The impact of early leaf removal on polyphenol/anthocyanin content and *in vitro* antioxidant potential of ‘Pinot Noir’ grapes from Vipava Valley

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Abstract

The impact of early leaf removal (at berry-set) on polyphenol and anthocyanin formation in grapes of Pinot Noir (*Vitis vinifera* L.) variety was investigated, aiming to improve their phenolic profile and colour characteristics for its later wine production. Therefore, the content of total phenol and total anthocyanin levels during grape maturation till the harvest time was monitored for both trial treatments of defoliated and non-defoliated (control) vines, and results were compared to an air/grapes surface temperature and precipitation data obtained. Phenolic compounds were analysed by HPLC-UV/VIS and Folin-Ciocalteu analysis, while extracts’ *in vitro* AOP characterization was based on scavenging of DPPH free radicals. Our results revealed a highly significant impact of early leaf removal on polyphenol/anthocyanin grape composition, where a much higher impact on total phenols content was observed in comparison to the one of anthocyanins. However, the highest concentrations were found in grapes sampled 9 days before the harvest time, where the levels of total phenol and anthocyanin concentrations reached the values of 1763 and 867 mg/kg, and hence increased their final grapes content for 24% and 25% in comparison to control, respectively. A similar trend was observed in the case of grapes *in vitro* antioxidant potential assessment, where the values of AOP have clearly correlated (*r* = 0.88) with the total phenol content, confirming the antioxidant activity of phenols present. Although both of them have later decreased by the time of harvest (22%), their final values were still significantly higher as compared to that of control (24%), suggesting the early leaf removal as successful tool for a phenol profile regulation of Pinot Noir grapes under conditions present.

Key words: early leaf removal, Pinot Noir, total phenols/anthocyanins, *in vitro* antioxidant (AOP) potential

Introduction

The wine colour is directly dependent on both quali- and quantitative profile of grape polyphenols such as anthocyanins, flavonols and hydroxycinnamic acids and/or on their enrolment into polymerization and co-pigmentation reactions (Tarara et al., 2008). While the latter is genetically defined by the cultivar itself, it can be modified/influenced also by the others *i.e.* environmental factors such as light, temperature, water stress and UV radiation exposure (Cortell et al., 2006).

Several viticulture practises have been already tested/proposed with an attempt to improve the polyphenol/anthocyanin grape composition, including leaf removal based on a canopy microclimate control, typically performed at veraison using manual or mechanical approaches (Poni et al., 2006). Although its application has been traditionally used for reducing grapes microbiological contaminations (*e.g.* *Botrytis cinerea*, Sour rot) and/or obtaining their better ripening characteristics, its potential at stage of berry set has not been yet fully exploited for improving the grapes polyphenol/anthocyanin profiles and hence their overall antioxidant (AOP) potential.
Pinot Noir \textit{cv.} is known as grape variety of low natural colour potential accompanied with low colour stability of its wines during aging/storage. In an effort to investigate the impact of early leaf removal on polyphenol/anthocyanin formation in grapes of Pinot Noir (\textit{Vitis vinifera} \textit{L.}) variety, the present study was carried out with an aim to improve the phenolic profile and its related colour characteristics for the later wine production.

\textbf{Materials and methods}

\textbf{Experimental plot}

The study was conducted in vintage 2009 within a 4-year old \textit{V. Vinifera} \textit{L.} Pinot Noir vineyard (Guyot trained, 6940 plants/he (0.8 m x 1.8 m), 220 m a.s.l.) located at Vipava Valley (Slovenia). A completely randomized experimental design was set up with 16 plots of 5 vines within a 4 rows with N-S orientation (Table 1). The early leaf removal (ELR) was performed at berry set (5\textsuperscript{th} June) removing the basal 5-6 leaves of all shoots manually, while the control (C) remained non-defoliated. Air temperature and precipitations data for the time of experiment were obtained from a local weather station (Zevs), while in situ measurements of berry surface temperatures were monitored using a Volt craft IR-380 thermometer.

