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Londrina
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Thesis Presented for Doctorate, Graduate Program in Agronomy, Londrina State University, Parana, Brazil.

Advisor: Prof. Dr. Sergio Ruffo Roberto.

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Thesis Presented for Doctorate, Graduate Program in Agronomy, Londrina State University, Parana, Brazil.

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Londrina, February 23, 2018.

DEDICATION

To my loving parents, who have helped me throughout my life.

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I am very thankful to the almighty ALLAH for without his grace and blessing, this work would not have been possible.

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RESUMO

A ‘Benitaka’ é uva de mesa importante, mas apresenta deficiência no desenvolvimento da cor vermelha devido à inibição da produção de antocianinas quando cultivada em áreas subtropicais. A aplicação exógena de reguladores de crescimento, como o ácido abscísico (ABA), pode ser um dos métodos utilizados para superar esse problema. O objetivo deste trabalho foi estudar a ação do isômero do ácido (*S*) -cis-abscísico (S-ABA) sobre ao desenvolvimento de cor, concentração de antocianinas e melhoria da qualidade da uva de mesa 'Benitaka', e determinar o tempo mais adequado para aplicação do *S*-ABA para esta cultivar. Os experimentos foram conduzidos em um vinhedo comercial localizado em Marialva, PR, e as videiras foram treinadas em um sistema de treliça aérea, espaçadas em 3,0 × 6,0 m. Os testes foram realizados durante duas safras consecutivas (safra regular de 2015 e fora de época de 2016), e os tratamentos incluíram aplicações de *S*-ABA 400 $mg.L^{-1}$, como segue: Controle (sem aplicação); Em pré-veraison (PRV) (7 dias antes de veraison); no momento do veraison (V); e no pós-veraison (POV) (7 dias após V). Uma segunda aplicação foi realizada para todos os tratamentos 10 dias após a primeira aplicação, exceto para o controle. O delineamento experimental foi em blocos ao acaso, incluindo: acúmulo total e diário de antocianinas, índice de cor de uvas vermelhas (CIRG) e desenvolvimento diário de CIRG, taxas semanais e diárias de acumulação de antocianinas, taxas semanais e diárias do CIRG, desenvolvimento da luminosidade (*L* *), croma (*C* *) e ângulo de matiz (*h* °), massa, comprimento, largura, firmeza, sólidos solúveis totais (SST), acidez titulável (TA), índice de maturação (SST / AT) e polifenóis totais de bagas . Os dados foram submetidos ao teste ANOVA e as médias comparadas pelo teste de Tukey a 5% de significância. A aplicação exógena de *S*-ABA no PRV ao POV pode aumentar significativamente os atributos de cor, bem como o teor total de antocianinas da uva de mesa 'Benitaka', mas as aplicações no PRV e no V fornecem uma maior resposta. Existe uma forte correlação entre o CIRG e a antocianina da casca das bagas, enquanto as características físico-químicas das bagas não são afetadas significativamente pelo uso de *S*-ABA. A firmeza da fruta varia ligeiramente em resposta à aplicação da *S*-ABA, mas não compromete a qualidade da baga para uso comercial. Durante as colheitas regulares e fora de época da uva de mesa 'Benitaka', a aplicação exógena de *S*-ABA em qualquer momento do V, especialmente em PRV ou V, pode melhorar significativamente a taxa semanal e diária de acumulação de antocianina, bem como cor no desenvolvimento das bagas. Outras propriedades químicas das uvas, isto é, a evolução de SST, AT e SST / AT, não são afetadas pelo uso de *S*-ABA e seguem um padrão previsível em relação aos dias de amadurecimento das bagas.

Palavras-chave: *Vitis vinifera* L. Antocianinas. Regulador de crescimento. Cor.

SHAHAB, Muhammad. **Dynamics of abscisic acid in relation to color and phenolic compounds of 'Benitaka' table grape.** 2018. 87 p. Thesis (Doctoral Degree in Agronomy) – Universidade Estadual de Londrina, Londrina, 2018.

ABSTRACT

'Benitaka' is an important table grape, but shows lack of color development due to anthocyanin inhibition when grown under subtropical areas. Exogenous application of growth regulators, like abscisic acid (ABA), is one of the methods used to overcome this problem. The aim of this work was to study the dynamics of (*S*)-*cis*-abscisic acid isomer (*S*-ABA) in relation to color development, anthocyanin concentration and quality improvement of 'Benitaka' table grape, and to determine the exact timing for *S*-ABA application that is best suited for this cultivar. The experiments were conducted in a commercial vineyard located at Marialva, PR, Brazil, and the vines were trained in an overhead trellis system, spaced at 3.0 × 6.0 m. Trials were carried out during two consecutive seasons (regular season of 2015 and off-season 2016), and treatments included applications of *S*-ABA 400 $mg.L^{-1}$, as follow: Control (no application); At pre-veraison (PRV) (7 days before veraison); At veraison (V); and At post-veraison (POV) (7 days after V). A second application was performed for all treatments 10 days after the first application, except for the control treatment. A randomized block design was used for both experiments and analysis included: total and daily anthocyanins accumulation, color index of red grapes (CIRG) and daily CIRG development, weekly and daily rates of anthocyanin accumulations, weekly and daily rates of CIRG development lightness (*L**), chroma (C*) and hue angle (*h*°), mass, length, width, firmness, total soluble solids (TSS), titratable acidy (TA), index of maturation (TSS/TA) and total polyphenols of berries. The data was subjected to ANOVA test and the means were compared using Tukey's test at 5% level of significance. The exogenous application of *S*-ABA from PRV to POV can significantly enhances the color attributes as well as total anthocyanin contents of 'Benitaka' table grape, but applications at PRV and at V provide a higher response. A stronger correlation exists between CIRG and anthocyanin of berry skin, whereas the physicochemical characteristics of berries are not affected significantly by the use of *S*-ABA. The berry firmness slightly varies in response to the *S*-ABA application, but not to the extent where it compromises the berry quality for commercial use. During both regular and off-season crops of 'Benitaka' table grape, the exogenous application of *S*-ABA at any time of V, especially at PRV or at V, can significantly improve the weekly and daily rate of anthocyanin accumulation as well as color development of the berries. Other chemical properties of grapes, i.e., TSS, TA and TSS/AT evolution, are not affected by the use of *S*-ABA, and follow a predictable pattern in relation to days of berries ripening.

Keywords: *Vitis vinifera* L. Anthocyanins. Growth regulator. Color.

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1. INTRODUCTION

Grapes are widely grown and consumed all over the world due to the beneficial effect on human health. Grapes hold an important position among the other fruit species because it is an important source of phenolic compounds that neutralize the free radicals, and thus, improves human health (ORAK, 2007; RASTIJA; SRECNIK; SARIC, 2009; WILDMAN, 2016). It is known that previously the production and export of grapes was exclusively controlled by traditional European countries, nevertheless, South American countries such as Chile and Brazil have shown a significant increase in growth and production of grapes in recent years, and in some cases, with two crops per year (RUIZ, 2011).

Table grapes grown in region of warm climate are usually affected by high temperatures which inhibit anthocyanin accumulation, thereby preventing adequate color development of some red and pink colored grapes. Grapes color holds important economic value from market point of view, and commercial value of grapes is mostly affected by its appearance, which includes color (PEPPI; FIDELIBUS; DOKOOZLIAN, 2006).

'Benitaka' (*Vitis vinifera* L.) is among the most important colored cultivar of table grapes grown in sub-tropical regions of South America. It was originated from a spontaneous somatic mutation from 'Italia' table grape, discovered in 1988 (SOUSA, 1996). This cultivar differentiates from the original 'Italia' grape because of the development of a dark red color, even in immature stages (CAMARGO, 1998), and has most attracted the interest of grape growers over the last few years (KISHINO; ROBERTO, 2007; LEÃO; SOARES.; RODRIGUES, 2009). However, lack of a pink and uniform color of berry skins is frequently observed when this cultivar is grown under warm climate, which may be due to the inhibition of anthocyanin accumulation (ROBERTO et al., 2012).

The color of the grapes is mostly influence by the amount and composition of anthocyanin contents (RIBÉREAU-GAYON, 1982; MAZZA, 1995). Anthocyanins (Greek *anthos* = flower, *kyanos* = blue) which are a type of phenolic compounds, present in the skin of berry and responsible for blue, purple and all tones of red colors in fruits, flowers, leaves and other parts. Anthocyanin accumulation in grape berries starts at veraison (color change or onset of ripening) (PIRIE; MULLINS, 1977; MARKAKIS, 1982; KELLER; ARNINK; HRAZDINA, 1998; LARRONDE et al., 1998; VITRAC et al., 2000; CASTELLARIN et al., 2007a,b; LECOURIEUX et al., 2014). Veraison (French *vérer* = to change) typically occurs during a period of 7-10 days with in grape bunches signaling a shift from photosynthetic to a heterotrophic metabolism (KELLER, 2015). Veraison can be identified from berry softening

and increase in sugar contents which is followed by a sudden increase in the color of the skin to purple, red or blue in dark skinned cultivars and yellowish in some white varieties (HUANG; HUANG, 2001).

During the developmental phase of the berries maturation in grapes, they go through a number of chemical and physiological modifications which are vital for the better fruit quality and which starts long before the ripening process (HRAZDINA; PARSONS; MATTICK, 1984; COOMBE, 1992; KANELLIS; ROUBELAKIS-ANGELAKIS, 1993; SARRY et al., 2004; BRUMMELL, 2006; CONDE et al., 2007; DEYTIEUX et al., 2007). It is strongly believed that in addition to sugar, abscisic acid plays a key role in bringing about these changes (GAMBETTA et al., 2010; KOYAMA; SADAMATSU; GOTO-YAMAMOTO, 2009; JIA et al., 2011). It is a well-known fact that abscisic acid (ABA) naturally accumulates in grape skins at the onset of ripening, a time when anthocyanin and other phenolic compounds also increase (COOMBE; HALE, 1973).

Further studies have shown that exogenous application of ABA to grapes also enhanced anthocyanin accumulation (LEE; TOMANA, 1980; KATAOKA et al., 1982; LEE et al., 1997), even under high temperature regimes that reduced the pigment accumulation in untreated grapes (TOMANA; UTSUNOMIYA; KATAOKA, 1979). Recently, it was possible to demonstrate that exogenous applications of the enantiomer (*S*)-*cis*-abscisic acid (*S*-ABA) at veraison increased the anthocyanin concentration in the skin of several grape cultivars more efficiently (ROBERTO et al., 2012; ROBERTO et al., 2013; KOYAMA et al., 2014; FERRARA et al., 2015; YAMAMOTO et al., 2015).

However, an important issue for investigations on veraison is to determine when this process exactly begins for each grape cultivar (GIRIBALDI; HARTUNG; SCHUBERT, 2010). Although several studies have advised the application of *S*-ABA at the veraison, but to determine precisely the beginning of this period is a difficult task, considering that this phenological stage is characterized by changes in the metabolism of berries and such changes occur differently among grape cultivars (ROBINSON; DAVIES, 2000).

In addition, weather conditions and the size of the area to be treated are factors that make it impossible to apply this plant growth regulator to only at certain stage of maturity in a limited period of time. In view of these facts, it is necessary to evaluate the efficiency of *S*-ABA application in a wider period of time e.g. before, during and after veraison to improve color of ‘Benitaka’ table grapes.

In light of the above facts, this study was designed with the main objective to study the dynamics of *S*-ABA in relation to color development, anthocyanin concentration and

quality improvement of ‘Benitaka’ (*Vitis vinifera* L.) table grapes, and to determine the exact timing for *S*-ABA application that is best suited for this grape cultivar.

The thesis contains two articles:

Article A: Exogenous *S*-ABA application: relationship between anthocyanins and skin color of ‘Benitaka’ table grape.

Article B: Rates of anthocyanins accumulation and color development in ‘Benitaka’ table grape treated with abscisic acid**.**

A general Review of Literature is presented before the articles.

2. REVIEW OF LITERATURE

2.1. THE GRAPEVINE AND THE VITICULTURE

The members of Vitaceae family are collectively termed as grapevines. The family contains approximately 1,000 species assigned to 17 genera that are typically shrubs or woody lianas that climb by means of their leaf-opposed tendrils - hence the name Vitaceae (Latin *viere* = to attach). All cultivated grapes belong to either the genus *Muscadinia* (2n = 40 chromosomes) or the genus *Vitis* (2n = 38 chromosomes) (KELLER, 2015).

The grapevine is a species of *Vitis* genus, native to the Mediterranean region, central Europe, and southwestern Asia, from Morocco and Portugal north to southern Germany and east to northern Iran. The Eurasian species *Vitis vinifera* (L.) gave rise to the overwhelming majority of grape varieties cultivated today (KELLER, 2015).

Viticulture has great economic and social importance, involving a large annual volume of business for the whole marketing chain, and it stands out among the crops as the one that presents the largest coefficient of generation of direct and indirect employments (LEÃO, 2003). Viticulture has high economic and social importance, through job creation and sustainability of small farms in a country (MELLO, 2013). Considered one of the major fruit grown in the world, the annual grape production in 2016 was 77.4 million tons, of which 14.8 million were produced by China, representing 19.1% of the world total production. Other countries, such as Italy, USA, France and Spain accounted for 10.6; 9.2; 8.0 and 7.6% of the total respectively, and together with China, contributed 51.1% of the world total grapes production. Brazil is the 17th largest producer of grapes, with 1.0 million tons harvested in 2016 (FAO, 2018).

In Brazil, viticulture began with the arrival of Portuguese settlers in the sixteenth century and remained a domestic practice until the mid-nineteenth century when Italian immigrants started to grown ‘Isabel’ grape (*Vitis labrusca*) introduced from USA (SOUSA, 1996). In the twentieth century, fine grapes returned for fresh consumption. High scale production of commercial table grapes in the northeastern semi-arid area started tropical viticulture in Brazil. Tropical viticulture gave a start to new production centers of table grapes in the northern regions of Paraná, Northwest of Sao Paulo and northern of Minas Gerais (LEÃO; POSSÍDIO, 2000; PROTAS; CAMARGO; MELLO, 2006). Viticulture has been continuously developing over the past years and has proved that it has a great potential for the development of the country (PROTAS; CAMARGO; MELLO, 2006).

Among the grape producing states in Brazil, Rio Grande do Sul is the top producer yeilding 413,640 tons, followed by Pernambuco, São Paulo, Bahia, and Paraná, with the production of 242,967; 144,110; 62,740 and 52,288 tons respectively. In addition the states of Santa Catarina and Minas Gerais have also produced 33,849 and 11,224 tons of grapes, respectively (IBGE, 2017).

In the State of Paraná there are two important regions of viticulture, the metropolitan region of Curitiba and the Northern area of the state. The northern region includes Londrina, Marialva, Uraí, Maringa, Rolândia and other adjacent areas. These countries dominate the production of vinifera table grapes. Viticulture in northern Parana was developed by japanese colony in the 1970s, with the production of fine table grapes mostly 'Italia' (*Vitis vinifera*). The diversification in the production took place with the growing of mutant clones derived from 'Italia' grape, such as 'Rubi' in the 1980s and later, 'Benitaka' and 'Brasil' (PROTAS; CAMARGO, 2011).

2.2. 'BENITAKA' TABLE GRAPE

'Benitaka' (*Vitis vinifera* L.) is among the most important colored table grapes cultivar grown in subtropical regions of South America (Figure 1). It was originated from a spontaneous somatic mutation from 'Italia' table grape, discovered in 1988 in Parana state, Brazil (SOUZA, 1996). This cultivar differentiates from the original 'Italia' grape because of the development of a dark rose color even in immature stages (CAMARGO, 1998), and has most attracted the interest of grape growers over the last few years (KISHINO; ROBERTO, 2007; LEÃO; SOARES; RODRIGUES, 2009).

'Benitaka' is a highly vigorous cultivar with medium to large leaves and large compact bunches that contains big ellipsoidal berries which can weight approximately 8-12 g each, while the bunches can weight around 400 g. The flesh is crunchy with a muscat flavor, and presents an excellent postharvest condition. The cultivar is highly resistant to powdery mildew and acid rot diseases while moderately resistant to anthracnose, botrytis, downy mildew and bacterial canker (EMBRAPA, 2015). Like 'Italia' grape, 'Benitaka' can be grafted on rootstocks 'IAC 572 Jales', 'IAC 766 Campinas' and 'Kober 5BB' (NACHTIGAL; CAMARGO, 2005). This cultivar can give up to two crops per year (regular and off-season crops) especially in subtropical and tropical areas if properly pruned. The vines are trained using overhead trellis system and under cane-prune technique. However, lack of a pink and uniform color of berry skins is frequently observed when this cultivar is

grown under warm climate, which is due to the inhibition of anthocyanin accumulation (ROBERTO et al., 2012).

Figure 2.2.1. ‘Benitaka’ table grape bunches (*Vitis vinifera* L.).

2.3. HEALTH BENEFITS

Several studies have shown that consumption of fruits and vegetables is associated with lower risk of developing chronic diseases such as cancer, cardiovascular disease, and hypertension, among others. This fact can be attributed to the high content of compounds with bioactive properties, known as phytochemicals, such as phenolic compounds, carotenoids, vitamins and endogenous metabolites in fruit and vegetables (GIUSTI; JING, 2007; HOWARD; HAGER, 2007; WANG, 2007).

Over the past 10 years, researchers and food manufacturers have become increasingly interested in polyphenols. The main reason for this interest is the recognition of the antioxidant properties of polyphenols, their inclusion in human diet, and their probable role in

the prevention of various diseases associated with oxidative stress, such as cancer and cardiovascular and neurodegenerative diseases (MANACH, 2004).

2.4 PHENOLIC COMPOUNDS IN GRAPES

Phenolic compounds are hydroxylated aromatic substances, with large structural diversity, ranging from one molecule to polymers, found naturally in grains, vegetables, fruits, tea, herbs, chocolate, coffee and grapes. About 8,000 kinds of phenolic compounds are known, being generally classified into simple phenols and polyphenols, based on the number of subunits (ARAÚJO, 2011).

