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Anthocyanin Levels and Expression Analysis of Biosynthesis-related Genes during Ripening of Sicilian and International Grape Berries Subjected to Leaf Removal and Water Deficit

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ABSTRACT

The imposition of managed water deficit and early leaf removal are strategies used to improve the grapes quality in terms of anthocyanin content. The aim of our work was to evaluate the change in total anthocyanin levels during the ripening of the Sicilian grapes (Nero d'Avola and Frappato) and of the international variety of Cabernet Sauvignon, subjected to two different levels of water deficit, 0% (NI) and 30% (I) of estimated crop evapotranspiration, and subjected to Early Leaf Removal (ELR) or Not (NLR). The expression of genes involved in anthocyanin biosynthesis, such as Phenylalanine Ammonia Lyase (PAL) and UDP-glucose-Flavonoid-Glucosyl Transferase (UFGT), was also monitored. Our results indicate that the amount of anthocyanin during the ripening process can be regulated by the application of the aforementioned agronomic practises. The anthocyanin content of Cabernet Sauvignon may be risen either by the simultaneous application of Early Leaf Removal and in water restitution regime (ELR-I) or by the association of water deficit and absence of defoliation (NLR-NI). The analysis of the total content of anthocyanin in Frappato variety has globally revealed that the maximum value in the levels of pigments is reached later than in the other autochthonous Nero d'Avola variety. This finding is of considerable interest since both the harvest time and product processing might be differentiated among varieties. Conversely, the variety Nero d'Avola was not affected by the experimental conditions and showed the highest level of pigments at fully ripe time.

Keywords: Anthocyanin, Early leaf removal, PAL, UFGT, *Vitis vinifera*, Water deficit.

INTRODUCTION

Anthocyanin pigments are present in the berry skin of grapevine as well as in wines fermented in the presence of red skins (Castellarin *et al.*, 2007a). The colour of wine is an essential feature for producing fine red wines. The first sensory contact with the wine is represented by the visual inspection that enables the establishment of an immediate judgment by the consumers about the quality of the product (Castellarin *et al.*, 2007a). The colour and flavour of red wines are closely related to their contents of anthocyanins, flavonols, and proanthocyanidins, substances generally referred to as polyphenols, which give the wines also significant health properties (Flamini *et al.*, 2013). Towards the end of the 20th century, epidemiological studies and associated meta-analyses strongly suggested that diets rich in plant polyphenol offer some protection against development of cancers, cardiovascular diseases, diabetes,

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osteoporosis and neurodegenerative diseases (Graf *et al.*, 2005). Among them, anthocyanins are the subject of increasing scientific interest because of their possible beneficial effects on human health (Mushtaq and Wani, 2013). Among all types of fruits, grapes and their derivatives are particularly rich in bioavailable anthocyanins. Although the basic aspects of their absorption and metabolism are not fully established (Kay, 2006¸ Fernandes *et al.*, 2014), it has been reported that they are easily absorbed in the intestine as they are (Bitsch *et al.*, 2004) and delivered to the brain within a few minutes from their ingestion (Passamonti *et al.*, 2005). Anthocyanins in grape are synthesized through a complex metabolic pathway at the beginning of berry ripening (Braidot *et al.*, 2008). The total amount of anthocyanins and the relative abundance of each type of anthocyanin are extremely variable among red and purple peel cultivars. Both traits, the total content and pigment composition, are strictly subjected to genetic control and are related to the development stages of the berry (Chaves *et al.*, 2010). Water stress is considered the main environmental factor limiting crop growth and yield, including grape in Mediterranean areas (Ghaderi et al., 2011). However, water deficit might represent an environmental factor on which it is possible to intervene in order to increase the anthocyanin accumulation. The analysis of the expression profile revealed that the increase in anthocyanin levels results from earlier and sharper expression of the genes controlling the flux through the anthocyanin biosynthetic pathway (Castellarin *et al.*, 2007b). The major anthocyanins synthesized in the berries under water deficit are peonidin 3-O-β-glucoside and malvidin 3-O-β-glucoside, because methoxylation of delphinidin rarely occurs to produce its derivate petunidin (Chaves *et al.*, 2010). Therefore, water stress seems to have a strong impact on the anthocyanin composition rather than on their total content. In addition, for the international varieties, it has not yet been clarified whether the increase of the anthocyanin content is simply due to the inhibition of berry growth, or to the concentration of solutes following the loss of water, or whether the water deficit directly induces the biosynthesis of all the phenolic substances. The few existing studies were carried out on "Cabernet Sauvignon" (Castellarin *et al.*, 2007a) and "Merlot" varieties (Bucchetti *et al.*, 2011) and suggest that the water deficit promotes fruit ripening and activates transcription of genes involved in the biosynthesis of anthocyanins. In our previous work, the effects on grape yield and quality of both managed water stress and early season partial defoliation were evaluated at fully ripe time in two autochthonous Sicilian grapes, Nero d'Avola and Frappato, and in an international grape variety, Cabernet Sauvignon. The results confirmed the effectiveness of early leaf removal in yield management that leads to smaller clusters (Nicolosi *et al.*, 2012; Ferlito *et al.*, 2014). Managed water stress reduces berry size and generally enhances anthocyanin accumulation (Ferlito *et al.*, 2014). The purpose of our research was to evaluate the change in levels of total anthocyanins during the development and ripening of the autochthonous Sicilian grapes Nero d'Avola and Frappato subjected to two different levels of water deficit (0 and 30% of estimated crop evapotranspiration) and subjected or not to early leaf removal. Furthermore, the content of these pigments was correlated with the expression of genes involved in the biosynthesis of anthocyanins. In particular, the expression of genes located both in the initial part of the metabolic pathway, such as phenylalanine ammonia lyase, and in the last reaction of anthocyanin biosynthesis, UDP-glucose-flavonoid-glucosyl transferase, was monitored. Along with the autochthonous varieties, the accumulation of anthocyanins and gene expression analysis were also evaluated in the international grape Cabernet Sauvignon grown under the same conditions.