\begin{table}[h]
\centering
\begin{tabular}{ |c|c|c|c|c|c|c|c| }
\hline
1\textsuperscript{st} row & C & ELR & C & ELR & C & ELR & C \\
2\textsuperscript{nd} row & C & ELR & C & ELR & C & ELR & C \\
3\textsuperscript{rd} row & C & ELR & C & ELR & C & ELR & C \\
4\textsuperscript{th} row & ELR & C & ELR & C & ELR & C & C & ELR \\
\hline
\end{tabular}
\caption{Experimental plot; early leaf removal (ELR) and non-defoliated control (C) vines}
\end{table}

\textbf{Grapes}

Random samples of 100 berries were collected with stalk peaces at weekly intervals of August 2009 (1, 8, 15, 23 and 24) immediately frozen and stored in freezer (\(-30^{\circ}\)C) prior to extract preparation.

\textbf{Phenols extraction}

Extracts were prepared according to Mattivi et al. (2006), where the skins of 20 frozen berries were peeled and subjected to extraction for 24 h in methanol (100 mL). Then, the extract was separated and 50 mL of methanol was added to the skins, which were subjected to further extraction for 2 h. Both extracts were combined and stored in freezer (\(-25^{\circ}\)C) until further HPLC-UV/VIS analysis.

\textbf{HPLC-UV/VIS analysis}

Extracts were diluted with 1\% TFA in H\textsubscript{2}O (1:1, v/v), filtered through 0.45 \textmu m PTFE filters (Macherey-Nagel, Germany) and analysed by HPLC-UV/VIS under chromatographic conditions, specified in Table 2.

\begin{table}[h]
\centering
\begin{tabular}{ |c|c|c|c|c|c| }
\hline
Table 2: HPLC-UV/VIS analytical conditions & HPLC-UV/VIS & \\
\hline
Instrument & Waters system; binary pump (510), autosampler (717+), UV/VIS detector (2487) \\
Column & Atlantis (150 x 3.9 mm, 3 \textmu m) \\
Flow rate & 0.5 mL/min \\
Vinj & 20 \mu L \\
Detection & UV/VIS (520 nm) \\
Mobile phase & A = H\textsubscript{2}O/CH\textsubscript{3}CN/TFA (89.8:10.0:0.2, v/v/v) \\
& B = H\textsubscript{2}O/CH\textsubscript{3}CN/TFA (49.8:50.0:0.2, v/v/v) \\
Gradient & 0 min (10\% B), 20 min (25\% B), 40 min (55\% B), 41 min (90\% B) \\
\hline
\end{tabular}
\end{table}
Identification of anthocyanins was obtained by comparison of $R_t$, UV-VIS and ESI-MS spectra with those of authentic standards when available, while the tentative identity of others was confirmed by comparison of UV-VIS and ESI-MS$^2$ spectra with those from the literature (Košir et al., 2004). Each of them was quantified separately based on external 6-point calibration of malvidin-3-glucoside (1–1000 μg mL$^{-1}$), summed and expressed as total anthocyanins in mg g$^{-1}$ of berry, respectively.

**Total phenol analysis**

Measurements were carried out according to a previously published protocol of Amerine and Ough (1988) employing Folin-Ciocalteu method. Absorbance at 765 nm was measured on HP UV/VIS spectrophotometer 8453 (Agilent Technologies, Santa Clara, USA) after 2 h reaction, and the final results were expressed in mg of gallic acid equivalents (GAE) per g of berries.

**In vitro antioxidant potential (AOP) assessment**

The samples AOP was evaluated according to Obied et al. (2007) with minor modifications as follows. Various aliquots of phenol extracts were diluted to final volume (20 μL) and added to a DPPH daily prepared meOH solution (80 μM, 2 mL) in small glass containers (3 mL). The samples were covered, well shaken, and kept in the dark for 60 min, and then the absorbance was measured at 515 nm. The scavenging% of DPPH was calculated according to $\% \text{DPPH}_{\text{rem}} = \frac{A_0 - A_{\text{sample}}}{A_0} \times 100$, where $A_0$ and $A_{\text{sample}}$ stand for absorbances of control and sample, respectively. The concentration resulting in 50% inhibition was referred as $EC_{50}$, and extracts’ AOP was calculated according to equation; $AOP = \frac{1}{EC_{50}}$.