Grapes contain various nutrient elements, such as vitamins, minerals, carbohydrates, edible fibers and phytochemicals. Polyphenols are the most important phytochemicals in grape because they possess many biological activities and health-promoting substances (SILVA, 1999; SHRIKHANDE, 2000; WADA et al., 2007). The phenolic compounds mainly include anthocyanins, flavanols, stilbenes (resveratrol) and phenolic acids (DOPICO-GARCIA et al., 2008; NOVAKA et al., 2008; SPACIL et al., 2008).

In nature, phenolic compounds are usually found combined with sugars and organic acids and are classified as flavonoids and non-flavonoids. All flavonoids have a basic structure containing two benzene rings linked to the heterocyclic pyran ring. On the other hand, non-flavonoids include more heterogeneous groups of compounds found in large quantity and in various chemical forms, including simple phenolics, phenolic acids (benzoic acid and hydrocinnamic acid), coumarins, stilbenes, tannins and lignins (ARAUJO, 2011; GUERRA, 2012).

Grapes accumulate a wide range of phenolic compounds, especially polyphenols. The basic composition of phenols produced in different varieties and different environment is almost the same. However, biosynthesis of these compounds is directly influenced by the cultivars and their genetic characteristics, temperature, humidity, sun exposure, soil type and fertilization, among other factors (PINTO et al., 2011; CASTELLARIN et al., 2012).

Total phenolic contents in ‘BRS Morena’ and ‘BRS Clara’ table grapes were recorded as 1,008 and 577 $mg.kg^{-1}$ of fresh fruit, respectively. For ‘BRS Morena’, 86.2% of the contents were distributed in skin while 13.85% in flesh, while for ‘BRS Clara’, the skin contained 76.5% and the flesh had 23.5% of the total. With regard to total antioxidant capacity, ‘BRS Morena’ and ‘BRS Clara’ grapes exhibited high values (39.62±1.11 and 15.93±0.24 $mmol.kg^{-1}$ as Trolox equivalents, respectively), that were mainly located at the skins (92.0% in ‘BRS Morena’ and 86.8% in ‘BRS Clara’) (LAGO-VANZELA et al., 2011).

The composition of grape seeds is basically (w/w) 40% fiber, 16% essential oil, 11% protein, 7% complex phenolic compounds like tannins, sugars, minerals, and other substances. Grape skin is a source of anthocyanidins and anthocyanins, natural pigments with antioxidant properties acting through inhibition of lipoperoxidation and which also have antimutagenic activities (KOTHAWADE; DHAKE; SHINDE, 2013).

2.4.1 Flavonoids

Flavonoids (or bioflavonoids) belong to a class of plant secondary metabolites. Flavonoids were referred to as vitamin P (BENTSATH; RUSZNYAK; SZENT-GYÖRGYI, 1937), probably because of the effect they had on the permeability of vascular capillaries, from the mid-1930s to early 1950s.

Chemically, they have the general structure of a 15-carbon skeleton, which consists of two phenyl rings (A and B) and heterocyclic ring (C). This carbon structure can be abbreviated as C6-C3-C6. According to the IUPAC nomenclature (MOSS; SMITH; TAVERNIER, 2009), the variation in the heterocyclic ring is the basis for classification of flavonoids in various subclasses: flavonols, flavones, flavanonols, isoflavones, flavanones and anthocyanidins. Over 5,000 types are known and are present in glycosylated form with different types of sugars. Flavonoids play various roles in plants, including pigmentation and defense (RIBEIRO; SERAVALLI, 2004; TAIZ; ZEIGER, 2009; ARAÚJO, 2011).

2.4.1.1. The flavanols (flavan-3-ols) and flavonols

The flavanols and flavonols, as anthocyanins, belong to the group of flavonoids. The flavanols gives a particular bitter and astringent taste, and are also involved in the development of oxidative browning and precipitates. The flavanols, also known as flavan-3-ols can be found as monomers, oligomers and polymers (BELITZ; GROSCH; SCHIEBERLE, 2009; TERRIER; PONCET-LEGRAND; CHEYNIER, 2009).

The most common flavanols in grapes are (+) - catechin, (-) - epicatechins, (-) - epicatechin gallate, and, less commonly, (-) - epigallocatechin. Catechins and epicatequinas are particularly abundant in seeds and grape skins (ABE et al., 2007; BELITZ; GROSCH; SCHIEBERLE, 2009; TERRIER et al., 2009).

Although present in small amounts, flavonols, play an important role in the development of the color and act as co-pigments along anthocyanins (ABE et al., 2007; CASTILLO-MUÑOZ et al., 2009).

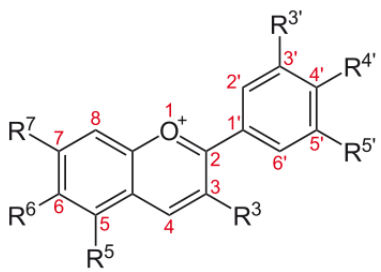
2.4.1.2. Anthocyanins

Anthocyanins are water-soluble vacuolar pigments that may appear red, purple, or blue depending on the pH. They belong to a parent class of molecules called flavonoids synthesized via the phenylpropanoid or falavnoid pathway; they are odorless and nearly flavorless, contributing to taste as a moderately astringent sensation. Anthocyanins are present in tissues of higher plants, including leaves, stems, roots, flowers, and fruits. Anthocyanins are derived from anthocyanidins by adding sugars (HE at al., 2010).

In red colored grapes, anthocyanins are usually present in the bark but may be also found in the flesh of some rich color varieties. The increase in the content of phenolic compounds in the bark occurs from the color change to full maturation. Anthocyanins appear to change color and accumulate during the maturation process, peaking at maturity (RIBÉREAU-GAYON et al., 2006).

The anthocyanins, anthocyanidins with sugar group(s), are mostly 3-glucosides of the anthocyanidins. The anthocyanins are subdivided into the sugar-free anthocyanidin aglycones and the anthocyanin glycosides (KONG; CHIA; GOH, 2003). Anthocyanins have planar structure containing two benzene rings separated by the heterocyclic ring (Figure 2.4.1.2.1). More than 600 anthocyanins are known by now. The most common sugars are glucose, galactose, arabinose, xylose and ramanose. The aglycone form is called anthocyanidin, and its basic structure flavylium is the core. Among the 20 naturally occurring anthocyanidins known, only six are most common: cyanidin, delphinidin, pelargonidin, malvidin, petunidin and peonidin. Among these, pelargonidin is not found in grapes (COULTATE, 2004; RIBEIRO; SERAVALLI, 2004; ARAÚJO, 2011).

Anthocyanins are very unstable and degraded during processing and storage due to sensitivity to light, temperature, pH, oxygen, among others. These pigments have better stability in acidic conditions (COULTATE, 2004; ARAÚJO, 2011).

Basic structure	**Anthocyanidin**	**$R_{3'}$**	**$R_{4'}$**	**$R_{5'}$**	**R_3**	**R_5**	**R_6**	**R_7**
	Aurantinidin	-H	-OH	-H	-OH	-OH	-OH	-OH
	Cyanidin	-OH	-OH	-H	-OH	-OH	-H	-OH
	Delphinidin	-OH	-OH	-OH	-OH	-OH	-H	-OH
	Europinidin	OCH3	-OH	-OH	-OH	OCH3	-H	-OH
	Pelargonidin	-H	-OH	-H	-OH	-OH	-H	-OH
	Malvidin	OCH3	-OH	OCH3	-OH	-OH	-H	-OH
	Peonidin	OCH3	-OH	-H	-OH	-OH	-H	-OH

	Petunidin	-OH	-OH	OCH3	-OH	-OH	-H	-OH
	Rosinidin	OCH3	-OH	-H	-OH	-OH	-H	OCH3

Figure 2.4.1.2.1. Basic structure of the main anthocyanidins and their radicals (HE et al., 2010).

2.4.1.3. Anthocyanins biosynthesis pathway

Anthocyanin biosynthesis involves the phenylpropanoid pathway, via flavanones (Figure 2.4.1.3.1). For the formation of anthocyanins, the chalcone synthase enzyme (CHS) catalyzes the condensation reaction of three molecules of malonyl-CoA with one molecule of p-coumaroyl-CoA to yield naringenin chalcone (tetrahidro chalcone), the first flavonoid formed in many plants. Subsequent enzymatic reactions of CHS to naringenin are catalyzed by chalcone isomerase (CHI) (HE et al., 2010).

The B ring of the naringenin can be further hydroxylated by flavonoid 3’-hydroxylase (F3’H) or flavonoid 3’5’-hydroxylase (F3’5’H) to produce eriodictyol or pentahydroxyflavanone, respectively (WATERS et al., 2005; BOGS et al., 2006) . An important step is carried out by the enzyme dihydroflavonol 4-reductase (DFR), which converts anthocyanidin precursors producing non-colored leucoantocyanidins, providing structure for anthocyanin biosynthesis (GOULD; DAVIES; WINEFIELD, 2009).

The conversion of leucoantocyanidins into anthocyanidins is performed by consecutive leucoanthocyanidin dioxygenase (LDOX) catalyzed reactions. The last step in the anthocyanin biosynthesis is performed by the enzyme UDP glucose: flavonoid 3-O-glycosyltransferase (UFGT), which carry out the anthocyanidin modifications for the different anthocyanins (ALI et al., 2011; GOULD; DAVIES; WINEFIELD, 2009; HE et al., 2010). Evidence suggests that in some plant species, only the last enzyme in the anthocyanin biosynthesis pathway, UFGT, acts specifically for the production of anthocyanins (ROUBELAKIS-ANGELAKIS, 2009).

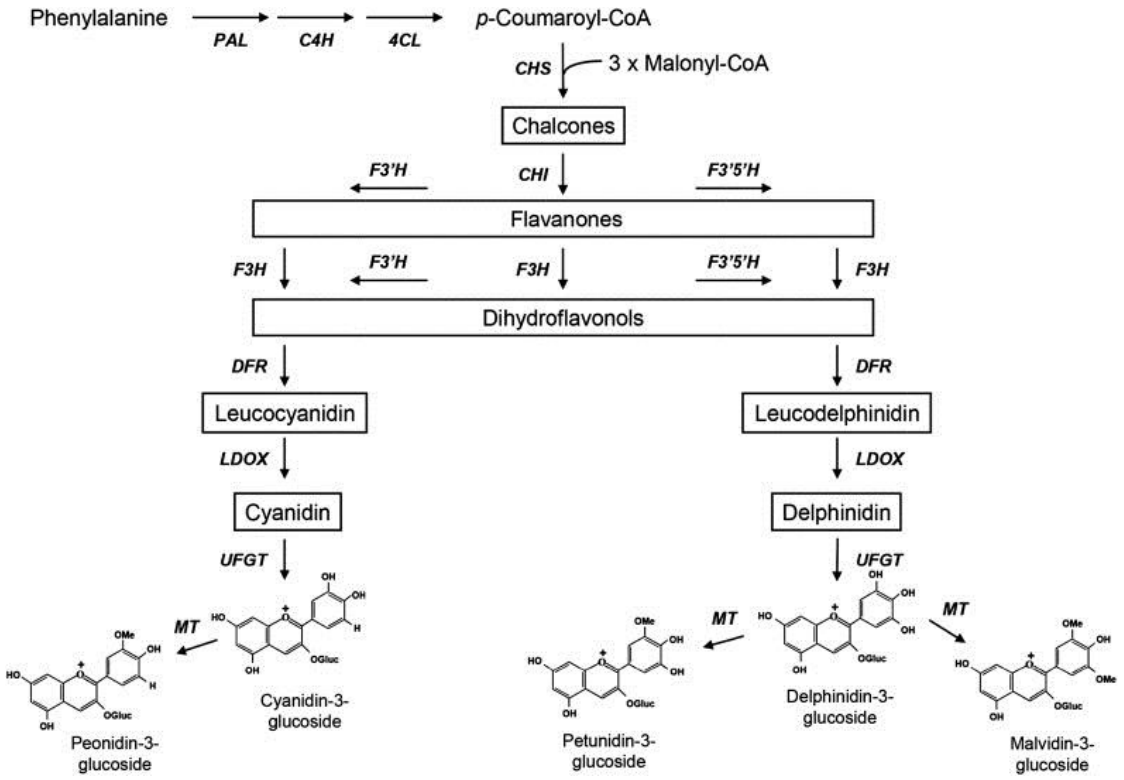


Figure 2.4.1.3.1: Biosynthetic pathway of flavonoids with prominence for the formation of anthocyanidins. CHS-chalcone synthase; CHI - chalcone isomerase; F3H-flavanone 3-hydroxylase; F3'H-flavonoid 3'-hydroxylase; F3'5'H - flavonoid 3 ', 5'-hydroxylase; DFR-dihydroflavonol reductase; LDOX - leucoantocyanidin dioxygenase; UFGT - UDP glucose: flavonoid 3-Oglycosyltransferases; MT-methyltransferases. Adapted from Rinaldo et al. (2015)

Although the color of grape berry is often characterized as a qualitative trait, there is quantitative variation within segregate populations for total anthocyanin and anthocyanin profile. Heritability is typically high for total anthocyanin content, and most of the phenotypic variation is due to MYB transcription factors (CHEN, 2015). The transcription factors VvMYBA1 and VvMYBA2 activate biosynthesis of anthocyanins in the vine (*V. vinifera*) and are not functional in cultivars of white grapes (Rinaldo et al., 2015).

In pigmented cultivars, the VvMYBA1 gene is expressed only after veraison, and is responsible for regulating anthocyanin biosynthesis, controlling the expression of biosynthetic genes, particularly UFGT. Other evidence suggests that VvMYBA1 is not expressed in young leaves, tendrils or stem of vines, being specific to berries tissues, whereas expression of VvMYBA1-2 genes has been shown to induce the expression of many of the genes within the

biosynthetic pathway of flavonoids (JEONG et al., 2004; KOBAYASHI; YAMAMOTO; HIROCHIKA, 2005).

2.6. ABSCISIC ACID

Plant growth regulators are organic or synthetic systematic compounds, which in lower concentrations promote, inhibit or modify morphological and physiological processes of the plant. This group includes auxins, cytokinins, gibberellins, abscisic acid (ABA), ethylene, brassinosteroids and jasmonic acid (CASTRO, 2006). Almost all aspects of plant growth and development undergoes hormonal control to a greater or lesser degree. One hormone can control many cellular processes, while a single process can be controlled by various hormones (SOARES; LEÃO, 2009).

ABA is a 15-carbon compound, and its *cis* and *trans* isomers are determined by the orientation of carboxyl group at carbon 2. Furthermore, it possesses an asymmetric carbon atom in position 1' of the ring, resulting in the *S* and *R* enantiomers (Figure 2). The *S*-enantiomer is naturally produced by plants and by some *Botrytis* fungi strains, and it is the most active form of ABA. The commercially available synthetic ABA for laboratory use is a mixture of approximately equal amounts of these *S* and *R* enantiomers (ZAHARIA et al., 2005; TAIZ; ZEIGER, 2009).

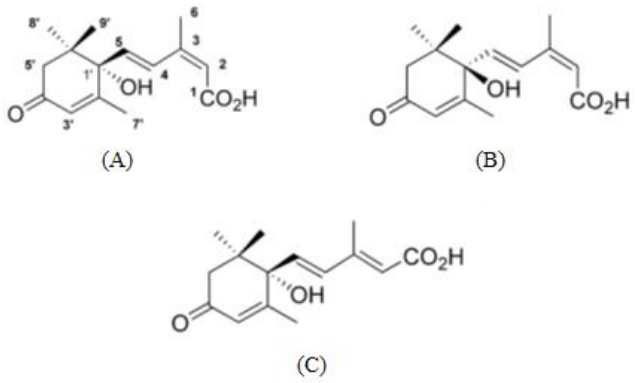


Figure 2.6.1. Chemical structure (*S*)-*cis*-ABA (A), (*R*)-*cis*-ABA (B) e (*S*)-2-*trans*-ABA (C).

In grapes, long before the berry ripening process begins, they undergo through a variety of phenological changes which are very important for the good quality of the fruit (HRAZDINA; PARSONS; MATTICK, 1984; COOMBE, 1992; KANELLIS; ROUBELAKIS-ANGELAKIS, 1993; SARRY et al., 2004; BRUMMELL, 2006; CONDE et al., 2007; DEYTIEUX et al., 2007). These effects are associated with genes which bring about

these changes (BOSS et al., 1996; DAVIES; ROBINSON, 2000; ROBINSON; DAVIES, 2000; GOES Da SILVA et al., 2005; TERRIER et al., 2005; CASTELLARIN; DI GASPERO, 2007; DELUC et al., 2007; PILATI et al., 2007). During this process, the expression of certain genes are suppressed while some other genes are promoted and it is strongly believed that ABA along with sugar contents play a key role in inducing those changes (KOYAMA; SADAMATSU; GOTO-YAMAMOTO, 2009; GAMBETTA et al. 2010; JIA et al., 2011; LIJAVETZKY et al., 2012).

ABA application can significantly increase the anthocyanin contents of the grapes with no negative affect on the bunch quality (HE et al., 2010). ABA not only promotes the anthocyanin contents of the grapes but also the expression of certain genes in the anthocyanins biosynthesis pathway and its regulatory factors. More specifically ABA enhances accumulation of mRNA for 7 different anthocyanin biosynthesis enzymes genes (JEONG et al., 2004). ABA sprayed at the time of veraison result in a long term effects. Anthocyanin synthesis results from the accumulation of *VvUFGT* transcripts which are triggered by the expression of *VvMybA1* transcription factor, while *S*-ABA concentration and the expression level of *VvMybA1* and *VvUFGT* are observed to be closely related (VILLALOBOS-GONZÁLEZ et al., 2016).