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MATERIALS AND METHODS

Site Description

The experiment was conducted in 2011 in a commercial *Vitis vinifera* L. vineyard located in the Ragusa district of Sicily (lat. 37° 01' 32" N; long. 14° 32' 50" W; elevation 220 m) bordered on all sides by other vineyards. The vines were planted in 2001 on a deep sandy soil with the following varieties: Frappato, Nero d'Avola, and Cabernet Sauvignon grafted onto 140 Ru. Rootstock. The vines were planted at a density of 4,444 vines per hectare (spaced 0.9 m in the row and 2.5 m between rows). The row direction was E-W. Vines were trained using a unilateral cordon system at a height of 0.5 m with the top of the canopy at approximately 1.60 m. Vines were spur-pruned to three to five nodes per vine, with two nodes per spur. All shoots derived from bourillon and adventitious buds were hand-pruned to retain six to ten shoots per vine. The shoots were vertically positioned and were not hedged during the growing season.

The experimental field was provided with irrigation system. The canopy and soil management practices were all mechanically performed, and standard cultural practices in the Mediterranean area were applied to all of the treatments (Ferlito *et al.*, 2014).

Early Leaf Removal, Irrigation Treatments and Experimental Design

The treatments of 20 parcels of five vine plots consisted of: (i) No Leaf Removal and rain-fed, with No Irrigation (NLR-NI); (ii) No Leaf Removal and watered at 30% of estimated crop Evapotranspiration (ETc) (NLR-I); (iii) Early Leaf Removal and rain-fed, with No Irrigation (ELR-NI), and (iv) Early Leaf Removal and watered at 30% of estimated *ETc* (ELR-I). The treatment plots were arranged in a completely randomized design. Leaves were removed by hand from the cordon up to the leaf of the last cluster per shoot. *ETc* was measured every 15 days. Crop evapotranspiration was estimated as a product of *ETc*, calculated by the Penman-Monteith equation, and crop coefficient (Kc) (Allen *et al.*, 1998). *Kc* values used were 0.30 from flowering to veraison and 0.15 from veraison to harvest (Intrigliolo and Castel, 2009). Each irrigated treatment was equipped with timing-valve assembly to control water delivery. Irrigation was applied every 15 days and started on July 1st. Water applications ended at the end of August, at the beginning of commercial maturity (85 of BBCH scale) (Lorenz *et al.*, 1994, Intrigliolo and Castel, 2009).

Sampling of the berries started at 33 DAA (Days After Anthesis) (81 of BBCH scale) and continued with fortnightly up to 108 DAA (89 of BBCH scale) (Lorenz *et al.*, 1994). Freshly harvested berries were washed with distilled water, gently dried with paper towels, and then berry skins were obtained by manually peeling with a scalpel, as reported in Zietsman *et al.* (2015). Samples were immediately frozen with liquid nitrogen at the time of collection and then stored at -80°C until being processed. Samples were split to obtain an aliquot for RNA extraction and an aliquot for anthocyanin extraction. Three biological replicates of five berries were collected at each stage and each replicate was collected by random sampling from five plants.

