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Research article

Relationship among color development, anthocyanin and pigment-related gene expression in 'Crimson Seedless' grapes treated with abscisic acid and sucrose

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ABSTRACT

'Crimson Seedless' is one of the most important table grape varieties in Chile, but under certain environmental conditions, the fruit exhibits inadequate red color development, causing economic losses due to lower product quality. The use of plant growth regulators, such as abscisic acid (ABA) and ethylene, during development increases the anthocyanin content of the skin, improving the color of the berry. Recently, sucrose has been identified as a signaling molecule capable of regulating the expression of genes of the anthocyanin biosynthesis pathway. The aim of this study was to analyze the effect of application of ABA and/or sucrose on color development and their relationship with anthocyanin metabolism. Applications of ABA (400 ppm or 200 ppm) and/or sucrose (90 mM) were performed close to the *véraison* stage. During development and at harvest, quality attributes such as berry firmness, total soluble solids and titratable acidity were not affected by these treatments. Increased red color development was observed in fruits treated with ABA and/or sucrose, due to accumulation of anthocyanins. Fruits subjected to sucrose treatment showed higher levels of anthocyanins than untreated fruits but lower levels than fruits treated with ABA. Increased expression of genes involved in anthocyanin biosynthesis was observed in ABA- and sucrose-treated fruits compared to untreated fruits. Based on these findings, we demonstrated that sucrose improved fruit color development by increasing synthesis and accumulation of anthocyanins, thus allowing earlier harvests and improving table grape quality.

1. Introduction

Chile is the major worldwide exporter of table grapes, with approximately 860 thousand tons (equivalent to 1.306 million dollars) destined for markets in North America (mainly the USA), Europe and Asia (Bravo, 2014). The main varieties produced in Chile are 'Red Globe' (RG), 'Thompson Seedless' (TS) and 'Crimson Seedless' (CS), the latter one being the third most important table grape cultivar, with 21% of total production (Bravo, 2014). 'Crimson Seedless' is a late-season, bright red and seedless table grape cultivar that was initially bred in California (Mohsen, 2011; Ramming et al., 1995). Its berries are firm, crisp and have good flavor; skin color can vary from cherry red to black (Cameron, 2001; Dokoozlian et al., 1995). In Chile, as well as in other countries such as Australia, the USA and Italy, obtaining an adequate red color for CS fruit at harvest is a major problem (Singh Brar et al., 2008; Cameron, 2001; Ferrara et al., 2013). At least 30% of the fruit produced by this variety may remain on the vine unharvested due to inadequate red color development, with negative consequences for the grower (Dokoozlian et al., 1995). Skin color is a key quality attribute required by the consumer. Fruit coloration is due to the

Abbreviations: ABA, abscisic acid; RG, Red Globe; TS, Thompson Seedless; CS, Crimson Seedless; PAL, phenylalanine ammonia-lyase; CHS, chalcone synthase; CHI, chalcone isomerase; F3H, flavonoid 3-hydroxylase; DFR, dihydroflavonol 4-reductase; LDOX, leucoanthocyanidin dioxygenase; UFGT, UDP glucose:flavonoid-3-O-glucosyltransferase; DAV, days after *véraison*; TSS, total soluble solids; TA, titratable acidity; CIRG, color index of red grapes; FW, fresh weight; HPLC, high performance liquid chromatography; qRT-PCR, Quantitative real-time PCR; PCA, principal component analyses; Pn, peonidin; Mv, malvidin; Cy, cyanidin; Dp, delphinidin; Pt, petunidin; CC, color coverage of cluster.

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presence of various pigments such as chlorophylls, carotenoids and anthocyanins (Singh Brar et al., 2008; Wei et al., 2011). The red color of the berry skin is a consequence of anthocyanin biosynthesis and accumulation in the cells (Yamane et al., 2006; Boss et al., 1996). The amounts of anthocyanins present in grape berry skins depend on the cultivar, seasonal conditions, phenological stage, and cultural practices (Fernández-López et al., 1998; Singh Brar et al., 2008; Liang et al., 2008). Poor color development in CS berry is likely a consequence of both summertime high temperatures, which inhibit the accumulation of anthocyanins, and a narrow day/night temperature range (Ferrara et al., 2013; Yamane et al., 2006; Dokoozlian et al., 1994; Spayd et al., 2002; Peppi et al., 2006). In red grapes, anthocyanin accumulation begins at the phenological stage of *véraison* and is regulated by a complex mechanism influenced by the plant hormone abscisic acid (Ferrara et al., 2015; Cantín et al., 2007).

Anthocyanins are synthesized by the phenylpropanoid pathway; several structural genes and encoding enzymes of this pathway have been well described (Villegas et al., 2016; Boss et al., 1996; Xie et al., 2011). The synthesis of anthocyanins begins with the conversion of phenylalanine to *4*-coumaroyl-CoA by the action of phenylalanine ammonia-lyase (PAL) (Xie et al., 2011). Chalcone synthase (CHS) forms naringenin-chalcone from 4-coumaroyl-CoA and malonyl-CoA (Xie et al., 2011). The naringenin chalcones formed are rapidly and stereospecifically isomerized to naringenin by chalcone isomerase (CHI), which is hydroxylated by flavonoid 3′-hydroxylase (F3H), transforming it into dihydrokaempferol (Xie et al., 2011; Del Valle et al., 2005; Boss et al., 1996). From this point, the synthesis of anthocyanins depends on oxidation and dehydration reactions catalyzed by dihydroflavonol 4-reductase (DFR) and leucoanthocyanidin dioxygenase (LDOX). Finally, anthocyanidins are glycosylated by glycosyl moieties from UDP-activated sugar donor molecules by the action of UDP glucose:-flavonoid-3-O-glucosyltransferase (UFGT); this gene is regulated by the MYBA1 transcription factor (Del Valle et al., 2005; Xie et al., 2011).