Historically, the cost of production of ABA was too high and did not justify its use in agriculture. However, the recent improvement in biological production methods of *S*-ABA through the fungus *Botrytis* sp. has made its use efficient enough to be reconsidered in viticulture (PEPPI; FIDELIBUS; DOKOOZLIAN, 2006, 2007a; CANTIN; FIDELIBUS; CRISOSTO, 2007). After that, several studies have shown the improvement of grape color by *S*-ABA exogenous application (ROBERTO et al., 2012; ROBERTO et al., 2013; KOYAMA et al., 2014; FERRARA et al, 2015; YAMAMOTO et al., 2015).

The effect of *S*-ABA application for the development of color may also be related to the time of application, since the grapes show more sensitivity to it for a number of days. So, choosing right time for the application is critical to evaluate its effect (GIRIBALDI; HARTUNG; SCHUBERT, 2010). Peppi et al. (2006, 2007b) evaluated the effect of ABA application on ‘Flame Seedless’ and ‘Crimson Seedless’ cultivars of grapes and concluded that application at veraison and after this phenological stage resulted in more colored grapes. Moreover, in an experiment performed by Giribaldi, Hartung and Schubert, (2010), the application before veraison was more effective for ‘Cabernet Sauvignon’ grape. Alonso et al. (2006) reported that ABA application at veraison and after 15 days of veraison increased total

anthocyanin significantly with a raise of about 15% in treated berries as comparison with control.

Although several studies have been conducted, it is a difficult task to determine precisely the beginning of veraison for the application of *S*-ABA, which can vary greatly between cultivars. The veraison is characterized by changes in metabolism of berries, which include the accumulation of sugar and softening, anthocyanins synthesis, the metabolism of organic acids, accumulation of aromatic compounds and changes in growth level. These events take place differently among different grape cultivars (ROBINSON; DAVIES, 2000).

In addition to these facts, the climatic conditions and size of the area to be treated are other factors that make it almost impossible to apply this plant growth regulator at a certain stage of maturity and in a short period time. So, it is necessary to evaluate the efficiency of *S*-ABA applied in a wider period of time of grape ripening, before, during and after veraison, especially in cultivars with poor color when cultivated in warm climate, like ‘Benitaka’.

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3. ARTICLE A – EXOGENOUS *S*-ABA APPLICATION: RELATIONSHIP BETWEEN ANTHOCYANINS AND SKIN COLOR OF 'BENITAKA' TABLE GRAPE

3.1 ABSTRACT

Grapes grown under warm climate condition tends to show less color development. This phenomenon is attributed to low anthocyanin accumulation of the berry skin in response to high temperature. 'Benitaka' (*Vitis vinifera* L.) table grape is one of those cultivars which show poor color development when grown under subtropical condition. The use of (*S*)-*cis*-abscisic acid (*S*-ABA) exogenous application is one of the recent techniques used to overcome this difficulty. The objective of this work was to evaluate the relationship between anthocyanins and skin color of 'Benitaka' table grape treated with *S*-ABA at different stages of berries ripening. The experiment was conducted during regular season of 2015 and 2016 off-season in a commercial vineyard of 11-year-old 'Benitaka' vines, grafted onto rootstock 'IAC 766 Campinas' in Marialva, state of Paraná (PR), Brazil,. The vines were trained on overhead trellis and spaced at 3.0 × 6.0 m apart. A randomized block design was used for the experiment and the treatments included the application of 400 mg L^{-1} of *S*-ABA, assigned as follows: Control (no application); At pre-veraison (PRV) (7 days before veraison); At veraison (V); and At post-veraison (POV) (7 days after V). A second application was performed for all treatments 10 days after the first application, except for the control. Berries analysis included, total and daily anthocyanins accumulation, color index of red grapes (CIRG) and daily CIRG development, lightness (L^*), chroma (C^*), hue angle ($h°$), mass, length, width, firmness, total soluble solids (TSS), titratable acidy (TA), index of maturation (TSS/TA) and total polyphenols of berries. The exogenous application of *S*-ABA from PRV to POV can significantly enhances the color attributes as well as total anthocyanin contents of 'Benitaka' table grape, but applications at PRV and at V provide a higher response. A stronger correlation exists between CIRG and anthocyanin of berry skin, whereas the physicochemical characteristics of berries are not affected significantly by the use of *S*-ABA. The berry firmness varies in response to the *S*-ABA application, but not to the extent where it compromises the berry quality for commercial use.

Keywords: *Vitis vinifera* L. Grapevines. Plant growth regulators. (*S*)-*cis*-abscisic acid. Quality attributes.

3.2 INTRODUCTION

Over the last few years, the growing area of ‘Benitaka’ table grape (*Vitis vinifera* L.) has increased in several subtropical regions due to its exceptional features (KISHINO; ROBERTO, 2007). It is an important colored cultivar originated from the somatic mutation of ‘Italia’ table grape (LEÃO; SOARES; RODRIGUES, 2009), however, lack of a pink and uniform color of berry skins is frequently observed when this cultivar is grown under warm climate (ROBERTO et al., 2012).

The appearance of the grapes highly influences its commercial value, and poor coloration of red/pink cultivars decreases its production efficiency (PEPPI; FIDELIBUS; DOKOOZLIAN, 2006). The color of the grapes is directly influenced by the amount and composition of anthocyanin contents present in the skin of the berry and are responsible for blue, purple and all tones of red colors inside fruits, vegetables and flowers (BAN et al., 2003; OWEN et al., 2009). Flavonols and phenolic acids act as co-pigments to stabilize color. These phenolic compounds are important secondary metabolites and present in different parts of grape berries (CHIRA at al., 2011; FLAMINI et al., 2013).

The accumulation of anthocyanins start at the time of verasion, which is regarded as the onset of ripening (CASTELLARIN et al., 2007a,b; LECOURIEUX et al., 2014). Veraison is typically prolonged for 7-10 days with in the grape bunches, signaling a shift from photosynthetic to a heterotrophic metabolism (KELLER, 2015), and can be identified from an increase in soluble solids contents, berry softening and a sudden increase in the skin color (HUANG; HUANG, 2001). This phenological stage can be divided into three distinct phases, such as pre-veraison, veraison and post veraison. Timing and lengths of these phases may vary among different cultivars in different climates. The time between the start of fruit set and the beginning of veraison can be regarded as a pre-veraison phase, while the pre-veraison stage can stretch up to 59 days starting from fruit set (ZAMBONI et al., 2010). Within few days from the start of verasion, the berries start to grow dramatically due to the accumulation of fructose and glucose, the time where the acids decline. All of these characters are associated with the engustment process, the time when sugar accumulation starts to soften the berries (ROBINSON, 2006). Although there is no specific time frame for the post-veraison stage, it may be explained as the time from the end of veraison until the start of harvest, whereas ripening process starts between 98 to 112 days after the fruit set (ZAMBONI et al., 2010).

Anthocyanins increases in the berries skin through variety of environmental stimuli such as developmental signals, environmental stresses (light, temperature, irrigation etc.) and plant growth regulators (BOSS; DAVIS, 2009). It is a well-established fact that abscisic acid

(ABA) naturally accumulates in grape skins at the onset of ripening, a time when anthocyanin and other phenolic compounds also increase. ABA stimulates the buildup of DNA coding for several enzymes that are responsible for anthocyanin accumulation in grapes, including UFGT gene (JEONG et al., 2004), which is responsible for the biosynthesis of anthocyanin in the grape berries (BOSS; DAVIS; ROBINSON ,1996a,b; KOBAYASHI et al., 2002). Although this gene is present among all type of grapes but it is expressed only in red color cultivars (BOSS; DAVIES; ROBINSON, 1996b). Several studies have demonstrated that multiple applications of the exogenous enantiomer (*S*)-*cis*-abscisic acid (*S*-ABA) can increase the anthocyanin concentration in grape skin more efficiently (ROBERTO et al., 2012, 2013; KOYAMA et al., 2014; YAMAMOTO et al., 2015; DOMINGUES NETO et al., 2017). However, for the effective improvement of color development in grape skin the application time and concentration are critically important (PEPPI; FIDELIBUS; DOKOOZLIAN, 2006, 2007a,b), but this may vary depending upon the cultivar and area of application.

Considering the facts above, applying *S*-ABA at the right time of verasion to improve grape color is a difficult task, especially when vines are grown in subtropical areas, where the weather conditions is inconsistent. Besides, the main changes in berry development at veraison, such as sugar accumulation, color and berry softening also rely on each grape cultivar. Thus, it is very important to identify the exact timing of verasion for ‘Benitaka’ table grape and to evaluate the *S*-ABA efficiency over a wider period, providing a longer range for its application.

The objective of this work was to evaluate the relationship between anthocyanins and skin color of ‘Benitaka’ table grape treated with *S*-ABA at different stages of berries ripening.

3.3 MATERIAL AND METHODS

3.3.1 Grapevines and growing conditions

The experiment was conducted in a commercial vineyard of 'Benitaka' table grape (*Vitis vinifera* L.) grafted onto 'IAC 766 Campinas' from 11-year-old vines in Marialva, state of Paraná (PR), Brazil (23º29'52, 8''S, 51º47'58''0 W, elevation 570 m), during 2 consecutive seasons, 2015 and 2016 (regular and off-season crops, respectively). The vines were trained on overhead trellis covered with a black plastic mash 18% and spaced at 3.0 × 6.0 m apart. According to Köppen classification, the climate of the area is type Cfa, i.e. subtropical with an average temperature below 18°C in winter, and average temperature above 22°C in summer. The average annual rainfall in the area is 1,596 mm, with most of the rainfalls occurring in summer (IAPAR, 2010). In both seasons, vines were cane pruned with 7-8 buds per cane, and hydrogen cyanamide 2.5% was applied to the terminal buds for uniform sprouting and flowering. Other cultural practices were carried out as usual (ROBERTO et al., 2012).

3.3.2 Treatments and experimental design

The treatments included the exogenous application of (*S*)-*cis*-abscisic acid (*S*-ABA) 400 mg.L^{-1}, as previously reported by Roberto et al. (2012, 2013). The *S*-ABA containing 100 g.L^{-1} of active ingredient was provided by Valent BioScienses® Corporation (Illinois, USA). The following *S*-ABA treatments, applied on different timings of veraison were tested: Control (no application); At pre-veraison (PRV) (at 7 days before veraison); At veraison (V); and At post-veraison (POV) (at 7 days after V). A second application was performed for all treatments 10 days after the first application, except for the control. For treatments application, a knapsack sprayer at a pressure of 568.93 psi (39.22 bar) was used, and the solution was applied onto bunches exclusively until run-off, avoiding the drift to the leaves. A non-ionic surfactant BreakThru® 0.3 mL.L^{-1} (Evonik Industries, Germany) was also added to the solution.

To identify the exact timing in which the PRV began, samples of 30 berries were collected three times per week from the experimental vineyard, starting 40 days after flowering, and once a week at 80 days after flowering up to ripening. The berry samples were evaluated regarding the total soluble solids content (TSS, °Brix), firmness (N) and color index of red grapes (CIRG), which suddenly changes at this phenological stage (KELLER, 2015). The detailed methodology used is described ahead. In addition, 'Benitaka' vines near the

experimental area, which were pruned earlier at different timings, were also closely observed about the days it took to reach veraison. At the beginning of the evaluation, the TSS contents were around 4 °Brix.

The first change observed was a slight increase in the TSS contents to 5.7 °Brix at 62 days after flowering (Figure 3.3.2.1). Apart from this, other variables remained the same, i.e., color was the same and berries were still as hard as recorded earlier. So, based on these data and observation of the nearby vines pruned earlier, it was confirmed that it would take at least a week for proper veraison to take place, and so this timing was considered as PRV. As expected, the berries showed signs of change exactly 7 days after that and was considered as V, where at least 50% of the berries showed a slight variation in color and firmness along with TSS.

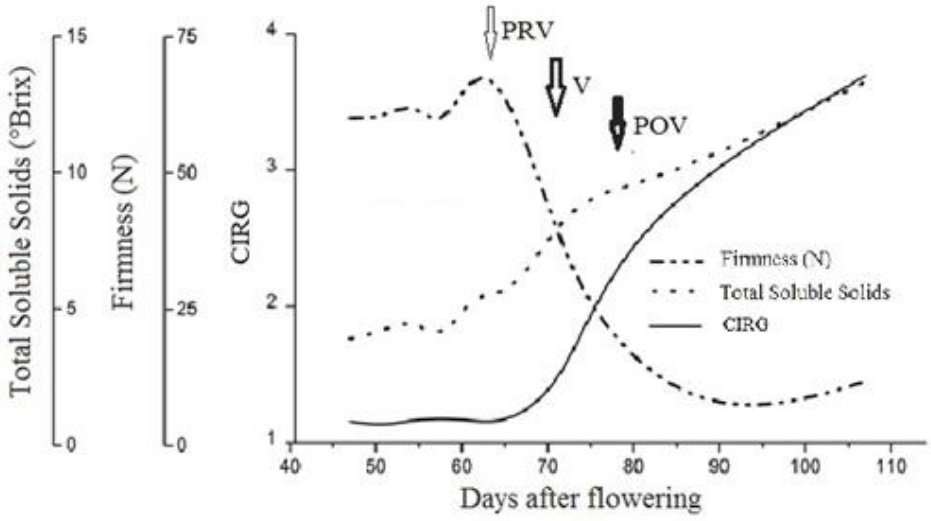

Figure 3.3.2.1. Evolution of total soluble solids (°Brix), firmness (N) and color index of red grapes (CIRG) of 'Benitaka' table grapes. Arrows indicate the timings where the treatments of *S*-ABA 400.mg L^{-1} were applied. PRV = Pre-veraison; V = Veraison and POV = Post-veraison.

The randomized block design was used as statistical model with four treatments and five replications. Each plot consisted of a single vine where ten bunches were marked for further analyses.

3.3.3. Berry sampling and fruits analysis

For both seasons, berries were harvested when the TSS stabilized around 14 °Brix, but not lower than 13 °Brix. For evaluation, 30 berries were selected from the 10 marked bunches, 3 from each bunch (from top, middle and bottom). The samples were than split into 3 subsamples (n=10 berries) for anthocyanins and polyphenols; weight, diameter, color and firmness; total soluble solids (TSS), titratable acidity (TA) and index of maturation (TSS/TA).

3.3.4. Total anthocyanins

The evaluation of total anthocyanin concentration in berry skin was performed according to Peppi; Fidelibus; Dokoozlian (2006). From each sample, 3 g of berry skin was peeled off and washed with distilled water followed by quick wash with de-ionized water and then dried with sterilized tissues. After that, 30 mL of acidified methanol (HCl 1% + methanol 99%) was added to the peeled berry skin and kept in dark for 48 hrs. The values were recorded using a spectrophotometer Genesys^{TM} 10S UV-VIS$^{®}$ (Thermo Scientific, USA) at 520 nm, and results were expressed as milligrams of total anthocyanins as malvidin-3-glucoside per gram of berry skin ($mg.g^{-1}$). The daily rate of anthocyanin accumulation was calculated by subtracting the former readings from the later and dividing it by the number of days, i.e., 7 days. The results are presented in milligram of malvidin-3-glucoside per gram of berry skin per day ($mg.g^{-1}.day^{-1}$).

3.3.5. Total polyphenols

The evaluation of total polyphenol concentration in the pulp and skin of berries was based on the Folin-Ciocauteau method (BUCIC-KOJIC et al., 2007; BORGES et al., 2013). The samples were macerated and then 5 g were homogenized with 50 mL of ethanol 50% in a blender during 2 min and centrifuged at 3,500 rpm during 5 min. An aliquot of 0.2 mL of the extract was mixed with 1.8 mL of distilled water and 10 mL of 10-fold diluted Folin-Ciocalteau reagent. After 30 s to 8 min, 8 mL of 7.5% of Na_2CO_3 solution was added. All test tubes with the mixture were shaken for 10 s on the vortex and kept in darkness during 2 hrs. Absorbance of each sample was measured after 15 min using a spectrophotometer Genesys^{TM} 10S UV-VIS$^{®}$ (Thermo Scientific, USA) at 765 nm against blank sample. Determination of total polyphenols was calculated from the calibration curve obtained with gallic acid, and readings were expressed as $mg.100g^{-1}$ of berries (gallic acid equivalents).

3.3.6. Color, mass, diameter and firmness of berries

Berry color was analyzed using a colorimeter CR-10 (Minolta®, USA) to obtain the following variables from the equatorial portion of berries (n=2 per berry): *L** (lightness), *C** (chroma) and *h*° (hue) (KOYAMA et al., 2014). Lightness values range from 0 (black) to 100 (white). Chroma indicates the purity or intensity of color, the distance from gray (achromatic) toward a pure chromatic color and is calculated from the *a** and *b** values of the CIELab scale system, starts from zero for a completely neutral color, and does not have an arbitrary end, but intensity increases with magnitude. Hue refers to the color wheel and is measured in angles; green, yellow and red correspond to 180, 90 and 0°, respectively (MCGUIRE, 1992; LANCASTER et al., 1997; PEPPI; FIDELIBUS; DOKOOZLIAN, 2006). The color index for red grapes (CIRG) was calculated using the formula: CIRG = (180-*h*°)/(*L**+*C**) (CARREÑO et al., 1995), and the 'Benitaka' berry color was then classified into 5 categories according to the CIRG values, i.e., green-yellow (<1.5), pink (1.5 to 2.5), red (2.5 to 3.5), violet (3.5 to 4.5), and dark violet (>5.0) (CARREÑO et al., 1998). The rate of daily color index of red grape (CIRG) was calculated by subtracting the final CIRG value from the initial readings, and then dividing it by the total number of days it took. The results were expressed as CIRG.day^{-1}.