RNA Isolation and cDNA Synthesis

Total RNA was extracted from 0.3 g of berry skin following the procedure described in Iandolino *et al.* (2004) and treated with 0.5 U g^{-1} RQ1 DNase (Promega). RNAs were routinely quantified using RNA Quant-it assay kit (Molecular Probes, Carlsbad, CA, USA) and the quality of total RNA was estimated by electrophoresis in 1.1% formaldehyde-agarose gels containing 0.5 μg mL^{-1} GelRed (Biotium, CA), loading 1 μg of each sample (Lo Piero *et al.,* 2014). Reverse transcription was achieved by using 2 μg of RNAs and the AMV Reverse Transcriptase (BD-clontech, USA). The

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synthesis of the second-strand was carried out using T4 DNA Polymerase (BD-clontech, USA) as described in Lo Piero *et al.* (2010).

Measurement of PAL and UFGT Expression by Real-Time Quantitative RT-PCR

Real-Time PCR was performed using the SYBR® Green JumpStart™ Taq ReadyMix (Sigma, USA) in a Smart Cycler II (Cepheid, Sunnyvale, CA, USA). PAL was monitored using the primer pair (For: TGGCAGCACCTCAATCTTC; Rev: TTTCCACTCTCCACCCCATC) described in Sparvoli *et al.* (1994) which amplifies the grape PAL deposited in NCBI under the Accession Number X75967. UFGT was monitored using the primer pair (For: AACTCATTGTGGGAAAGCG; Rev: TCACTCCAATCTCCAAAACATC) described in Sparvoli *et al.* (1994) which amplifies the grape UFGT deposited in NCBI under the Accession Number X75968. The relative quantitation of gene expression between the samples was calculated using the Comparative Threshold (CT) method (Heid *et al.*, 1996). The housekeeping gene Elongation Factor 1-alpha (EF-1-alpha), was retrieved from the literature (Faccioli *et al.*, 2007) and the primers used were: For: ATGATTCCCACCAAGCCCAT and Rev: ACACCAACAGCCACAGTTTGC (Faccioli *et al.*, 2007). This value was calculated for each sample, and then, the comparative expression level of the single genes was given by the formula $2^{-\Delta\Delta CT}$ where $\Delta\Delta CT$ was calculated by subtracting the baseline's ΔCT from the sample's ΔCT and where the baseline represents the expression level of the first sampling (33 DAA). Three independent triplicates of quantitative PCR experiments were performed for each gene to generate an average *CT* and to calculate standard deviation. The dynamic range of PAL, UFGT and the housekeeping gene was determined by monitoring the variation of ΔCT with template dilution; the efficiency was found to be very similar for each pair of primers. To each triplicate, 5 ng of cDNA was added to a final volume of 25 µl with a final concentration of 1X Platinum two-step qRT-PCR master mix, 100 nM per primer. Thermal cycling conditions were 95°C for 10 minutes followed by 95°C for 30 seconds, 58°C for 30 seconds, and 65°C for 60 seconds for 40 cycles. Each cDNA sample was run in duplicate. Negative controls without reverse transcriptase were routinely included. The experiment was repeated at least three times on independently isolated RNA preparations.

Total Anthocyanins Content

Anthocyanin determination was performed by pH-differential spectrophotometry according to a slight modification of the method described in Rapisarda *et al.* (2000) as slightly modified in Crifò *et al.* (2012). Briefly, aliquots (1 g) of berry skin were frozen in liquid nitrogen, powdered by mortar and pestle, and successively extracted with 1 ml of water by vigorous shaking for 1 hour at 4 °C. Samples were centrifuged at 12,000×g 20 minutes, then the supernatant was recovered and analysed for anthocyanin content by dissolving aliquots of fresh berry skin extract respectively in buffer 1 (55 mM KCl, 0.145N HCl pH 1.0) and buffer 2 (0.2 M CH_3COONa pH 4.5) and measuring the absorbance of samples both at 510 and 700 nm. Anthocyanin content was calculated by the following formula: $p/p = [(\Delta Abs/\varepsilon \times L) \times MW \times DF \times (V/W_t)] \times 100\%$, where: $\Delta Abs = (Abs_{510\ nm}\ pH\ 1.0 - Abs_{700\ nm}\ pH\ 1.0) - (Abs_{510\ nm}\ pH\ 4.5 - Abs_{700\ nm}\ pH\ 4.5)$; ε= Malvidin-3-O-glucoside molar absorbance coefficient (28,000); *MW*= Malvidin-3-O-glucoside molecular Weight (493.43), *DF*= Dilution Factor, *V*= Final Volume (ml), W_t= Sample Weight (mg), *L*= Cell path Length (usually 1 cm).

