoot length, shoot tip death, low fruit set, and tiny berries are all common symptoms of B deficiency (Fleischer et al., 1998; Ruiz et al., 1998; Ebadive et al., 2001; Brown et al., 2002; Christensen et al., 2006; Singh et al., 2012). Several studies have demonstrated that B application increased the yield in grapes

(Mostafa et al., 2006; Er et al., 2011; Ally et al., 2015; Asci et al., 2017). However, although there is limited information on the effect of B on the antioxidant components of plants and there is no information about grapes. Therefore, the major objective of this study was to determine the effect pre-harvest B application on the accumulation of some antioxidant components (total phenolics, ascorbic acid, β-carotene, anthocyanins) in 'Alphonse Lavallée' grape cultivars.

MATERIALS AND METHODS

The experiment was performed on 9-year old *Vitis vinifera* L. cv. 'Alphonse Lavallée' grafted on 41 B.M.G. planted at 2 x 3 m spacing trained on a bilateral cordon system in a commercial vineyard in the Senirkent-Isparta Province in the Mediterranean region of Turkey (lat. 38º 11' 8" N, long. 30º 40' 55" E and elevation 981 m). Eight vine at similar conditions were used. Boric acid (BA) was sprayed at concentrations of 0, 250, 500, 750 and 1000 mg/L. Tween 20 at the rate of 0.1% (v/v), as a surfactant was added to BA solution. The prepared solutions were sprayed directly onto the vine (1 L per vine) at blooming and at blooming + fruit set stages 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 phenolic content was estimated by the Folin-Ciocalteu colorimetric method, based on the procedure of Singleton and Rossi (1965), using gallic acid as a standard phenolic compound. The reduction of the Folin-Ciocalteu reagent by phenolic compounds under alkali conditions, which resulted in the development of a blue colour, was measured at 765 nm using a spectrophotometer. The total phenolic content was calculated with the use of a calibration curve and results were expressed as mg gallic acid equivalent per g dry weight. L-Ascorbic acid content was determined spectrophotometrically at 520 nm using a standart curve according to the procedure described by Pearson and Churchill (1970). Each determination was carried out in triplicate. β-carotene content of the sample was measured using the method of Association of Official Analytical Chemists (AOAC, 1980). The absorbance of the extracts was measured at 436 nm wavelength by using a spectrophotometer. The concentration of β-carotene was calculated using Bear-Lamberts Law. Total anthocyanin content determination was based on a pH differential method and expressed as malvidin 3-glucoside equivalents (Wrolstad, 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 2 (application time; at blooming, at blooming + at fruit set) × 5 (BA concentration; 0, 250, 500, 750 and 1000 mg/L) factorial arrangements with completely randomized block design. Each treatment had three replications of 8 vines. Statistical analyses were performed with LSD multiple range test. Differences were considered statistically significant at the $p \leq 0.05$ levels. Statistical analyses were performed by using JMP 8 (SAS Institute, Inc., Cary, NC).

RESULTS AND DISCUSSIONS

In concerning with influences of BA spraying on antioxidant properties of Alphonse Lavallée grape, data was shown in Table 1 and Table 2. Data showed that the interaction effects of BA concentrations and application periods had a significant ($p \leq 0.0001$) influence on total phenolic, β-carotene and anthocyanin contents. Grapes tretaed with 250 and 750 mg/L BA at blooming and at fruit set stages produced more total phenolic compound (16.84 mg/g and 16.32 mg/g, respectively) compared to control and the other applications. Similarly, the highest anthocyanin content was observed with 250 and 750 mg/L BA at blooming and at fruit set stage, too (66.89 and 66.55 mg/100 g) (Table 1).

As shown Table 2, β-carotene content was also higher 250 mg/L BA at blooming and at fruit set stage (698.32 μg/kg) followed by 750 mg/L BA at blooming and at fruit set stage (422.75 μg/kg). Data showed that there was no significant difference observed among the treatments for ascorbic acid content. Ascorbic acid content varied from 1.97 to 2.73 mg/100 g.

Table 1. Response of BA spraying at different stage on total phenolic (mg/g DW) and anthocyanins (mg/100 g FW) of 'Alphonse Lavallée'

Application period (AP)	Concentrations (C) (mg/L)	Total phenolics (mg/g DW)	Anthocyanins (mg/100 g FW)
Blooming (B)	0	7.59 f	35.24 de
	250	11.33 e	46.67 b
	500	14.40 b	30.33 e
	750	12.13 cde	42.03 bc
	1000	13.03 c	35.78 cde
Blooming + Fruit set (B+FS)	0	7.59 f	35.24 de
	250	16.84 a	66.89 a
	500	12.73 cd	41.60 bcd
	750	16.32 a	69.55 a
	1000	11.88 de	37.39 cd
Main effect (Stages)			
B		11.70	38.01
B + FS		13.07	50.13
	Main effect (Concentrations)		
	0	7.59	35.24
	250	14.09	56.78
	500	13.56	35.96
	750	14.22	55.79
	1000	12.46	36.59
p values			
AP		<0.0001	<0.0001
C		<0.0001	<0.0001
AP x C		<0.0001	<0.0001

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

Also, the main effect of BA application periods and concentrations on antioxidant properties were shown in Table 1, too. When the main effects of BA application periods and concentrations examined, they were obtained that at blooming + at fruit set periods and 250 mg/L BA concentrations had the highest total phenolics, ascorbic acid, anthocyanins and β-carotene contents (Tables 1 and 2).