The diameter (mm) and mass (g) of berries were measured with a digital caliper and a digital scale, respectively. The grapes berry firmness was determined using a texture analyzer TA.XT plus (Stable Micro Systems, Surrey, U.K.) with a cylindrical probe (35 mm diameter, P35). The berries were compressed in their equatorial diameter at 1 mm.s^{-1} (probe speed), and deformation rate of 20% of their equatorial diameter, and the firmness was expressed as the force (N) to deform the berries (LIJAVETZKY et al., 2012).

3.3.7. Total soluble solids (TSS), titratable acidity (TA) and maturation index (TSS/TA) of berries

The TSS contents were calculated using a digital refractometer DR301-95 (Krüss Optronic, Germany) by crushing the berries of each plot, and the results were expressed in °Brix. The TA was evaluated by titrating the solution extracted from the berries with standardized 0.1N NaOH in semi-automatic titrator. The titration was stopped when the pH of the solution reached 8.2, and the results were expressed as a percentage of tartaric acid (INSTITUTO ADOLFO LUTZ, 2008). The maturation index was obtained from the ratio of TSS/TA.

3.3.8. Statistical analysis

The data was submitted to analysis of variance and means were compared by Tukey's test at 5% probability (GOMES, 2009). In addition, a simple correlation analysis was also carried out between CIRG and total anthocyanins.

3.4. RESULTS AND DISCUSSION

3.4.1. Anthocyanins response to *S*-ABA application at different timings of verasion

Anthocyanin contents of 'Benitaka' table grapes during regular season of 2015 as well as off-season 2016 were significantly higher in *S*-ABA treated berries as compared to non-treated ones (Table 3.4.1.1). Anthocyanin contents were almost three-fold higher in *S*-ABA treated berries when compared with control. Application of *S*-ABA resulted in higher anthocyanin contents of the 'Benitaka' berries. However, results derived from these treatments are statistically at par with each other, meaning a longer window for application, from PRV to POV. This increase of anthocyanin is explained by the fact that application of *S*-ABA at different timings of maturation enhances levels of secondary metabolites in grapes, more specifically by triggering the biosynthesis of anthocyanins (TECCHIO et al., 2017).

The results also show that daily rate of anthocyanin accumulation followed the same pattern as the total anthocyanin contents of the 'Benitaka' grapes. The daily rates of anthocyanin accumulations during 2015 and 2016 were significantly higher among *S*-ABA treated berries at different timings of verasion. During 2015, PRV treatment was superior with higher daily anthocyanin accumulation, statistically similar to that of at V and POV, all of which were significantly higher than control. However during 2016, application of *S*-ABA at V was recorded with the highest daily anthocyanin accumulation with a very slight edge over treated berries at PRV. Whereas, *S*-ABA application at POV resulted in low rate of daily anthocyanin accumulation as compared to berries treated at PRV and V.

Untreated berries were recorded with lowest rate of daily anthocyanin accumulation among all treatments. There is a substantial difference between the daily rates of anthocyanin accumulation during 2015 and 2016 due to the fact that that during regular season of 2015, berries reached to full ripe after 41 days of their first treatment, whereas during off-season 2016 it took only 34 days (Table 3.4.1.1).

Table 3.4.1.1. Total anthocyanins and daily rate of anthocyanins accumulation of 'Benitaka' grape berries (*Vitis vinifera* L.) subjected to different treatments with (*S*)-*cis*-abscisic acid (*S*-ABA) at different timings. Regular crop 2015 and off-season crop 2016.

Treatments (timings of *S*-ABA 400 mg.L^{-1} application)[1]	Total anthocyanins (mg.g^{-1} of skin)		Daily rate of anthocyanins accumulation (mg.g^{-1} of skin day^{-1})	
	2015	2016	2015	2016
Control	0.8±0.1 b	1.2±0.4 b	0.027±0.01 b	0.032±0.01 c
Pre-veraison (PRV)	3.4±0.2 a	3.0±0.2 a	0.114±0.01 a	0.082±0.01 a
Veraison (V)	3.0±0.7 a	3.1±0.2 a	0.101±0.02 a	0.084±0.01 a
Post-veraison (POV)	2.3±0.6 a	2.5±0.3 a	0.078±0.02 a	0.068±0.01 b
F	33.37*	33.66*	17.85*	33.69*

[1]A second application of *S*-ABA 400 mg.L^{-1} was performed for all treatments 10 days after the first application, except for the control. Means within columns followed by different letters differ significantly by Tukey's test (p<0.05). *: significant (p<0.05).

Although *S*-ABA exogenous application at V significantly improved the anthocyanin contents of 'Redglobe' and 'Crimson seedless' but for more effective improvement of anthocyanin contents among different grapes cultivars this time may vary (PEPPI; FIDELIBUS; DOKOOZLIAN, 2007a,b). Some cultivars respond well to even a single application of *S*-ABA for anthocyanin accumulation, while others may require multiple applications to gain such benefits. This increase in the anthocyanin contents of 'Benitaka' table grapes when *S*-ABA is applied at PRV and V can be explained by the fact that grapes tissues are more responsive to the ABA stimuli for anthocyanin biosynthesis when they are still in early veraison stages (YAMAMOTO et al., 2015). ABA plays a key role in regulating a number of genes at onset of veraison, including those in anthocyanin biosynthesis and their signaling pathway (GAMBETTA et al., 2010). This plant growth regulator reinforces the accumulation of transcription factor *VvMYBA1*, regulator of UFGT gene expression (BOSS; DAVIES; ROBINSON, 1996b; KOBAYASHI et al., 2002; JEONG et al., 2004; AZUMA et al., 2007), which interacts with the promoter regions of UFGT and other genes involved in anthocyanin biosynthesis.

In 'Kyoho' grapes, the application of *S*-ABA at veraison increased UFGT gene expression one week after application, raising the concentration of anthocyanins in the berries (BAN et al., 2003). In 'Crimson Seedless', *S*-ABA stimulated the expression of the UFGT gene one day after its application, and the UFGT levels of the berries were slightly more pronounced. One week after the application, berries treated with 300 mg L^{-1} of *S*-ABA showed values six times greater than untreated berries. During maturation to harvest, the *S*-

ABA treated bunches showed significantly more intense berries, which were attributed to changes in transcription levels of the UFGT gene (PEPPI; WALKER; FIDELIBUS, 2008).

3.4.2. Berry color response towards *S*-ABA application at different timings of veraison

For both seasons evaluated, 2015 and 2016, the CIRG of 'Benitaka' grape berries treated with *S*-ABA at different timings was higher in comparison to untreated ones (Table 3.4.2.1). According to the grape color classification of Carreño et al. (1998) based on CIRG values, berries treated in season 2015 with *S*-ABA at any timing of veraison (PRV, V and POV) were recorded as dark violet, whereas control berries showed red color. Similarly, results from the season of 2016 demonstrated that *S*-ABA treated berries presented dark violet color, regardless of the application time at veraison (PRV, V and POV), and even though control berries showed a violet color in this season, they were still inferior to *S*-ABA treated berries in terms of color development. The CIRG values of all *S*-ABA treated berries showed statistically similar result to each another except for control. However, during 2016 season, *S*-ABA applied at PRV and V presented statistically similar results, but not compared to POV and control. Treatments of *S*-ABA at different timings of veraison enhanced CIRG more quickly as compared to control. Even at the time of harvest, untreated berries had green patches among the berries, whereas the color coverage of *S*-ABA treated bunches were more uniform (Figures 3.4.2.1 and 3.4.2.2).

Table 3.4.2.1. Color index of red grapes (CIRG) and daily rate of CIRG of 'Benitaka' grape berries (*Vitis vinifera* L.) subjected to different treatments with (*S*)-*cis*-abscisic acid (*S*-ABA) at different timings. Regular crop 2015 and off-season crop 2016.

Treatments (timings of *S*-ABA 400 mg.L^{-1} application)[1]	CIRG		Daily rate of CIRG (CIRG day^{-1})	
	2015	2016	2015	2016
Control	3.1±0.7 b	4.6±0.6 c	0.06±0.02 b	0.09±0.09 c
Pre-veraison (PRV)	5.6±0.5 a	5.8±0.5 a	0.14±0.02 a	0.13±0.07 a
Veraison (V)	5.4±0.4 a	5.6±0.4 ab	0.14±0.02 a	0.12±0.06 b
Post-veraison (POV)	5.4±1.2 a	5.4±0.4 b	0.14±0.04 a	0.12±0.05 b
F	107.01*	61.29*	104.71*	61.05*

[1]A second application of *S*-ABA 400 mg.L^{-1} was performed for all treatments 10 days after the first application, except for the control. Means within columns followed by different letters differ significantly by Tukey's test (p<0.05). *: significant (p<0.05).

In regards to daily rate of CIRG or daily color development, for 2015 season, *S*-the means observed for ABA treated berries, either at PRV, V or POV, were higher to control, but at par among themselves, whereas, during off-season 2016, treated berries at PRV showed higher daily CIRG values among all other treatments (Table 3.4.2.1).

Starting right from the first application, the development of anthocyanins and color was closely related. Application of *S*-ABA at PRV, V or POV with an additional second application significantly increased anthocyanin contents as well as color of the 'Isabel' grapes (YAMAMOTO et al., 2015). Multiple applications of *S*-ABA improve the color more rapidly and strongly as compared to single application in table grapes (ROBERTO et al., 2013). In general, *S*-ABA improves the quick pigmentation of the berry skin by accelerating anthocyanin accumulation via increasing the expression of some structural genes and transcriptional factors in the phenylpropanoid pathway (PEPPI; FIDELIBUS; DOKOOZLIAN, 2008, GAGNÈ et al., 2011). From our results, it has been noted that berries applied with *S*-ABA at PRV and V were more responsive to treatments as compared to POV, suggesting that genes responsible for the anthocyanin biosynthesis in 'Benitaka' table grapes can be triggered more efficiently during the early stage of berry maturation.

Moreover, we demonstrated that good results of berry color improvement can be achieved when *S*-ABA is applied within a longer range of time, from PR to POV, which represents a period of three weeks. This is an important observation since some weather

conditions such rainy or windy days, as well a large area of vineyards can make the job difficult in a short period of time.

Figure 3.4.2.1. Bunches of 'Benitaka' table grape subjected to different treatments with (*S*)-*cis*-abscisic acid (*S*-ABA 400 mg.L^{-1}) at different timings. A: Control (no application); B: At pre-veraison; C: at veraison; D: at post-veraison. A second application of *S*-ABA 400 mg.L^{-1} was performed for all treatments 10 days after the first application, except for the control. Regular crop 2015.

Figure 3.4.2.2. Bunches of 'Benitaka' table grape subjected to different treatments with (*S*)-*cis*-abscisic acid (*S*-ABA 400 mg.L^{-1}) at different timings. A: Control (no application); B: At pre-veraison; C: At veraison; D: at post-veraison. A second application of *S*-ABA 400 mg.L^{-1} was performed for all treatments 10 days after the first application, except for the control. Off-season crop 2016.

The darker color of the grape berries is associated with higher contents of anthocyanins present in the skin (OLIVARES et al., 2017). It is well known that anthocyanins are associated to berry color, and this codependence was clearly demonstrated when the correlation analysis between CIRG and total anthocyanins accumulation was carried out (Figures 3.4.3.3A and B), where during both season (2015 and 2016), the relationship between these two variables was highly significant. Nevertheless, high total anthocyanin contents are responsible for the darker color of grape berries and this analysis supports that hypothesis.

These results show a strong linear relationship between both variables, which was highly significant. These findings indicate that right after the application of *S*-ABA, enhanced anthocyanin accumulation as well as color. These analyses can serve as base of understanding

as how do these variables develop through the course of time and how *S*-ABA affects the development.

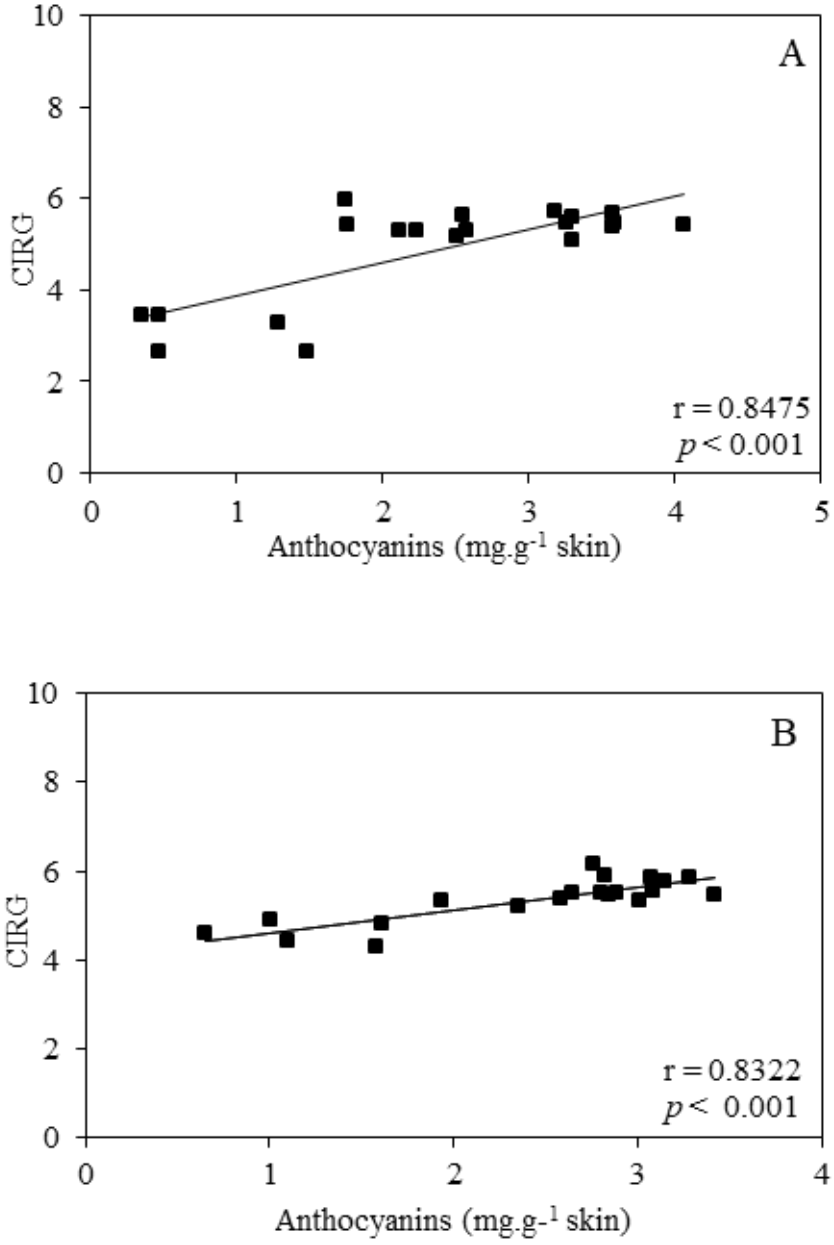

Figure 3.4.2.3. Simple linear correlation between color index of red grapes (CIRG) with total anthocyanins accumulation (mg.g^{-1} of skin) of 'Benitaka' grape berries (*Vitis vinifera* L.) subjected to different treatments with (*S*)-*cis*-abscisic acid (*S*-ABA 400 mg.L^{-1}). A: Regular crop 2015; B: Off-season crop 2016.

3.4.3. Lightness (*L**), chroma (*C**) and hue angle (*h*°) of berries

Color quality analysis from both seasons suggests that lightness values among all the grapes decreased over time, starting from V until harvest (Table 3.4.3.1). However, it has been confirmed that two applications of *S*-ABA at different timings of V significantly decrease *L** values (TECCHIO et al., 2017).

After applying *S*-ABA to the grapes, the *L** decreased sharply among all the treatments regardless of the application timings. Whereas, untreated berries were recorded with significantly high *L** as compared to the treated grapes (OLIVARES et al., 2017). Lower *L** values suggest a darker color among the *S*-ABA treated berries than untreated berries which have higher *L** readings (ROBERTO et al., 2012; DOMINGUES NETO et al., 2017; TECCHIO et al., 2017).

Lower *C**, as a result of *S*-ABA application (Table 3), indicates low color purity (CANTIN et al., 2007; FERRARA et al., 2013, 2015). However, it is worth mentioning here that this slight change in color purity is undetectable to the unaided eye and has no negative effect on the market value of the grapes (OLIVARES et al., 2017). Some studies have reported no effect of *S*-ABA over *h*° (YAMAMOTO et al., 2015; TECCHIO et al., 2017).

However, in 2015 season, the *h*° decreased along with *L** (Table 3), implying a diversion from the green towards red color (OLIVARES et al., 2017). Meanwhile, during 2016 season, the *h*° differed slightly from the pattern observed in 2015. This phenomenon may have occurred because of the difference in weather conditions occurred during ripening between both seasons. It is widely known that anthocyanin biosynthesis is favored by lower night temperatures during off-season crop, where grape ripening takes place in autumn instead of summer in regular season crop (KOSHITA et al., 2007; RICCE et al., 2013; FERRARA et al., 2015).

Table 3.4.3.1. Lightness (*L**), chroma (*C**) and hue angle (*h*°) of 'Benitaka' grape berries (*Vitis vinifera* L.) subjected to different treatments with (*S*)-*cis*-abscisic acid (*S*-ABA) at different timings. Regular crop 2015 and off-season crop 2016.

Treatments (timings of *S*-ABA 400 mg.L^{-1} application)[1]	*L**		*C**		*h*°	
	2015	2016	2015	2016	2015	2016
Control	29.6±2.6 a	23.1±2.2 a	9.47±1.0 a	8.6±1.2 a	61.0±20.9 a	35.8±9.1 b
Pre-veraison (PRV)	21.0±9.7 b	19.6±9.7 c	3.99±0.8 d	4.4±1.0 d	41.3±9.7 b	41.1±9.7 a
Veraison (V)	21.0±12.2 b	20.6±7.1 bc	4.70±1.9 c	5.3±1.4 c	41.9±12.2 b	36.7±7.1 ab
Post-veraison (POV)	21.7±5.9 b	20.8±6.3 b	6.09±1.1 b	6.5±1.5 b	33.0±5.9 c	34.0±6.9 b
F	177.00*	42.20*	193.82*	103.32*	38.41*	6.75*

[1]A second application of *S*-ABA 400 mg.L^{-1} was performed for all treatments 10 days after the first application, except for the control. Means within columns followed by different letters differ significantly by Tukey's test (p<0.05). *: significant (p<0.05).