**Results and discussion**

Both extracts of defoliated (ELR) and non-defoliated control (C) vines exhibited similar qualitative phenol profile (Figure 1) composed of five known anthocyanins present in Pinot Noir grape skins namely; delphinidin 3-glucoside (Del-3-Glu), cyanidin 3-glucoside (Cy 3-Glu), petunidin 3-glucoside (Pet 3-Glu), peonidin 3-glucoside (Peo 3-Glu) malvidin 3-glucoside (Mal 3-Glu).

![Figure 1: HPLC-UV/VIS chromatogram of grape skin extracts cv. Pinot Noir monitored at 520 nm.](image)

Though the phenolic profile was qualitatively maintained among the extracts analysed, a highly significant ($P \leq 0.001$) differences were observed at the quantitative level (Figure 2) where the amount of phenols recovered from ELR was notably larger than those from control (C). As seen, the concentrations of both groups studied i.e. total anthocyanins and polyphenols were strongly affected by berry-set leaf removal, suggesting its positive impact on grapes phenols formation/accumulation. While the increases were on average lower for total anthocyanins (15%), the latter were much higher in the case of total polyphenols (29%), reaching maximum in the mid of August with up to 50% higher yields (1763 mg kg$^{-1}$) obtained vs. control (1196 mg kg$^{-1}$). Early leaf removal is known to ensure a longer and higher canopy light exposure, easily reaching clusters and hence mostly likely attributing to higher/better synthesis of phenols, if not to extreme temperatures are attained (Tarara et al., 2008).

However, from a harvest date perspective, a typical maturation time - phenol content dependent curve was obtained for both trials performed (ELR vs. C), where the levels of total anthocyanins and polyphenols have gradually increased till the mid of August (15$^{th}$ August), followed by a sudden drop before the time of harvest.
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(24th August). This type of biphasic behaviour is rather usual for grape maturation, and although maximum yields were not consistent with fruits harvest time, the final phenol concentrations obtained were significantly larger than those in control. In other words, early leaf removal has promoted the phenols yields rise of both i.e. total anthocyanins and polyphenols at harvest time for 25% and 24% on average, confirming its positive impact on a phenol grape accumulation.

A similar trend was also observed in case of extracts’ in vitro antioxidant potential (AOP) assessment, where the latter have clearly correlated \(r = 0.88\) with the total phenols content, confirming antioxidant activities of the phenols present (Figure 2, right). Although trend of AOP results were less comparable to anthocyanin and/or more to polyphenol content, the former have importantly contributed to overall AOP via high free radical scavenging abilities and the fact that anthocyanins present more than half (54%) of grapes total phenol content.

As the phenol profile can be influenced by environmental conditions (Cortell et al., 2006), we consequently followed an air temperature/precipitation behaviour in addition to a grapes surface temperature monitoring during experimental trial (Figure 3). As seen from results, the maximum air temperature data were not
comparable with berries temperatures measured, which is in line previous report of Tarara et al. (2006). In fact, the temperatures of grapes were consistently higher (4°C on average) in comparison to atmospheric ones (Figure 3, right), but has in spite of high extremes attained (> 35°C) apparently not affected synthesis of phenols, since the increases of both studied groups (total anthocyanins/polyphenols) till the mid of August were observed. Interestingly, their concentrations have decreased afterwards, most likely due to a precipitation related grape phenols dilution effect.

Conclusions

The early leaf removal (at berry set) has shown to be a highly efficient tool for improving anthocyanin/polyphenol grapes composition in terms of higher yields (15/29% on average) and antioxidant activities (53% on average) obtained for Pinot Noir grapes of Vipava Valley vineyards (Slovenia, 2009), and is therefore recommended for further field experiments of more seasons and vineyard location/exposition tests prior to its extrapolation to other Pinot Noir vineyards grown under similar geo-climatic conditions.

References


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