Statistical Analysis

The results are presented as mean value of three replicates and sample variability was

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calculated using standard deviation. Statistical analysis was performed with SPSS software package (SPSS, Chicago, Illinois, USA). Statistical differences between 33 DAA samples and further sampling were analyzed by Student's T test.

RESULTS AND DISCUSSION

The purpose of this research was to evaluate both the change in levels of anthocyanins and the expression of genes involved in pigment biosynthesis during the ripening of grapes from Sicilian vineyards subjected to different levels of water deficit (0 and 30% of estimated crop evapotranspiration) as well as to early leaf removal. Along with two autochthonous varieties, Frappato and Nero d'Avola, the changes in anthocyanins and the levels of gene expression were also evaluated in the international grapes Cabernet Sauvignon, grown under the same conditions of native grapes. Figure 1 shows the results related to the international variety Cabernet Sauvignon. In the case of samples subjected to the restitution of 30% of evapo-transpired (NLR-I), the maximum levels of anthocyanin were reported at 78 DAA, and then they decreased steadily until the end of the experimental period. The samples subjected to 0% of water restitution (NLR-NI) show instead, a peak in the level of anthocyanin pigments at 93 DAA. Furthermore, at this stage the samples subjected to 0% of water restitution (NLR-NI) reach levels of anthocyanin significantly higher (450 mg 100 g^{-1}) than those of samples subjected to water restitution (NLR-I) (100 mg 100 g^{-1}). Therefore, the state of water deficit induces both a forward reposition of about 15 days of the maximum levels of pigments and an increase in their contents. It is interesting that in both experimental conditions (NLR-I and NLR-NI) there is a marked decrease of anthocyanin pigments at 108 DAA, which suggests that delayed grape harvest time negatively affects the amount of berry pigments, likely because of the pigment's degradation. These results are in accordance to those obtained by Castellarin *et al.* (2007a) which showed that the water deficit promotes higher concentration of anthocyanin in the Cabernet Sauvignon red wine grapes. In Figure 1, the anthocyanin levels of Cabernet Sauvignon subjected to

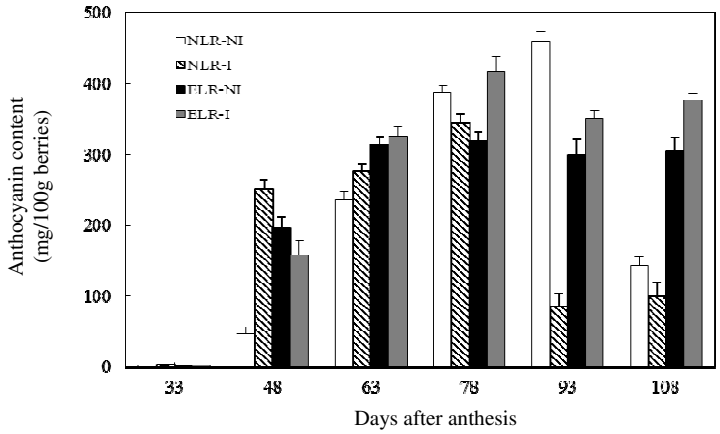

Figure 1. Total anthocyanin content during development and ripening of the Cabernet Sauvignon grape subjected to two regimes of water supply: 0% (NI) and 30% (I) of estimated crop evapotranspiration, in samples Not subjected to Leaf Removal (NLR); 0% (NI) and 30% (I) of estimated crop evapotranspiration, in samples subjected to Early Leaf Removal (ELR). Data are mean±SD (n= 3).

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Early Leaf Removal (ELR) are also shown. In both thesis, 0% (ELR-NI) and 30% (ELR-I) of water restitution, the level of pigments increases and reaches a plateau level (Figure 1). In samples subjected to water restitution (ELR-I), the levels of anthocyanin reached a peak at 78 DAA and they are slightly higher than those of samples subjected to water deficit (ELR-NI) (Figure 1), as well as higher than those of samples Not subjected to early Leaf Removal (NLR-I) (Figure 1). The decrease of anthocyanin recorded at the end of the experimental period is less drastic than that observed in NLR samples. The effects of defoliation upon pigment content are more evident on samples not subjected to water restitution (NI). In fact, they reach a peak at 63 DAA in ELR samples (Figure 1), long before the not defoliated samples (NLR). Moreover, the content of anthocyanin of ELR-NI samples at 63 DAA is 320 mg 100 g^{-1}, suggesting that water deficit associated with the early leaf removal induces an anticipation of the maturation process, but adversely affects the total levels of pigments of the berries. The expression profiles in Cabernet Sauvignon grapes of PAL (Phenylalanine Ammonia Lyase) and UFGT (UDP-glucose Flavonoid GlucosylTransferase), respectively the first enzyme of phenylpropanoid pathway and the last enzyme in the biosynthesis of anthocyanin, are shown in Figure 2. PAL is expressed at almost constant levels in all the samples. However, consistent with anthocyanin levels, in the samples Not subjected to early Leaf Removal and subjected to water deficit (NLR-NI), a slight enhancement of the PAL expression was recorded (Figure 2). Similarly, the water deficit in the samples Not subjected to Leaf Removal (NLR-NI) strongly enhances UFGT gene expression reaching a peak (3 fold higher) at 93 DAA (Figure 2) along with the increase of anthocyanin content (Figure 1). On the contrary, UFGT is expressed at almost constant levels in all the other samples. These expression profiles in grapes Cabernet Sauvignon are in accordance to those reported by Castellarin *et al.* (2007a) showing that the expression of most structural genes of the anthocyanin biosynthesis are induced by water deficit.