3.4.4. Mass, length, width and firmness of berries

Various studies have shown that *S*-ABA treatments for the improvement of color and anthocyanin do not affect other physico characteristics of berries (KISHINO; ROBERTO, 2007; SANDHU et al., 2011; ROBERTO et al., 2012, 2013; KOYAMA et al., 2014; YAMAMOTO et al., 2015). The same can be observed from the results of current experiment where there was no significant effect of *S*-ABA over weight of the 'Benitaka' berries (Table 3.4.4.1).

Diameter of the berries, however, showed a little different behavior where during 2015, both length wise and width wise diameter were different among treated berries. However, during 2016 no such effect was detected (Table 3.4.4.1). This behavior can be explained by the fact that although *S*-ABA does not influence the length and width of the berries but the weather conditions of specific season can alter these variables (YAMAMOTO et al., 2015). It can also be observed from the results of this trial that berry firmness decreased in response to *S*-ABA application (Table 3.4.4.1).

Plants secondary metabolites such as anthocyanins (color pigments) are stored inside the cell vacuole, transported through ATP-binding cassette (ABC) transporters (TAIZ; ZEIGER, 2000). The fact that *S*-ABA application improved anthocyanin accumulation means that more of these pigments are stored inside cell vacuole thus increasing the solute concentration. As the water flow from the location of lower solute concentration towards high solute concentration (LODISH et al., 2000), cell vacuole must have absorbed more water from the surrounding cytoplasm and caused a decrease in the cell turgidity. This might be the reason why firmness of grape berries decreased in response to *S*-ABA treatments. It is also important to mention that the berry surface was firm and no cracking or tendency toward cracking was observed at the time of harvest. Even though *S*-ABA decreased the berry firmness, there is no evidence that it negatively affected the quality or commercial value of the product.

Table 3.4.4.1 Mass, length, width and firmness of 'Benitaka' grape berries (*Vitis vinifera* L.) subjected to different treatments with (*S*)-*cis*-abscisic acid (*S*-ABA) at different timings. Regular crop 2015 and off-season crop 2016.

Treatments (timings of *S*-ABA 400 mg.L^{-1} application)[1]	Mass (g)		Length (mm)		Width (mm)		Firmness (N)	
	2015	2016	2015	2016	2015	2016	2015	2016
Control	10.4±0.7	8.6±0.6	28.9±3.2 b	27.3±1.3	23.6±1.2 b	23.2±1.9	11.7±1.4 a	10.5±1.2 a
Pre-veraison (PRV)	11.6±1.1	9.2±0.6	30.7±1.8 a	27.9±1.3	24.7±1.4 a	23.3±3.0	9.6±1.4 c	8.8±1.1 c
Veraison (V)	11.8±0.9	9.8±0.5	30.2±1.6 a	28.0±1.4	24.7±1.4 a	24.0±1.5	9.9±1.1 c	9.3±0.8 bc
Post-veraison (POV)	10.1±0.6	8.5±0.8	29.6±1.5 ab	27.6±3.4	23.3±1.1 b	23.8±1.2	10.8±1.6 b	9.5±1.3 b
F	4.14^{ns}	1.5 ^{ns}	9.79*	1.1 ^{ns}	16.13*	2.0 ^{ns}	24.63*	22.72*

[1]A second application of *S*-ABA 400 mg.L^{-1} was performed for all treatments 10 days after the first application, except for the control. Means within columns followed by different letters differ significantly by Tukey's test (p<0.05). *: significant (p<0.05). ns: non-significant.

3.4.5. Total soluble solids (TSS), titratable acidity (TA), index of maturation (TSS/TA) and total polyphenols of berries

Regarding TSS, TA and maturation index (TSS/TA) of both evaluated seasons, there has been a little difference in the pattern followed by these variables (Table 3.4.5.1). Although TSS contents can be influenced by exogenous plant growth regulator treatments (LUAN, 2014; JU et al., 2016), but here the difference in the TSS contents suggest that the change may have occurred in response to environmental stimuli rather than *S*-ABA treatment. However, the 2016 crop did not show any such response, thus confirming our hypothesis (KOYAMA et al., 2014; YAMAMOTO et al., 2015; ZHU et al., 2016). TA and TSS/TA also followed the same pattern where a slight change was observed during 2015 season in these variables, while during 2016 no difference among treatments was noticed (Table 3.4.5.1).

It is important to mention here that certain quality parameters can be affected by specific environmental conditions (JU et al., 2016). So, this might be the reason why TA and TSS/TA also varied like TSS during the season of 2015 but not in 2016. Total polyphenols of 'Benitaka' table grape didn't show any difference in response to the *S*-ABA treatments during 2015 season. However, they varied a little during the season of 2016 but the margin of variation is very small. The response of phenolic acid and antioxidant activity depends mostly on internal factors, more importantly its genotype (XU et al., 2014). That is the reason why among some cultivars, *S*-ABA can increase the total polyphenols e.g. muscadine (OLIVARES et al., 2017), 'Malbec' (BERLI et al., 2011) and 'Cabernet Sauvignon' grapes (DEIS et al., 2011; JU et al., 2016), whereas other remain unaffected by its treatment e.g. 'Isabel' grapes (KOYAMA et al., 2014; YAMAMOTO et al., 2015).

As a general view, the exogenous application of *S*-ABA show a very significant effect on the anthocyanin contents as well as berry color of 'Benitaka' table grapes when applied around the time of veraison. It has been also observed that early application leads to high rate of daily anthocyanin accumulation and increase the total anthocyanin by almost three folds as compared to control treatment. The right time of *S*-ABA application is a key toward achieving these goals when genes responsible for anthocyanin biosynthesis are receptive to these hormones, and better results were obtained when *S*-ABA was applied at PRV or at V. Sugar and ABA levels are believed to be the reason behind triggering anthocyanins accumulation in berries skin, by activating the genes expression involved in anthocyanin biosynthesis (KELLER, 2015). Applying *S*-ABA around veraison time, when the genes are already active, increases their efficiency. A study performed on 'Crimson Seedless' showed that three weeks

after the application of *S*-ABA, UFGT levels of the treated grapes decreased (PEPPI; WALKER; FIDELIBUS, 2008). So, a second application of *S*-ABA at a time when the concentrations of endogenous ABA are close to the maximum levels or beginning to decrease, possibly contribute to the increase in the gene expression. A stronger correlation exists between anthocyanin and color development, as well as on daily basis. TSS, TA, index of maturation and total polyphenols seems not to be affected significantly by the use of *S*-ABA. Similarly, other physical attributes like length, width and berry mass do not show any significant change. However, berry firmness slightly varies in response to these treatments, but not to the extent where it compromises the berry quality for commercial use. There is a slight difference in terms of berry color of the two seasons evaluated, and this difference is probably due to the different weather conditions in which ripening takes place in both seasons.

3.5. CONCLUSIONS

The exogenous application of *S*-ABA from pre to post-veraison can significantly enhances the color attributes as well as total anthocyanin contents of ‘Benitaka’ table grape, but applications at pre-veraison and at veraison provide a higher response.

A stronger correlation exists between anthocyanin and color index of berry skin, as well as on daily basis, whereas the physicochemical characteristics of berries are not affected significantly by the use of *S*-ABA.

The berry firmness varies in response to the *S*-ABA application, but not to the extent where it compromises the berry quality for commercial use.

Table 3.4.5.1. Total soluble solids (TSS), titratable acidy (TA), index of maturation (TSS/TA) and total polyphenols of 'Benitaka' grape berries (*Vitis vinifera* L.) subjected to different treatments with (*S*)-*cis*-abscisic acid (*S*-ABA) at different timings. Regular crop 2015 and off-season crop 2016.

Treatments (timings of *S*-ABA 400 mg.L^{-1} application)[1]	TSS (°Brix)		TA (% of tartaric acid)		Index of maturation (TSS/TA)		Total polyphenols (mg.100g^{-1})	
	2015	2016	2015	2016	2015	2016	2015	2016
Control	13.4±0.4 b	14.3±1.4	0.74±0.08 a	0.67±0.05	18.4±2.5 c	21.4±3.2	0.25±0.01	0.14±0.01 b
Pre-veraison (PRV)	13.5±0.4 ab	15.0±0.3	0.53±0.05 b	0.66±0.07	25.8±3.1 ab	21.7±1.7	0.24±0.03	0.16±0.02 ab
Veraison (V)	14.1±0.5 a	15.7±0.8	0.52±0.07 b	0.73±0.01	27.5±4.7 a	21.5±1.3	0.23±0.04	0.19±0.03 ab
Post-veraison (POV)	13.2±0.3 b	15.4±0.5	0.63±0.05 ab	0.73±0.02	21.1±1.6 bc	21.4±1.0	0.22±0.01	0.20±0.02 a
F	6.80*	2.40 ^{ns}	12.17*	4.26 ^{ns}	14.89*	0.58 ^{ns}	0.87^{ns}	6.59*

[1]A second application of *S*-ABA 400 mg.L^{-1} was performed for all treatments 10 days after the first application, except for the control. Means within columns followed by different letters differ significantly by Tukey's test (p<0.05). *: significant (p<0.05). ns: non-significant.

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4. ARTICLE B – RATES OF ANTHOCYANINS ACCUMULATION AND COLOR DEVELOPMENT IN 'BENITAKA' TABLE GRAPE TREATED WITH ABSCISIC ACID

4.1. ABSTRACT

In colored grapes cultivars, usually the anthocyanin contents are inhibited by the high temperature during ripening and the berries suffer a loss in color, thus affecting its market value. In order to overcome this issue, a research was planned to evaluate the influence of (*S*)-*cis*-abscisic acid (*S*-ABA) on rates of anthocyanins accumulation in 'Benitaka' table grapes when applied at different timings of veraison, and to quantify the gradual increase of berry color on daily basis. Vines of 'Benitaka' table grape (*Vitis vinifera* L.), 11 years of age, grafted on rootstock 'IAC 766 Campinas' were selected for this work, located in a commercial vineyard at Marialva city, state of Parana, Brazil. The trials were carried out during two consecutive seasons i.e. regular season of 2015 and off-season of 2016. The vines were spaced at a distance of 3.0 x 6.0 m and cane-pruned. The experiments were conducted in a randomized block design, and the treatments were replicated five times. The treatments used for the experiments contained *S*-ABA 400 $mg.L^{-1}$ applied at different timings of veraison, as follow: Control (with no application); At pre-veraison (PRV); At veraison (V); and At post-veraison (POV). For all *S*-ABA treatments, a second application was performed 10 days after the first application. Berries were analyzed for weekly and daily anthocyanin accumulations, weekly and daily CIRG development, total soluble solids content (TSS), titratable acidity (TA) and maturation index (TSS/TA). During both regular and off-season crops of 'Benitaka' table grapes, two applications of *S*-ABA at any time of veraison, especially at PRV or at V, can significantly improve the weekly and daily rate of anthocyanin accumulation as well as color development of the berries. Other chemical properties of grapes, i.e., TSS, TA and TSS/AT evolution, are not affected by the use of *S*-ABA, and follow a predictable pattern in relation to days of berries ripening.

Keywords: *Vitis vinifera* L.; *S*-ABA; rate of anthocyanin accumulation; CIRG; bioactive compounds.

4.2 INTRODUCTION

Table grapes are a rich source of phenolic compounds with antioxidant and anti-inflammatory properties, which are helpful in preventing several diseases (PEZZUTO, 2008; KATALINIC et al., 2010; NIXDORF; HERMÓSÍN-GUTIÉRREZ, 2010). These secondary metabolites are present in different parts of berries, where skin is enriched with anthocyanins, pigments responsible for the red, pink or black color (LACAMPAGNE; GAGNÉ; GÉNY, 2010; FLAMINI et al., 2013), and in some cultivars, these pigments can also be found in the flesh (OWEN et al., 2009; KOYAMA; SADAMATSU; GOTO-YAMAMOTO, 2010).

However, when colored table grapes are grown in subtropical areas, high temperatures during ripening may inhibit anthocyanins accumulation and prevents color development, negatively affecting the market value of table grapes since the skin color is a very important economic feature (PEPPI; FIDELIBUS; DOKOOZLIAN, 2006; KOYAMA et al., 2010; ROBERTO et al., 2012).

‘Benitaka’ (*Vitis vinifera* L.) is one of the most important colored table grape developed from somatic mutation of ‘Italia’ grape (LEÃO; SOARES; RODRIGUES, 2009). Interest in growing this cultivar has been increasing due to its dark pink color and uniform, large and crunchy berries (KISHINO; ROBERTO, 2007). However, lack of color is an issue while growing this cultivar in subtropical warm climate (ROBERTO et al., 2012).

In grapes, the anthocyanin starts accumulating at the time when abscisic acid (ABA) also starts to increase in berries, and reportedly being responsible for the anthocyanin biosynthesis (OWEN et al., 2009; KOYAMA; SADAMATSU; GOTO-YAMAMOTO, 2010). In the last years, it has been demonstrated that applications of the enantiomer (*S*)-*cis*-abscisic acid (*S*-ABA) increase the anthocyanin contents of grapes and improve its color (PEPPI; FIDELIBUS; DOKOOZLIAN, 2007a,b; OWEN et al., 2009; KOYAMA; SADAMATSU; GOTO-YAMAMOTO, 2010; DEIS et al., 2011; ROBERTO et al., 2012, 2013; YAMAMOTO et al., 2015).

Exogenous application of *S*-ABA is effective around the time of verasion (onset of ripening), a time when physiological changes starts to appear in grapes, such as increase of soluble solids, berry softening, and coloring (YAMAMOTO et al., 2015, KOYAMA et al., 2014). However, in most of the cases, application of *S*-ABA at the time of verasion is a difficult task, especially because the onset of these changes does not occur simultaneously and may widely vary among cultivars (GIRIBALDI; HARTUNG; SCHUBERT, 2010). Besides, large growing areas and unfavorable climatic conditions, such as prolonged rainfall periods, turn difficult to apply this plant growth regulator at veraison in a short period of time in the

whole area, since it is a time-consuming operation and only the bunches are subjected to the application.

Considering these aspects, the evaluation of *S*-ABA application over a longer period of time, i.e., from pre to post-veraison on color development hasn´t been explored yet, especially regarding the weekly and daily rates of anthocyanin development, what could provide a better understanding about the response of berries towards such treatments in different circumstances.

In order to overcome this issue, a research was planned to evaluate the influence of (*S*)-*cis*-abscisic acid (*S*-ABA) on rates of anthocyanins accumulation in 'Benitaka' table grapes when applied at different timings of veraison, and to quantify the gradual increase of berry color on daily basis.

4.3 MATERIAL AND METHODS

4.3.1 Experimental area and pre-conditions

Vines of 'Benitaka' table grape (*Vitis vinifera* L.), 11 years of age, grafted on rootstock 'IAC 766 Campinas' were selected for this work, located in a commercial vineyard at Marialva city, state of Parana, Brazil (23º29'52, 8''S, 51º47'58''0 W, elevation 570 m). The climate of this area has been classified as Cf*a* by Köppen i.e. subtropical humid with winter mean temp below 18 ºC, summer mean temperature above 22 °C and 1,596 mm of rainfall mostly during summer (CAVIGLIONE et al., 2000). The trials were carried out during two consecutive seasons i.e. regular season of 2015 and off-season of 2016. The vines were spaced at a distance of 3.0 x 6.0 m and cane-pruned with 7-8 buds per cane. For uniform bud sprouting, 2.5 % of hydrogen cyanamide was applied on the terminal buds. Other practices like fertilizer application, weed control, pest and diseases management were carried out according to the local practices used (ROBERTO et al., 2012).

4.3.2 Treatments and statistical design

The isomer (*S*)-*cis*-abscisic acid (*S*-ABA) was provided by Valent BioScienses® Corporation (Illinois, USA), containing 100 $g.L^{-1}$ of active ingredient. The experiments were conducted in a randomized block design, where the treatments were replicated five times, an each plot consisted of one single vine. Ten bunches per each plot were marked for further analysis. The treatments used for the experiments contained *S*-ABA 400 $mg.L^{-1}$ (ROBERTO et al., 2012; 2013) applied at different timings of veraison, as follows: Control (with no application); At pre-veraison (PRV); At veraison (V); and At post-veraison (POV). For all *S*-

ABA treatments, a second application was performed 10 days after the first application in order to potentialize anthocyanin accumulation (ROBERTO et al., 2012; 2013). Application timings were identified considering the total soluble solids (TSS) of berries. The first treatment (at PRV) was applied when the berry TSS contents suddenly jumped from 4.0 to 5.7 °Brix. Similarly for the second treatment (at V), TSS was 7.3 °Brix and at least 50% of the berries showed change in color, performed 7 days after PRV. The last treatment (at POV) was also applied 7 days after V, where the TSS was 9.5 °Brix.

For *S*-ABA application, bunches of the 'Benitaka' table grape 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 $L.ha^{-1}$ in a complete and uniform way. In addition, 0.3 $mL.L^{-1}$ of BreakThru® (Evonik Industries, Germany), a non-ionic surfactant, was added to all treatments for uniformity of the treatment.

4.3.3. Sampling and analyses

During both evaluated seasons, anthocyanin contents, color development, TSS, titratable acidity and maturation index were evaluated on weekly basis starting right from the application of first treatment (at PRV). For this purpose, 30 berries were randomly selected from each plot i.e. 3 from each marked bunch with one from top, one from the middle and one from the bottom of each bunch. These samples were then split into three subsamples (n=10) for further evaluation. For both seasons, bunches were harvested when TSS of the berries stabilized around 14.0 °Brix.