Gene expression and activation of enzymes for anthocyanin biosynthesis are influenced by three main factors: (i) climatic conditions, (ii) cultural practices and (iii) phenological stages. i) Anthocyanin accumulation is suppressed by high temperature and low light intensity (Spayd et al., 2002). For instance, temperatures of 30 °C inhibit anthocyanin accumulation in berries (Spayd et al., 2002; Yamane et al., 2006; Cantín et al., 2007). Grapes grown in warm regions develop less red color than those from cooler regions (Yamane et al., 2006; Peppi et al., 2006). ii) Careful canopy and crop management, as well as application of ethephon, optimize the color of CS grapes (Dokoozlian et al., 1994). To obtain homogeneous color throughout clusters, plant growth regulators (PGR), such as abscisic acid (ABA) and 2-chloroethylphosphonic acid (ethephon), are permitted under national regulations in some countries, such as the USA, Australia, Chile and Italy (Ferrara et al., 2015). Ethephon is usually applied to red table grapes to improve berry color, but its effects on color are inconsistent and can cause berry softening (Jensen et al., 1975, 1982; Szyjewicz et al., 1984; Peppi et al., 2006, 2007). Other studies showed that exogenous ABA treatment could increase skin anthocyanin concentrations in grapes, but the high cost of ABA has precluded the development of practical applications for viticulture (Peppi et al., 2006). iii) In regards to phenological stages, sugar is one of the main factors that contributes to color change. Sucrose is known to be an activator of gene expression for enzymes involved in anthocyanin biosynthesis, and sugar deficiency can delay pigmentation of fruits (Dai et al., 2014; Ferrara et al., 2015). Sugar begins to accumulate at a higher rate at the beginning of *véraison*, and some studies have reported that increases in the concentrations of both sugar and ABA were correlated with grape ripening (Zhang et al., 2009a). Sugars have been studied not only as an energy source but also as signaling molecules able to control gene expression (Lecourieux et al., 2013). The accumulation of anthocyanins in grapes has been shown to be stimulated by exogenous application of sugars to grape cell suspensions and tissue cultures, probably because of osmotic stress (Gambetta et al., 2010; Ferri et al., 2011).

The objective of this study was to analyze the effect of the application of abscisic acid and/or sucrose on color development and their relationship with anthocyanin and chlorophyll metabolism.

2. Material and methods

2.1. Plant material

Ten-year-old plants of table grapes cv. 'Crimson Seedless' were obtained from a commercial vineyard located in Los Andes (Aconcagua Valley, 32°52′27″ S and 70°38′26″ W), Chile. Plants were spaced 4 × 3 m grown on their own roots using an overhead trellis system and watered with drip irrigation. Vineyard operations such as plant fertilization, pest control, and other crop management were carried out according to local practices, without nitrogen additions during the season. A randomized block design with four blocks and six treatments was used. The treatments were Control; ABA (ProTone®, Valent BioSciences) at a concentration of 200 ppm (ABA200); ABA at a concentration of 400 ppm (ABA400); sucrose (Merck, Germany) 90 mM; ABA 200 ppm + sucrose 90 mM (ABA200 + sucrose); and ABA 400 ppm + sucrose 90 mM (ABA400 + sucrose). Four replicates per treatment were used, one replicate corresponded to one vine with 60 bunches. All treatments were applied directly to the 60 bunches with a hand-held sprayer at 13 days after *véraison* (DAV). *Véraison* was established when 50% of the berries in the cluster were soft, which actually corresponded to 51.11 ± 0.77% of berry softening. Grapes were sampled weekly, from the start of *véraison* (January 15, 2014, 0 DAV) until the time of harvest (March 20, 2014, 63 DAV). All grapes were harvested when 15% of the bunches from the control treatment had reached class 5 according to the visual color scale (Fig. 1). At each sampling time, 50 berries were obtained from 16 homogeneous clusters within each treatment. Immediately after sampling, grape berries were transported under refrigerated conditions to the Postharvest Laboratory at INIA-La Platina, Santiago (Chile) for evaluation of maturity parameters and metabolites and for molecular assays. For the molecular assays, twenty whole berries were frozen in liquid nitrogen and stored at −80 °C until further use.

2.2. Maturity parameters

Total soluble solids content (TSS) was measured with a manual temperature-compensated refractometer (ATC-1E, Atago, Tokyo, Japan) and the results were expressed as percentage (%). Titratable acidity (TA) was obtained by titrating 10 mL of juice from a representative sample of fruit with 0.1 N NaOH until neutralization of organic acids at a pH of 8.2. In this case, the results were expressed as a percentage of tartaric acid equivalents. Additionally, berry firmness was assessed by a Firmtech 2 texture analyzer (Bioworks, KS, USA), and the results were expressed in g mm^{-1}. For TSS and berry firmness, a total of 20 berries per replicate were considered, and for TA a composite sample was used for each replicate.

2.3. Color development and color quality assessments

Color development of sixteen clusters was assessed as percentage of color coverage by using a visual color scale with five