Figure 3 shows the results related to the autochthonous variety Frappato. The amount of the anthocyanin levels in the berries of the samples subjected to water restitution (NLR-I) shows a peak at 78 DAA (308 mg 100 g^{-1}). The samples subjected to water deficit (NLR-NI) show an increasing content of anthocyanin during the entire experimental period and reach a peak at 93 DAA, this value (508 mg 100 g^{-1}) being almost two times higher than that registered in the samples subjected to water restitution (NLR-I). The different anthocyanin contents between the samples subjected and not subjected to water deficit (NI and I) are maintained in the samples harvested after 108 DAA. In the sample not subjected to water restitution (NLR-NI) the levels of anthocyanin (436 mg 100 g^{-1}) are about seven times higher than that recorded in the sample subjected to irrigation (NLR-I) (61 mg 100 g^{-1}). Figure 3 shows the trend of the total anthocyanin content in the samples subjected to early leaf removal at the two schemes of water supply (0 to 30%). Defoliation does not affect the anthocyanin contents in the samples not subjected to irrigation (ELR-NI) (Figure 3). In fact, they show an increasing content of anthocyanins reaching a peak at 93 DAA, in correspondence to which the content of the pigments (530 mg 100 g^{-1}) is similar to that of the samples not defoliated (NLR-NI) (Figure 3). On the contrary, in the defoliated and irrigated samples (ELR-I), the anthocyanin contents increase during the experimental period, reaching higher values (436 mg 100 g^{-1}) than those detected in the samples subjected to water restitution (335 mg 100 g^{-1}) at 108 DAA (Figure 3). Therefore, defoliation does not influence the total content of anthocyanin in the samples subjected to water deficit (NI). On the contrary, in the samples subjected to Early Leaf Removal and to water restitution (ELR-I), the content of anthocyanin increases during the experimental period, reaching at

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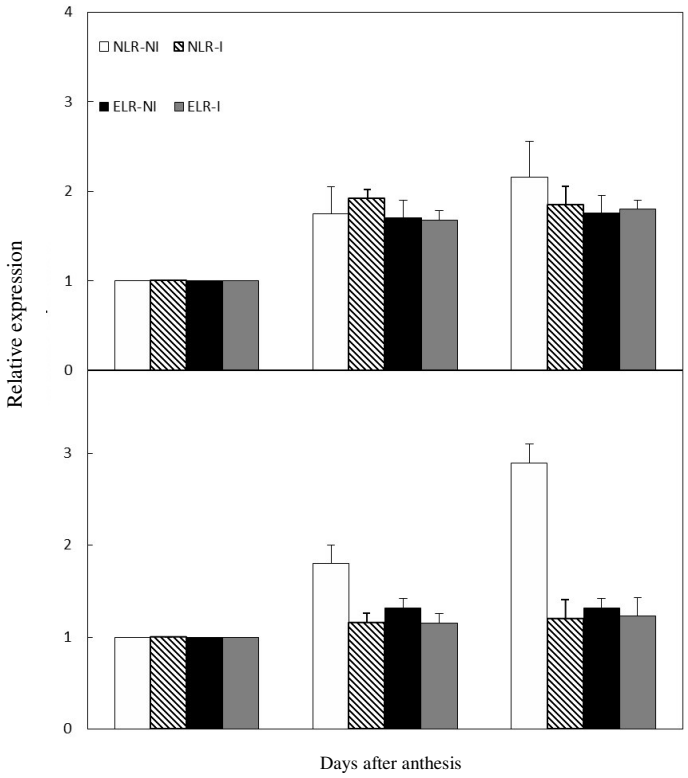

Figure 2. Expression pattern of anthocyanin biosynthetic genes in the Cabernet Sauvignon grape subjected to two regimes of water restitution, 0% (NI) to 30% (I) estimated crop evapotranspiration in samples subjected to (ELR) and Not subjected (NLR) to early Leaf Removal. The relative quantitation of genes expression was calculated by real time RT-PCR using the Comparative Threshold (CT) method, as described in the Materials and Methods. Data are mean±SD (n= 4). Asterisks depict significant differences at $P \leq 0.05$; (**a)** PAL (Phenylalanine Ammonia Lyase), (**b)** UFGT (UDP-glucose Flavonoid GlucosylTransferase).