4.3.4 Anthocyanins evaluations

To determine the anthocyanin content in berries, from each plot, samples of 3 g of berry skin were used, which were gently separated from the flesh using sterile blade, and washed with distilled and de-ionized water. The skins were than dried with sterilized tissue, and added with 30 mL of acidified methanol (HCl 1% + methanol 99%) and left in dark for 48 hrs. Spectrophotometer Genesys™ 10S UV-VIS® (Thermo Scientific, USA) at 520 nm was used for evaluating the samples, whereas results were expressed in milligrams of total anthocyanins as malvidin-3-glucoside per gram of berry skin ($mg.g^{-1}$) (PEPPI; FIDELIBUS; DOKOOZLIAN, 2006). For evaluating the weekly rate of anthocyanin accumulation, readings from earlier sample were subtracted from the later ones and divided by the total number of days, i.e., 7 days, and the results were expressed as milligram of malvidin-3-glucoside per gram of berry skin per day ($mg.g^{-1}$.skin).

4.3.5 Skin color evaluations

A colorimeter CR-10 (Minolta®, USA) was used for skin color evaluation. For each plot, 10 berries were analyzed for color development, by recording its *L** (lightness), *C** (chroma) and *h*° (hue angle). The values of light may range from 0 (black) to 100 (white). Chroma is calculated from the *a** and *b** of the CIELab scale system. Chroma signifies color purity or color intensity from achromatic (grey) towards chromatic color that starts from zero without any possible end point but the intensity increases with magnitude. Hue angle refers to the color wheel i.e. green, yellow and red in regards to the values of 180, 90 and 0°, respectively (PEPPI; FIDELIBUS; DOKOOZLIAN, 2006; 2007a,b; KOYAMA et al., 2014). For color index for red grapes (CIRG), the formula CIRG = (180-*h*°)/(*L**+*C**) was used (CARREÑO et al., 1995). The weekly rate of color index of red grape (CIRG) was calculated by subtracting the final CIRG value from the initial readings, and then dividing it by the total number of days it took, and the results were expressed as CIRG.

4.3.6 Total soluble solids (TSS), titratable acidity (TA) and maturation index (TSS/TA)

A digital refrectometer DR301-95 (Krüss Optronic, Germany) was used for the TSS evaluation. For this purpose, juice was extracted from 10 berries of each plot, and the results were expressed as °Brix. For titratable acidity (TA) determination, a semi-automatic titrator was used where juice extracted from the berries was titrated with 0.1N NaOH. The results were presented as percentage of tartaric acid (INSTITUTO ADOLFO LUTZ, 2008). The maturation index was calculated from the ratio of TSS and TA.

4.3.7 Statistical analysis

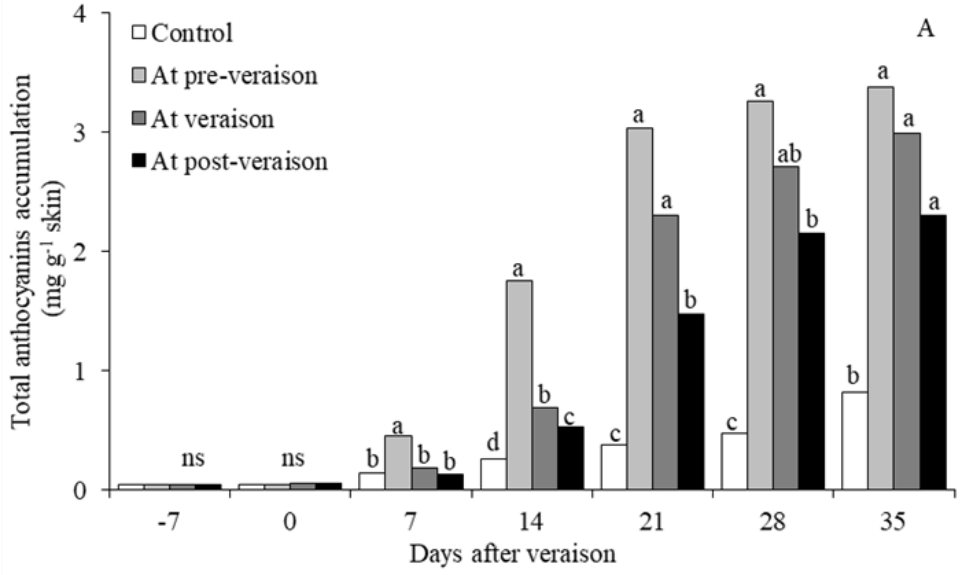
The collected data was analyzed using analysis of variance (ANOVA) whereas the means were compared using Tukey's test with at least 5% level of probability (GOMES, 2009). Furthermore, regression analyses were carried out for total soluble solids, titratable acidity and maturation index.

4.4. RESULTS AND DISCUSSION

4.4.1. Total and weekly rate of anthocyanin accumulation

Total anthocyanin accumulation was significantly affected in both seasons by the use of exogenous *S*-ABA applied at different timings of verasion. After two weeks of the application at PRV, the grape berries started to show increase in anthocyanin concentration of its skin (Figures 4.4.1.1A and B). During both seasons, all treatments, regardless of their application timing, i.e., at PRV, at V and at POV, presented a significant increase throughout the berry ripening until harvest. This increase was superior among *S*-ABA treated berries in comparison with control treatments from the start of veraison until harvest. It has been observed that during both seasons, although at PRV and at V presented higher means as compared with at POV during the process of berries ripening, in both cases the final means of these treatments were statistically similar to each other and significantly higher than control treatment.

Moreover, regardless of the different timings of *S*-ABA application, it is clear the significance of the second application (i.e. 10 days after first one) to keep the anthocyanin accumulation over time, and this behavior was observed during both growing seasons. Some cultivars may respond well to a single application of *S*-ABA such as 'Crimson Seedless' (PEPPI; FIDELIBUS; DOKOOZLIAN, 2007b), whereas others, like 'Rubi' and 'Niagara Rosada' may need multiple applications of to get such benefits (DOMINGUES NETO et al., 2017; TECCHIO et al., 2017).



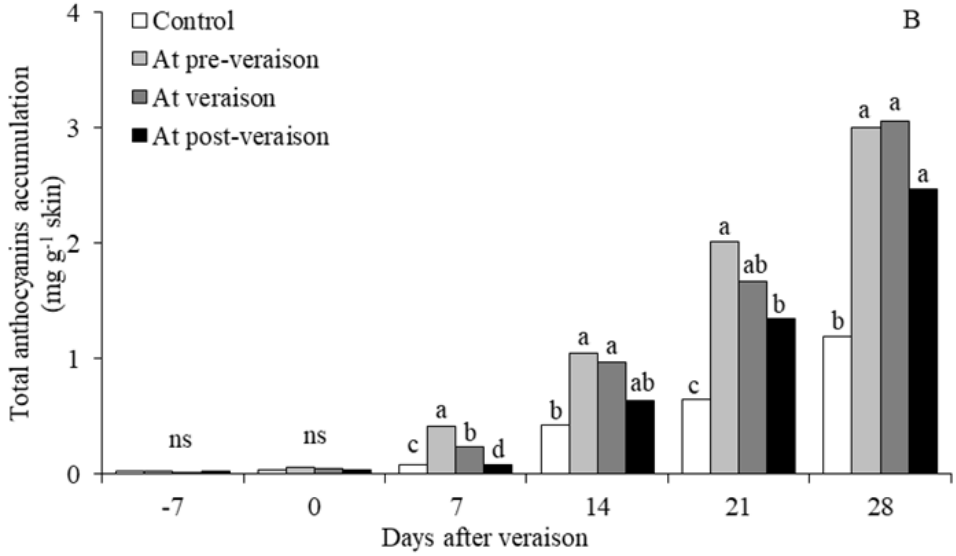


Figure 4.4.1.1. Total anthocyanins accumulation (mg.g^{-1} of skin) of 'Benitaka' berries (*Vitis vinifera* L.) subjected to (*S*)-*cis*-abscisic acid (*S*-ABA 400 mg.L^{-1}) at different timings of veraison. A second application of *S*-ABA 400 mg.L^{-1} was performed for all treatments 10 days after the first application, except for the control. A: Regular crop 2015; B: Off-season crop 2016. Means within columns for the same letter followed by different letters differ significantly by Tukey's test (p<0.05). ns: non-significant.

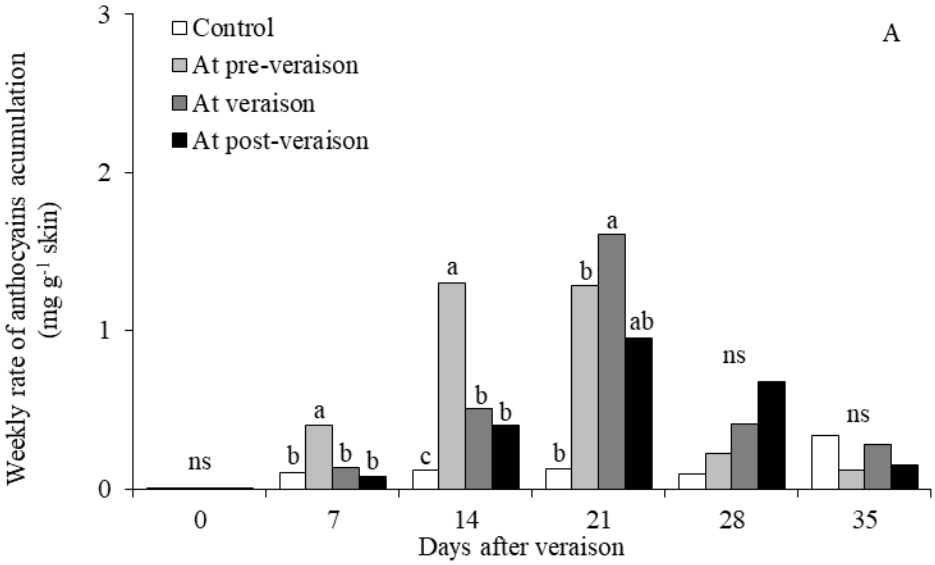
It has been demonstrated that application of *S*-ABA around the time of veraison increases the anthocyanin contents in the berries skin of several grape cultivars (PEPPI; FIDELIBUS; DOKOOZLIAN, 2006, 2007a,b; LEÃO et al., 2014; YAMAMOTO et al., 2015). This can be attributed to the effects of *S*-ABA on the expression of genes related to anthocyanin biosynthesis and accumulation of metabolites in grape berries (GIRIBALDI; HARTUNG, SCHUBERT, 2010; KOYAMA; SADAMATSU; GOTO-YAMAMOTO, 2010; VILLALOBOS-GONZÁLEZ et al., 2016). The use of ABA enhances the accumulation of the transcription factor *VvMYBA1*, regulator of *UFGT* gene expression (BOSS; DAVIES; ROBINSON, 1996; KOBAYASHI et al., 2002; JEONG et al., 2004; AZUMA et al., 2007), whereas the *UFGT* acts specifically for the production of anthocyanins (ROUBELAKIS-ANGELAKIS, 2009).

Although the final means of total anthocyanin were almost similar during both seasons, the development pattern of anthocyanin accumulation was slightly different. During 2015 season, the anthocyanin accumulation was fast after the application of *S*-ABA, but at the end of the cycle, the increase seemed to stabilize. On the other hand, during 2016 season, this

behavior varied, where the anthocyanin buildup was initially slow but got a momentum, and even at harvest, berries showed a tendency towards producing more anthocyanin contents, unlike 2015 where anthocyanin accumulation stabilized at the time of harvest.

This phenomenon can be more clearly observed from weekly rate of anthocyanin accumulation (Figures 4.4.1.2A and B). It can be observed that during 2015 season, the weekly rate of anthocyanin accumulation during early ripening stages (14 and 21 DAV) was faster as compared to later stages (28 and 35 DAV), where this development stabilized. On the other hand, during 2016 season, the rate of weekly anthocyanin accumulation was not significantly high during early stages (14 DAV) but gradually increased and was still increasing at harvest. The rate of weekly anthocyanin accumulation during 2016 was more efficient compared to 2015 seasons, whereas during both seasons berries treated with *S*-ABA at PRV showed a significantly higher accumulation rate followed by at V application.

Hasty anthocyanin accumulation was observed among *S*-ABA treated 'Cabernet Sauvignon' berries during early stages of ripening. Also, the anthocyanin synthesis differed among two growing season of the trial which can be attributed to the climatological differences among the two seasons (GAGNÉ et al., 2006).



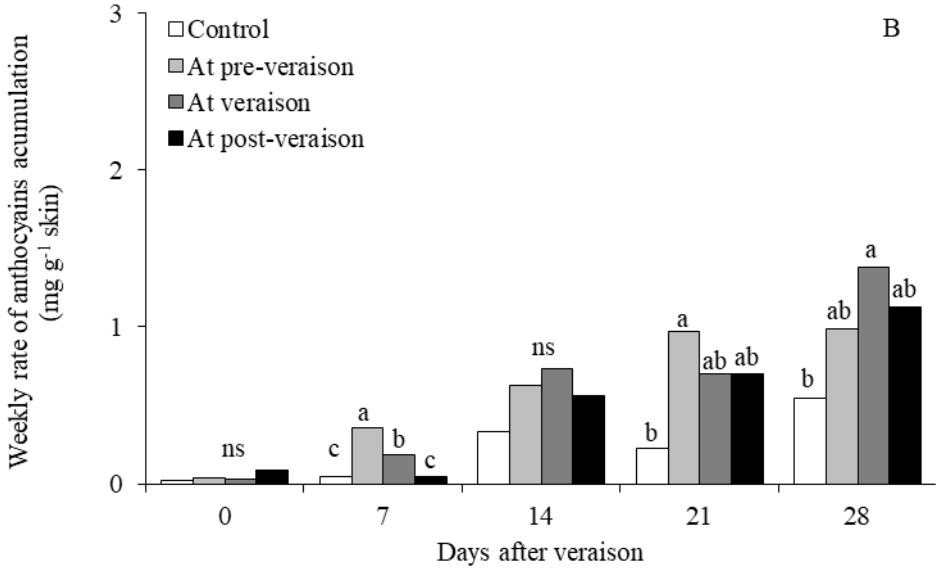


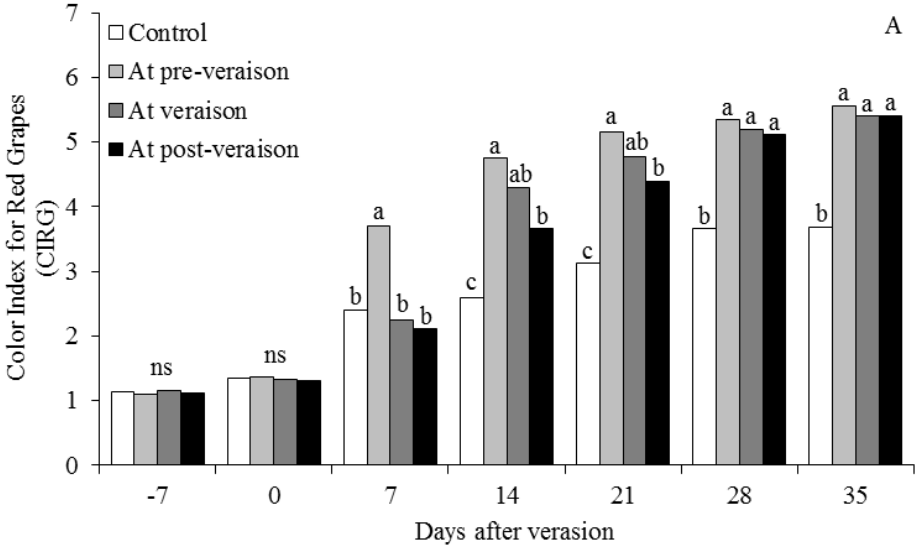
Figure 4.4.1.2. Weekly rate of anthocyanin accumulation (mg.g^{-1}.skin) of 'Benitaka' berries (*Vitis vinifera* L.) subjected to (*S*)-*cis*-abscisic acid (*S*-ABA 400 mg.L^{-1}) at different timings of veraison. A second application of *S*-ABA 400 mg.L^{-1} was performed for all treatments 10 days after the first application, except for the control. A: Regular crop 2015; B: Off-season crop 2016. Means within columns for the same letter followed by different letters differ significantly by Tukey's test (p<0.05). ns: non-significant.

Several environmental factors influence the anthocyanin accumulation in grapes skin including temperature, solar radiation and the interaction between temperature and solar radiation (TARARA et al., 2008). So, the different pattern of anthocyanin accumulation during two seasons may have occurred due to difference in climatic condition of the two seasons especially the temperature and its variation during day and night. The early increase in anthocyanin contents may have occurred due to the fact that less mature berries respond well to *S*-ABA as compared to more ripe berries, in terms of anthocyanin accumulation and color development (YAMAMOTO et al., 2015). However, some cultivars respond well to late *S*-ABA application as well (PEPPI; FIDELIBUS; DOKOOZLIAN, 2007a,b). Meanwhile, in current study, the timing of applying *S*-ABA had a longer range, where starting from PRV to POV all the treatments showed significant improvement in berries anthocyanin contents, thus allowing a longer period of time for applying the plant growth regulator (YAMAMOTO et al., 2015).

4.4.2 Berries color and weekly rate of color development

Color development followed the same pattern observed for total anthocyanin accumulation, with a very slight variation during 2016 season. Like anthocyanin color development, it started to increase more quickly in *S*-ABA treated berries as compared with non-treated berries (Figures 4.4.2.1A and B). During both seasons, right after the application of *S*-ABA, the treated berries showed significantly darker color in comparison to control with application at PRV and at V, being the superior treatments in terms of color development (Figures 4.4.2.2 and 4.4.2.3). Multiple *S*-ABA applications showed similar effect on berries color development as anthocyanin concentration, as previously discussed.