Antioxidants, such as total phenolics and anthocyanins, are important indicators in grape and wine, and their contents are considered as the most important nutritional value parameters. B is one of the nutrients responsible for the changes in concentration and metabolism of phenolic compounds in vascular plants. It is well known that B deficiency causes an accumulation of phenolics through the stimulation of the enzyme phenylalanine-ammonium lyase (PAL) (Cakmak et al., 1995). B is rapidly absorbed by flowers (Sarrwy et al., 2012) and the B application effects can be attributed to its effect on fruit set and development or other metabolic processes such as carbohydrate transport, which are enhanced by its application (Marschner, 2012; Davarpanah et al., 2016). Due to the fact sucrose is a positive regulator of the biosynthesis of phenolics, the improvement of B treatment on photosynthesis and sugar accumulation could possibly enhance the biosynthesis of phenolics (Solfanelli et al., 2006). To our knowledge, there is very little studies examining the relationship between phenolics and B. There were no comprehensive and detailed studies about the effect of sprayed B on the whole development process included antioxidant properties of grapes. But some investigators have observed a positive effect of B on total phenolic contents in fruits. For instance, foliar sprays of B increased total phenolic compounds in olive (Saadati et al., 2013) and in pungent pepper (Manas et al., 2014). The authors indicate that the increase was due to an increased expression of genes responsible to phenolic compound biosynthesis.

Table 2. Response of BA spraying at different stage on ascorbic acid (mg/100 g FW) and β-carotene (µg/kg FW) of 'Alphonse Lavallée'

Application period (AP)	Concentrations (C) (mg/L)	Ascorbic acid (mg/100 g FW)	β-carotene (µg/kg FW)
Blooming (B)	**0**	1.97	289.65 bcd
	250	2.60	321.04 bcd
	500	2.62	352.34 bcd
	750	2.69	281.56 cd
	1000	1.79	392.61 bc
Blooming + Fruit set (B+FS)	**0**	1.97	289.65 bcd
	250	2.73	698.32 a
	500	2.19	273.21 cd
	750	2.52	422.75 b
	1000	2.25	245.90 d
Main effect (Stages)			
B		2.33	327.44
B + FS		2.33	385.96
	Main effect (Concentrations)		
	0	1.97	289.65
	250	2.66	509.68
	500	2.41	312.78
	750	2.60	352.15
	1000	2.02	319.25
p values			
AP		0.9965	0.0626
C		0.1000	0.0010
AP x C		0.6670	0.0001

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

Ascorbic acid acts as an antioxidant in plants. According to some researchers its levels are responsive to a variety of environmental or stress factors such as light, temperature, salt and drought, pollution, metals or herbicides (Hancock and Viola, 2005; Nicolle et al., 2004). Lukaszewski and Blevins (1996) reported that adequate level of B is required for the accumulation of ascorbic acid. Although there was no significant difference observed among the B treatments for ascorbic acid content in our investigation, Mondi and Munsi (1993) reported that applied B significantly increased the ascorbic acid concentration in potatoes. According to the study, since B plays an important role in the translocation of carbohydrates from leaves to other portions of the plant, greater concentrations of ascorbic acid may have been translocated to the tuber. Also, Govindan (1950) reported that the ascorbic acid concentration of tomatoes rose with increased B uptake in the plant. Cakmak and Romheld (1997) have shown that the decline in vitamin C in some plant parts such as shoot tips and young leaves of sunflower under B deficient conditions.

It is known that B is important for the structural and functional integrity of plasma membranes. The lack or additional of B may also influence efflux of other macro and micro elements (Camacho-Cristobal et al., 2008; Hajiboland and Farhanghi, 2010). Singh et al. (2012) found that the accumulation of P, K, Mg, S, Na, Al and Mn in the carrot storage roots increased when additional B was not supplied to the plants. This alteration of mineral levels could either directly or indirectly affect the accumulation of phytonutrients, such β-carotenes.

CONCLUSIONS

Previous studies were mostly concerned the effect of B on the quality of grapes but, there were no comprehensive and detailed studies about the effect of sprayed B on the whole development process included antioxidant properties of grapes. Obtained results are important to increase the antioxidant properties of 'Alphonse Lavallée' grapes. As a result of investigations, the better results were obtained with 250 mg/L BA applicated at blooming and at fruit set periods and B application can be recommended for 'Alphonse Lavallée' grape cultivar.

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