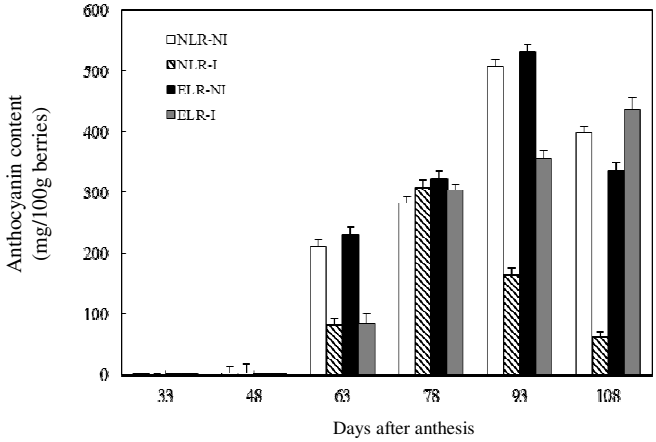

Figure 3. Total anthocyanin content during development and ripening of the Frappato grape subjected to two regimes of water restitution: 0% (NI) and 30% (I) estimated crop evapotranspiration, in samples Not subjected to Leaf Removal (NLR);a0% (NI) and 30% (I) estimated crop evapotranspiration, in samples subjected to Early Leaf Removal (ELR). Data are mean±SD (n= 3).

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108 DAA values higher than those measured in the samples Not subjected to early Leaf Removal (NLR-I). Consequently, leaf removal represents the agronomical practice that positively influences the total content of anthocyanins but only in the samples subjected to water restitution (I). As shown in Figure 4, the transcription of PAL and UFGT increases along the experimental period accordingly to the anthocyanin levels reaching their maximum values in both NLR-NI and ELR-NI at 93 DAA (Figure 4).

Figure 5 shows the data related to the native variety Nero d'Avola. The anthocyanin content of the theses subjected to the different water supply (NI and I) reaches similar values at 63 and 78 DAA (620 and 570 mg 100 g^{-1}), respectively. In the samples subjected to early leaf removal (Figure 5), the trends related to the anthocyanin content are very similar and both reach a maximum value at 63 DAA in the range of 620 and 658 mg 100 g^{-1}. In the Nero d'Avola autochthonous variety the different water restitution regime does not markedly affect the levels of anthocyanin accumulating in the fruits. The positive influence of early leaf removal on anthocyanin content is more pronounced than that attributable to the water deficit; in fact, starting from the 78 DAA, anthocyanin sharply decreases in the irrigated thesis (NLR-I). Finally, in Figure 6 the expression pattern of PAL and UFGT genes in the Nero d'Avola variety is shown. The results suggest that gene expression is strictly consistent with anthocyanin accumulation. In particular, PAL transcripts are strongly