During 2015 season, early *S*-ABA applications (at PRV and at V) were recorded with higher anthocyanin accumulation throughout the ripening of berries, where at harvest, all treatments were significantly at par with each other, including at POV, except control. On the other hand, for 2016 season, the same behavior was observed for the treatments, but with a little difference, where at harvest, *S*-ABA applied at POV treatment showed significantly similar results to that of at V application, but lower to at PRV. Overall, all the treatments during off-season were higher than control, but the treatments applied at PRV and at V were recorded with higher values.



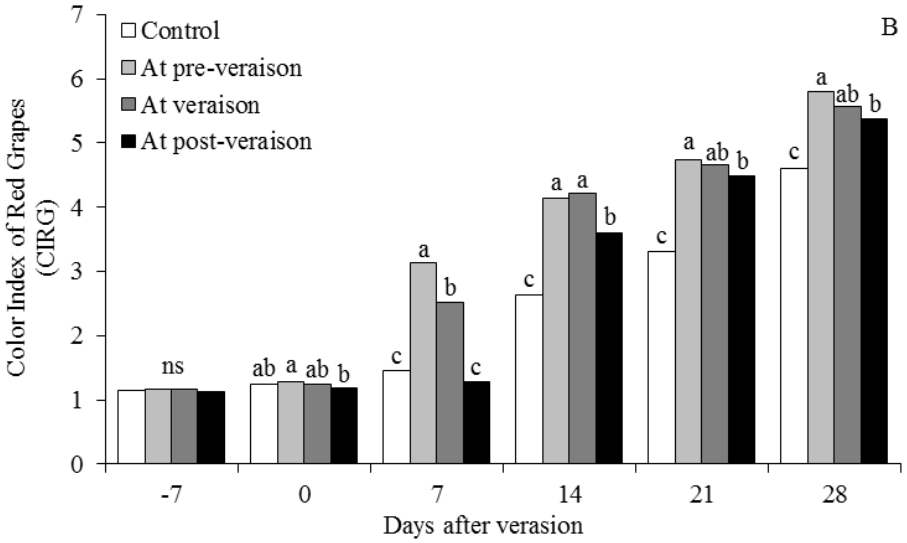


Figure 4.4.2.1. Color index of red grapes (CIRG) of 'Benitaka' berries (*Vitis vinifera* L.) subjected to (*S*)-*cis*-abscisic acid (*S*-ABA 400 mg.L^{-1}) at different timings of veraison. A second application of *S*-ABA 400 mg.L^{-1} was performed for all treatments 10 days after the first application, except for the control. A: Regular crop 2015; B: Off-season crop 2016. Means within columns for the same letter followed by different letters differ significantly by Tukey's test (p<0.05). ns: non-significant.

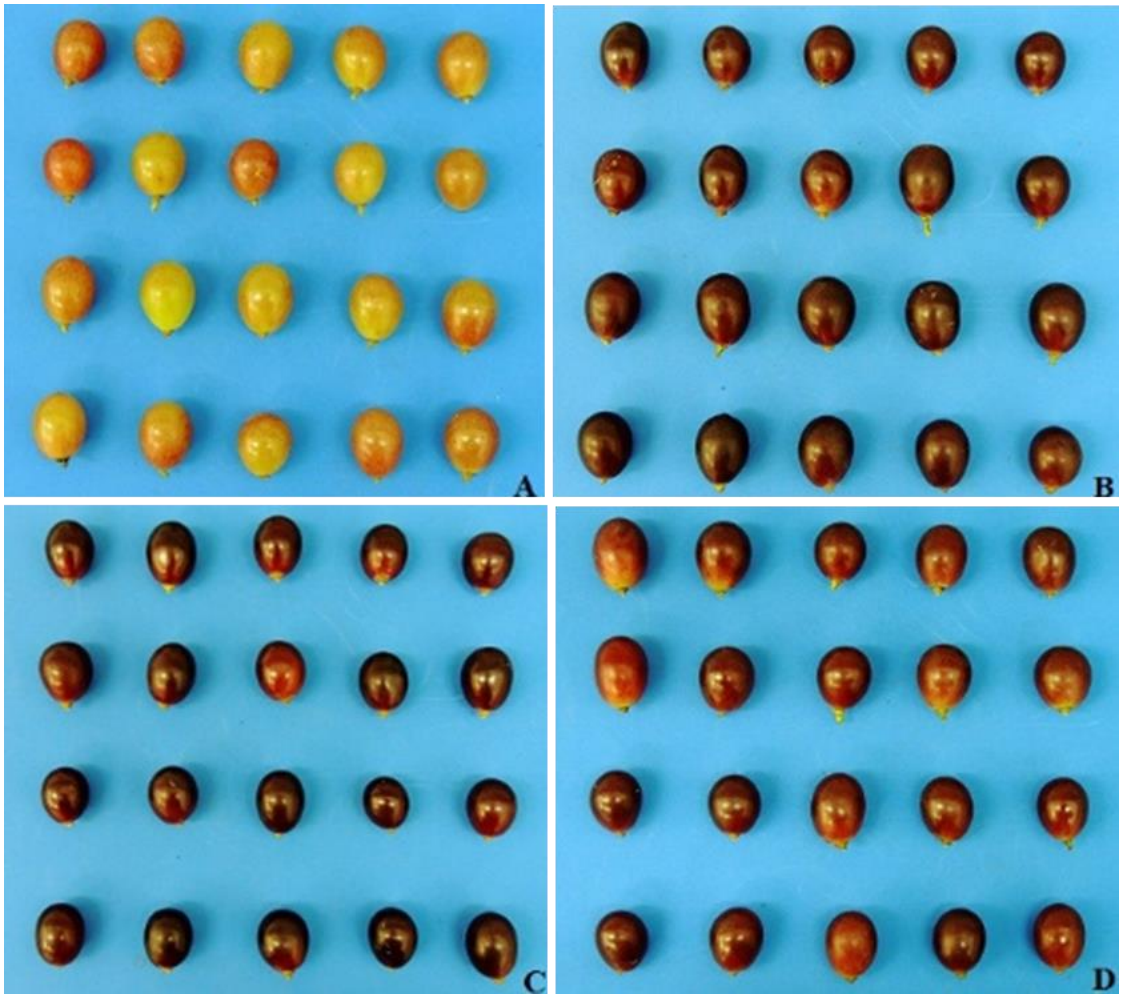


Figure 4.4.2.2. Berries of 'Benitaka' table grape subjected to different treatments with (*S*)-*cis*-abscisic acid (*S*-ABA 400 mg.L^{-1}). A: Control (no aplication); B: At pre-veraison; C: At veraison; D: At post-veraison. A second application of *S*-ABA 400 mg.L^{-1} was performed for all treatments 10 days after the first application, except for the control. Regular crop 2015.

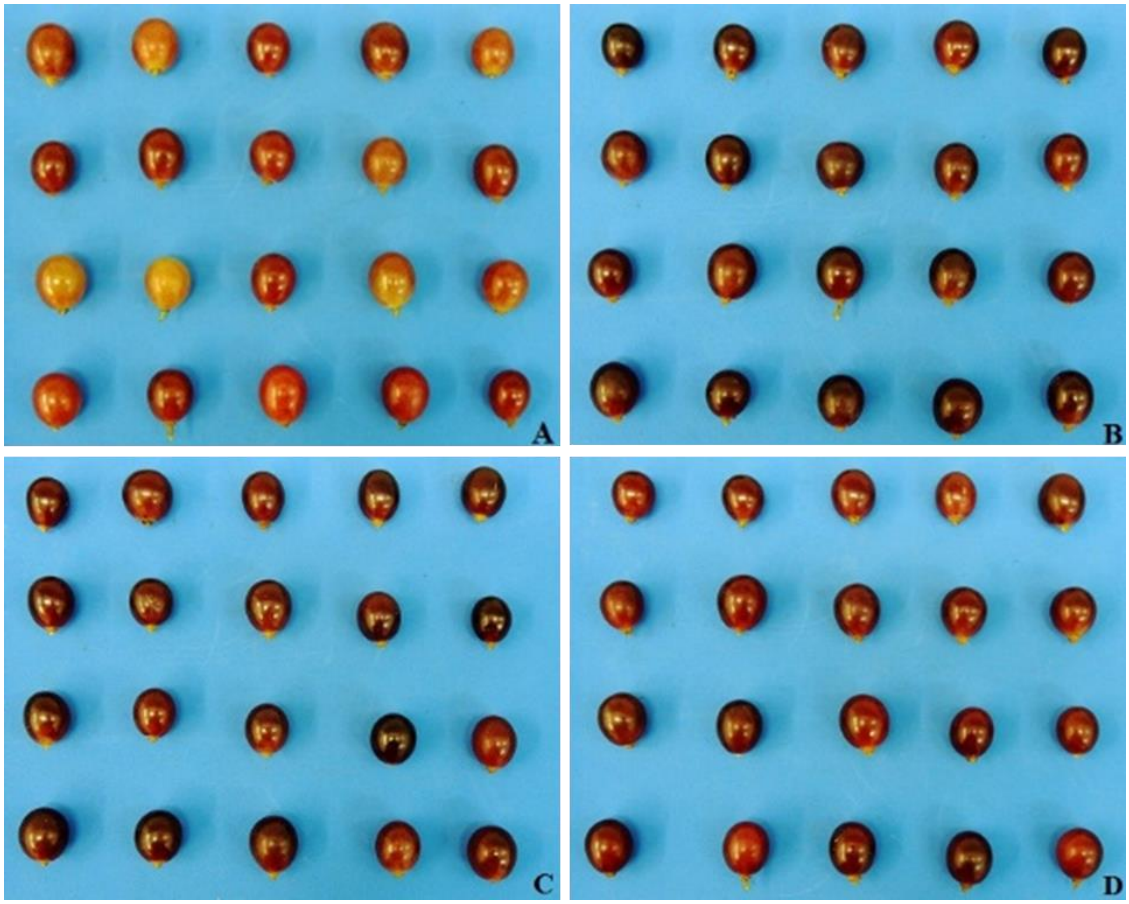


Figure 4.4.2.3. Berries of 'Benitaka' table grape subjected to different treatments with (*S*)-*cis*-abscisic acid (*S*-ABA 400 mg.L^{-1}). A: Control (no application); B: At pre-veraison; C: At veraison; D: At post-veraison. A second application of *S*-ABA 400 mg.L^{-1} was performed for all treatments 10 days after the first application, except for the control. Off-season crop 2016.

Regarding weekly rate of color development (Figures 4.4.2.4A and B), treated berries tend to develop color faster. It can also be observed that berries which produce high color during early stages tend to produce lower daily color development during later stages of berries ripening. Decrease in berry color at later stages may be attributed to the degradation of anthocyanins by glycosidases and peroxidases (GAGNE et al., 2006). During both seasons, the same pattern was observed, where the earlier *S*-ABA application (at PRV) showed a fast increase during early stages, but decreased during over time, and similarly after each application, the rate of color development increased in such manner. This behavior is related to the development of anthocyanins development in the early stages of ripening. Since berries produced fast accumulation of anthocyanins at early stages of berries ripening due to early veraison applications (at PRV and at V) (YAMAMOTO et al., 2015; TECCHIO et al., 2017), this is the reason why the color development was also fast during early maturation stages.

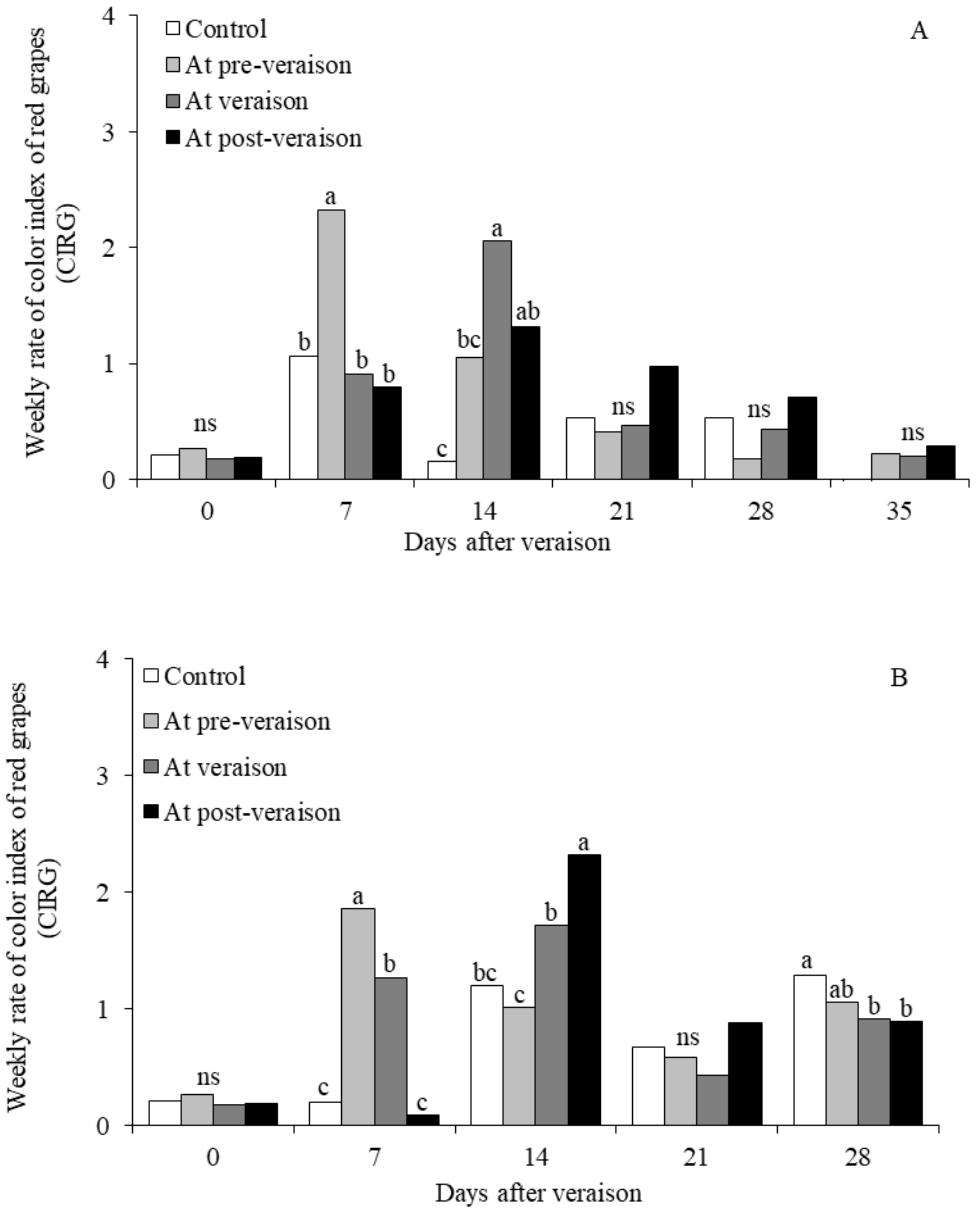


Figure 4.4.2.4. Weekly rate of color index for red grape (CIRG) of 'Benitaka' berries (*Vitis vinifera* L.) subjected to (*S*)-*cis*-abscisic acid (*S*-ABA 400 mg.L^{-1}) at different timings of veraison. A second application of *S*-ABA 400 mg.L^{-1} was performed for all treatments 10 days after the first application, except for the control. A: Regular crop 2015; B: Off-season crop 2016. Means within columns for the same letter followed by different letters differ significantly by Tukey's test (p<0.05). ns: non-significant.

4.4.3 Total soluble solids (TSS), titratable acidity (TA) and index of maturation (TSS/TA)

Regression analysis show that TSS contents of ‘Benitaka’ berries (Figures 4.4.3.1A and B) developed with no effect from the *S*-ABA application. TSS development through the course of ripening showed a linear regression with days of berries ripening (Tables 4.4.3.1 and 4.4.3.2). All of the samples reached maximum TSS around 14 °Brix during both seasons with no influence from the use of the plant growth regulator. The berries showed a predictable pattern of TSS improvement during both season. No effect of *S*-ABA was observed over the TSS contents of ‘Crimson Seedless’ grapes, which ranged from 14 to 15 °Brix (FERRARA et al., 2015). The TSS of grape is not usually influenced by the use of *S*-ABA (PEPPI; FIDELIBUS; DOKOOZLIAN, 2007a; FERRARA et al., 2013), but rather on the environmental condition and cultural practices (KELLER, 2015). As sugar is the dominant component (90%) of TSS (KELLER, 2015) its accumulation is mostly dependent on photosynthesizing leaves and woody storage parts (REBUCCI et al., 1997) rather than use of *S*-ABA, and that is why a linear behavior was observed in TSS development of ‘Benitaka’ berries.

Regression analysis of TA showed a negative polynomial behavior through the course of berries ripening (Figures 4.4.3.2A and B) in both seasons. Like TSS, TA was also not influenced by the use of *S*-ABA exogenous application to the berries. Application of *S*-ABA at different concentrations around veraison did not alter the chemical characteristics of ‘Sovereign Coronation’ grape berries (REYNOLDS et al., 2016). The TA of ‘Crimson Seedless’ decreased during the course of berry ripening but with no influence from the *S*-ABA treatments (FERRARA et al., 2015). The same can be observed for the maturation index of ‘Benitaka’ berries, where the regression analysis showed a positive polynomial regression as the berries matured (Figures 4.4.3.3A and B).

Contrary to TA, maturation index increased in the same gradual manner that TA decreased, and with high maturation index at harvest of ‘Monastrell’ (RUIZ-GARCÍA et al., 2013) as well as ‘Chambourcin’ grapes (ZHANG; DAMI, 2012), where no effect of ABA application was found on the physicochemical properties of the berries. Several factors can influence the development of these variables and the results can vary according to the cultivar and the environmental conditions in the bunch ripening (JACKSON, 2008). In berries of ‘Flame Seedless’, application of exogenous *S*-ABA reduced the TA of berries (PEPPI; WALKER; FIDELIBUS, 2008).