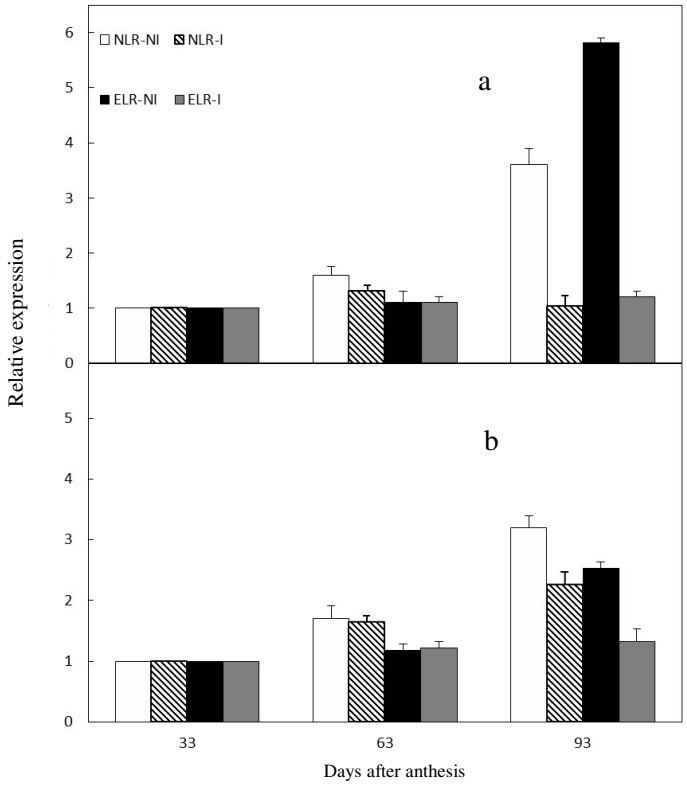

Figure 4. Expression pattern of anthocyanin biosynthetic genes in the Frappato grape subjected to two regimes of water restitution, 0% (NI) to 30% (I) in samples subjected to (ELR) and Not subjected (NLR) to early Leaf Removal. The relative quantitation of genes expression was calculated by real time RT-PCR using the Comparative Threshold (CT) method, as described in the Materials and Methods. Data are mean±SD (n= 3). Asterisks depict significant differences at $P \leq 0.05$; (**a**) PAL (Phenylalanine Ammonia Lyase), (**b**) UFGT (UDP-glucose Flavonoid GlucosylTransferase).

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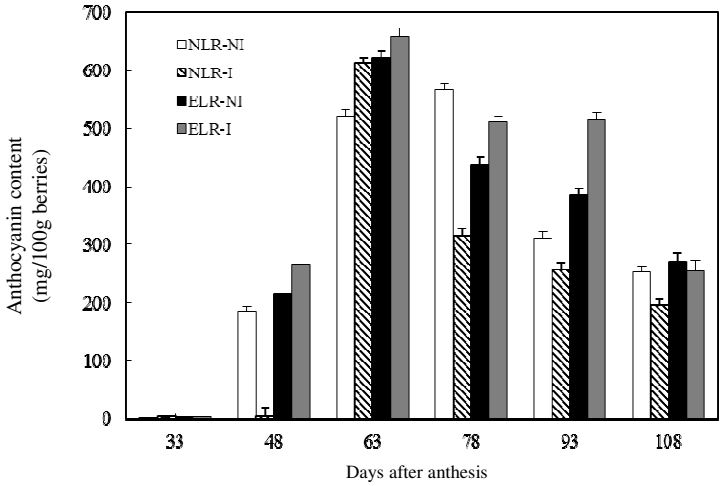

Figure 5. Total anthocyanin content during development and ripening of the Nero d'Avola grape subjected to two regimes of water restitution: 0% (NI) and 30% (I) estimated crop evapotranspiration, in samples Not subjected to Leaf Removal (NLR); 0% (NI) and 30% (I) estimated crop evapotranspiration, in samples subjected to Early Leaf Removal (ELR). Data are mean±SD (n= 3).

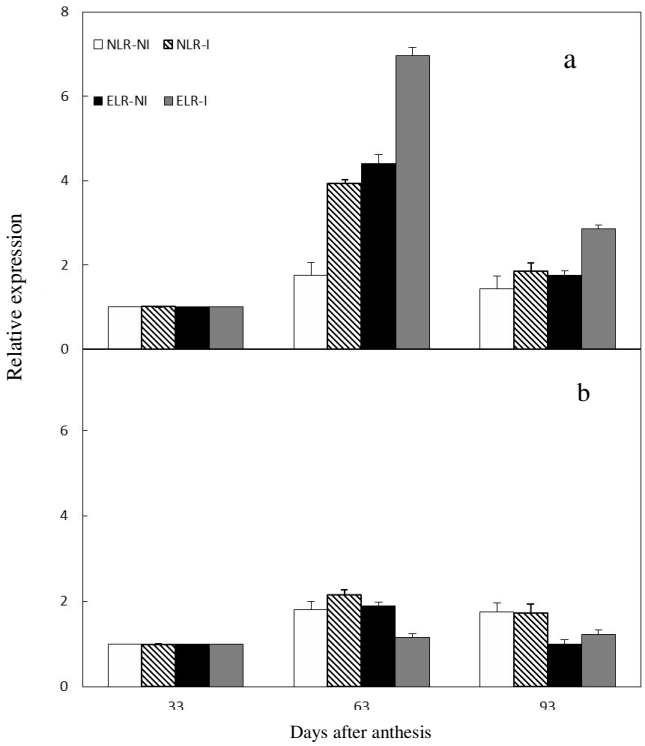

Figure 6. Expression pattern of anthocyanin biosynthetic genes in the Nero d'Avola grape subjected to two regimes of water restitution, 0% (NI) to 30% (I) in samples subjected to (ELR) and Not subjected (NLR) to early Leaf Removal. The relative quantitation of genes expression was calculated by real time RT-PCR using the Comparative Threshold (CT) method, as described in the Materials and Methods. Data are mean±SD (n= 4). Asterisks depict significant differences at $P \leq 0.05$; (**a**) PAL (Phenylalanine Ammonia Lyase), (**b**) UFGT (UDP-glucose Flavonoid GlucosylTransferase).

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induced at 63 DAA in the Nero d'Avola grape subjected to defoliation in both regimes of water supply (ELR-I and ELR-NI), reporting 7 and 4 times higher values than those registered at 33 DAA, respectively (Figure 6). Similarly, the samples Not subjected to Leaf Removal (NLR) show an induction of PAL mRNAs in both water supply conditions. Lastly, the UFGT is expressed in all analyzed samples showing insignificant differences among the theses.

CONCLUSIONS

In this work, we show that early leaf removal and water deficit might be used singularly or in association both to modify the amount of berry anthocyanin during the ripening process and to discriminate the harvest time among the varieties. Overall, our results show that the samples richer in anthocyanin pigments in the variety Cabernet Sauvignon may be arisen by the simultaneous application of water deficit and absence of defoliation (NLR-NI) or by the association of Early Leaf Removal and water restitution (ELR-I). In the Cabernet Sauvignon, the aforementioned protocols lead to similar levels of anthocyanin but at different times, after 78 DAA in the ELR-I samples and after 93 DAA in the NLR-NI samples, thus allowing to plan differentiated and flexible harvest periods. The analysis of the total content of anthocyanin in the variety Frappato has revealed that the increase in the levels of pigments is late compared to the Nero d'Avola variety. This finding is of considerable interest to the farmers so that they can differentiate either the harvest time or product processing in the attempt to optimize the process timing and the quality of the products. In the variety Frappato, the irrigation does not lead to the increase of pigment levels that are instead higher in conditions of water stress (NI), regardless of applying or not applying defoliation. The variety Nero d'Avola has not been affected by the various imposed experimental conditions, and shows the highest levels of pigments at fully ripe time. The maximum levels of anthocyanin are reached at 63 DAA, earlier than in the other varieties under investigation. Finally, it should be noted that a sharp decrease of the pigment content is detected after reaching the maximum peak at 63 DAA. This finding suggests not delaying the harvest time of Nero d'Avola variety beyond this period as it would adversely affect the quantities of berry pigments and, as a consequence the amount of anthocyanin of deriving musts and wines.

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بررسی سطوح آنتوسیانین و بیان ژن های مربوط به بیوسنتز در زمان رسیدن انگورهای سیسیلیایی و بین المللی تحت شرایط حذف برگ و کمبود آب

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چکیده

تحمیل شرایط کمبود آب مدیریت شده و حذف برگ، روشهایی هستند که برای بهبود بخشیدن به کیفیت انگورها در بخش محتوای آنتوسیانین به کار برده می شوند. هدف این تحقیق بررسی تغییرات سطح آنتوسیانین در کل در زمان رسیدن انگورهای سیسیلیایی (نرو دآوولا و فراپاتو Nero d'Avola and Frappato) از گونه ی بین المللی Cabernet Sauvignon است، که تحت شرایط دو سطح مختلف از کمبود آب، 0% (NI) و 30% (I) از تبخیر و تعرق محصول و همچنین شرایط حذف برگ (ELR) یا حذف نکردن برگ (NLR) قرار گرفتند. بیان ژن های دخیل در بیوسنتز آنتوسیانین، مانند Phenylalanine Ammonia Lyase (PAL) و UDP-glucose-Flavonoid-Glucosyl Transferase (UFGT) نیز مورد سنجش قرار گرفتند. نتایج حاکی از این است که میزان آنتوسیانین در پروسه ی رسیدن را می توان با استفاده از عملیات کشاورزی ذکر شده تنظیم کرد. میزان محتوای آنتوسیانین در انگور Cabernet Sauvignon ممکن است با کاربرد همزمان حذف برگ و رژیم بازگردانی آب (ELR-I) و یا با ترکیب کمبود آب و عدم حذف برگ ها (ELR-NI) افزایش پیدا کند. بررسی محتوای کلی آنتوسیانین در گونه ی فراپاتو به طور جهانی مشخص کرده است که در این گونه بیشترین میزان رنگدانه دیرتر از دیگر گونه های نرو دآوولا به دست خواهد آمد. این یافته بسیار مورد توجه بوده زیرا زمان حاصل دهی وتولید محصول ممکن است در بین گونه های مختلف متفاوت باشد. از طرفی دیگر گونه ی نرو دآوولا تحت تأثیر شرایط آزمایشگاهی قرار نگرفت و بالاترین سطوح رنگدانه را در زمان رسیدن کامل داشت.

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