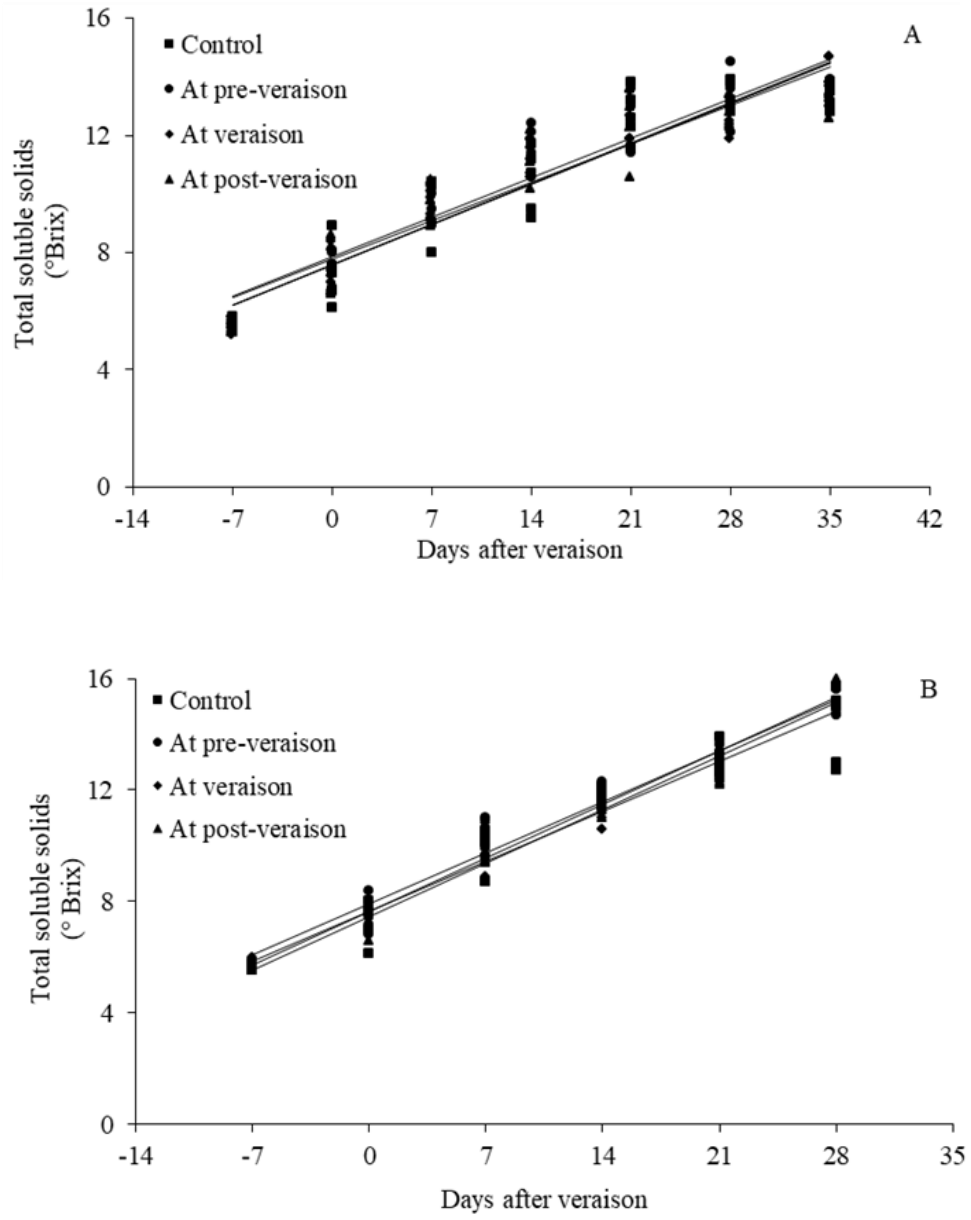


Figure 4.4.3.1. Evolution of total soluble solids - TSS (°Brix) of 'Benitaka' berries (*Vitis vinifera* L.) subjected to (*S*)-*cis*-abscisic acid (*S*-ABA 400 mg.L^{-1}) at different timings of veraison. A second application of *S*-ABA 400 mg.L^{-1} was performed for all treatments10 days after the first application, except for the control. A: Regular crop 2015; B: Off-season crop 2016.

Table 4.4.3.1. Regression equations for total soluble solids (TSS), titratable acity (TA) and TSS/AT evolution of 'Benitaka' berries (*Vitis vinifera* L.) subjected to (*S*)-*cis*-abscisic acid (*S*-ABA 400 mg.L^{-1}) at different timings of veraison. Regular crop 2015.

Variable	Treatment	Equation	R^2	*p*-value
TSS	Control	0.1971x + 7.57	0.8849	< 0.001
	At pre-veraison	0.1934x + 7.85	0.8888	< 0.001
	At veraison	0.1976x + 7.59	0.9276	< 0.001
	At post-veraison	0.1877x + 7.77	0.8726	< 0.001
TA	Control	$0.0021x^2$ - 0.1295x + 2.7473	0.9774	< 0.001
	At pre-veraison	$0.0023x^2$ - 0.1368x + 2.6083	0.9909	< 0.001
	At veraison	$0.0019x^2$ - 0.1298x + 2.7745	0.9910	< 0.001
	At post-veraison	0.0021x2 - 0.1316x + 2.7223	0.9872	< 0.001
TSS/TA	Control	$0.0038x^2$ + 0.3284x + 3.1165	0.9400	< 0.001
	At pre-veraison	$0.0063x^2$ + 0.4276x + 3.4882	0.9580	< 0.001
	At veraison	$0.0124x^2$ + 0.2491x + 2.6589	0.9425	< 0.001
	At post-veraison	$0.0044x^2$ + 0.3600x + 3.2644	0.9688	< 0.001

Table 4.4.3.2. Regression equations for total soluble solids (TSS), titratable acity (TA) and TSS/AT evolution of 'Benitaka' berries (*Vitis vinifera* L.) subjected to (*S*)-*cis*-abscisic acid (*S*-ABA 400 $mg.L^{-1}$) at different timings of veraison. Off-season crop 2016.

Variable	Treatment	Equation	R^2	*p*-value
TSS	Control	$0.2570x + 7.65$	0.9369	< 0.001
	At pre-veraison	$0.2625x + 7.89$	0.9709	< 0.001
	At veraison	$0.2763x + 7.62$	0.9588	< 0.001
	At post-veraison	$0.2742x + 7.46$	0.9811	< 0.001
TA	Control	$0.0002x^2 - 0.1319x + 2.7694$	0.9886	< 0.001
	At pre-veraison	$0.0028x^2 - 0.1477x + 2.6334$	0.9830	< 0.001
	At veraison	$0.0029x^2 - 0.1523x + 2.6783$	0.9865	< 0.001
	At post-veraison	$0.0023x^2 - 0.1392x + 2.7596$	0.9851	< 0.001
TSS/TA	Control	$0.0141x^2 + 0.2715x + 2.6601$	0.9640	< 0.001
	At pre-veraison	$0.0112x^2 + 0.3782x + 3.3297$	0.9857	< 0.001
	At veraison	$0.0107x^2 + 0.3651x + 3.1775$	0.9843	< 0.001
	At post-veraison	$0.0131x^2 + 0.3023x + 2.7419$	0.9936	< 0.001

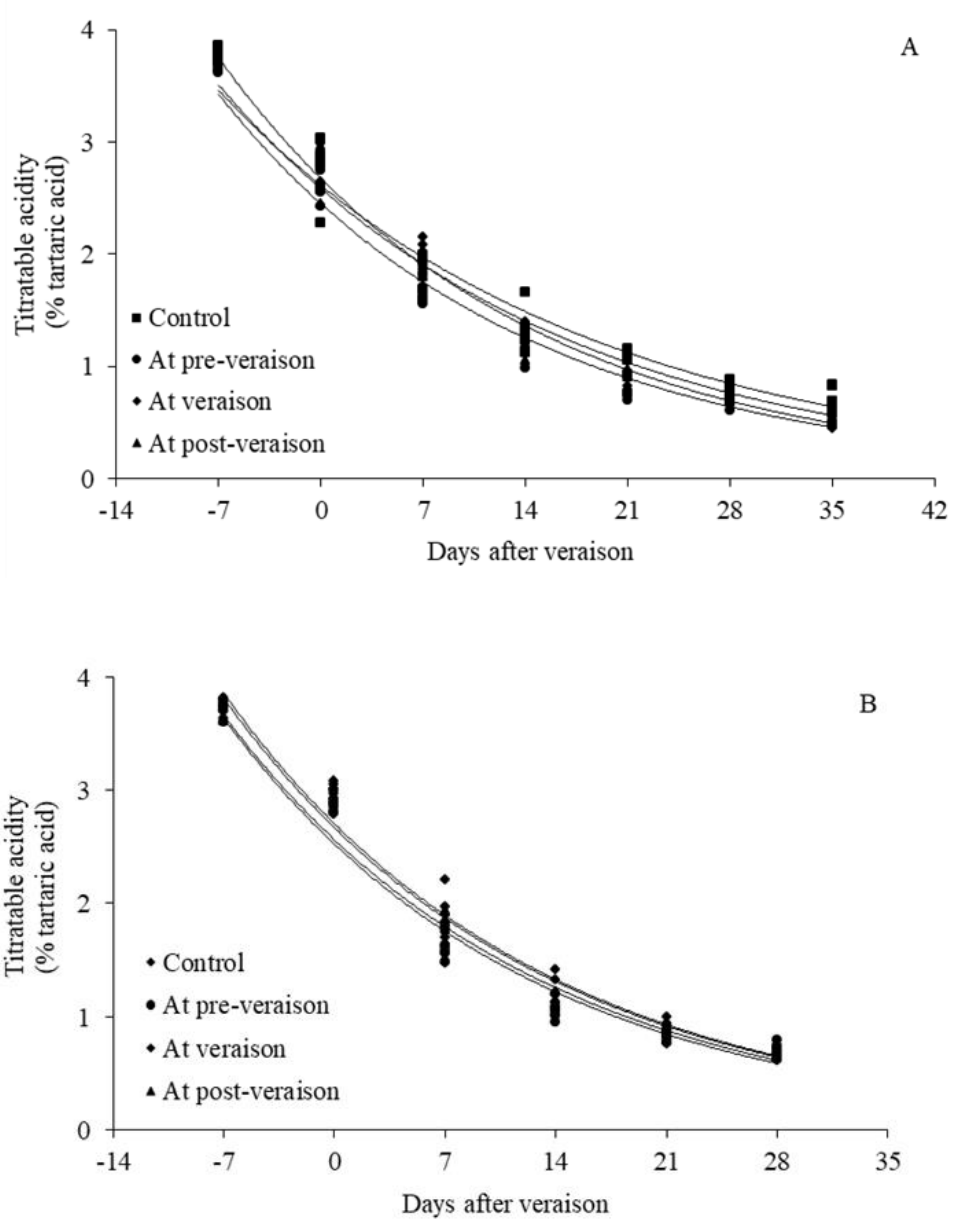


Figure 4.4.3.2. Evolution of titratable acidity - TA (% of tartaric acid) of 'Benitaka' berries (*Vitis vinifera* L.) subjected to (*S*)-*cis*-abscisic acid (*S*-ABA 400 mg.L^{-1}) at different timings of veraison. A second application of *S*-ABA 400 mg.L^{-1} was performed for all treatments 10 days after the first application, except for the control. A: Regular crop 2015; B: Off-season crop 2016.

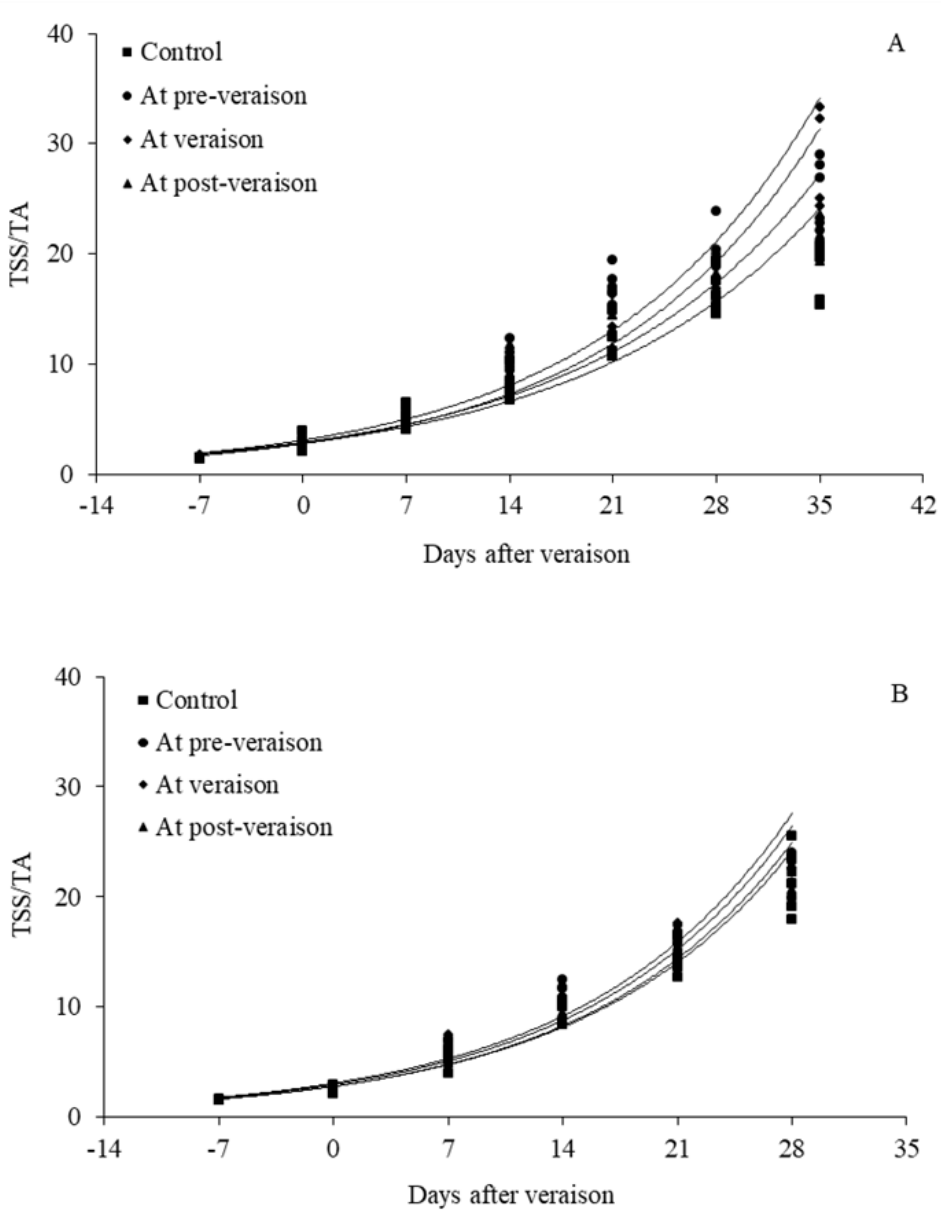


Figure 4.4.3.3. Evolution of index of maturation **(**TSS/TA) of 'Benitaka' berries (*Vitis vinifera* L.) subjected to (*S*)-*cis*-abscisic acid (*S*-ABA 400 mg.L^{-1}) at different timings of veraison. A second application of *S*-ABA 400 mg.L^{-1} was performed for all treatments 10 days after the first application, except for the control. A: Regular crop 2015; B: Off-season crop 2016.

This work was focused on investigating the anthocyanin as well color development during the course of berry ripening, in response to *S*-ABA treatments at different timings of veraison. The study also focused on new aspects of anthocyanin and color development such as weekly rate of anthocyanin accumulation and weekly rate of CIRG, which have never been explored before. It has been observed that *S*-ABA significantly improved the color development and anthocyanin accumulation in 'Benitaka' table grapes. Early application of the regulator produced faster anthocyanin accumulation during early weeks of veraison, which stabilized at the time of harvest.

During 2016 season, this behavior slightly differed but the role of *S*-ABA in development of anthocyanin and color development was the same in both cases. Daily rates of anthocyanin accumulation and CIRG followed the similar pattern. These analyses implicate that after the application of *S*-ABA, the rate of daily anthocyanin peaked in response to the treatments, whereas control treatment did not show any such response. A second application of *S*-ABA provided the anthocyanin accumulation over time, as well as the color development of the 'Benitaka' grape. The berries response towards *S*-ABA clearly showed that multiple applications of *S*-ABA are necessary to get such effects. The weekly rate of color and anthocyanin development gives us an idea how much anthocyanin accumulates in berries skin on daily basis and helps us understand more the behavior of anthocyanin accumulation and its effect on color development of berries skin. Similar to total anthocyanin accumulation and CIRG, the weekly rates of both variables also varied slightly between two seasons, but this difference can be more attributed to the climate rather than the use of regulator itself where a slight change in weather and climate can cause a significant difference in observations (KELLER, 2015). The regression analysis of physicochemical properties i.e. TSS, TA and maturation index reveals that these variables depends on the natural phenology of the vine, as well as other factors including environment, genotype, cultural practice etc. However, there has no influence observed from the use of *S*-ABA on these variables, which followed a predictable pattern that is usually followed by this cultivar in same environmental conditions.

5. CONCLUSIONS

During both regular and off-season crops, treating ‘Benitaka’ grape berries pre or at veraison with *S*-ABA can significantly improve the weekly and daily rate of anthocyanin accumulation as well as color development of the berries.

Other chemical properties of grapes, i.e., TSS, TA and TSS/AT, are not affected by the use of *S*-ABA, and follow a predictable pattern in relation to days of berries ripening.

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