Flavonoid profiles of Sicilian grape skin cells might diverge from a commonly accepted paradigm

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ABSTRACT

We have studied the flavonoid profiles of grapes cultivated in the Sicilian environment (Nero d'Avola, Syrah and Nerello Mascalese), focusing on the synthesis reactions occurring in the grape skin cells, from véraison to harvest. Throughout two years we have observed that the accumulation curve of total flavonoids (mainly flavanols and anthocyanins, determined by 280_{nm} absorbance) had a higher slope of that of total anthocyanins. These observations hints at the possibility that in the (semi arid) Sicilian environment, the synthesis of proanthocyanidins and/or their flavanol precursors in the skin cells, might continue even after véraison.

RIASSUNTO

Abbiamo studiato i profili dei flavonoidi di uve coltivate in ambiente siciliano (Nero d'Avola, Syrah e Nerello Mascalese), concentrandoci sulle reazioni di sintesi che si verificano nelle cellule della buccia, dall'invaiatura fino alla raccolta. In due anni abbiamo osservato che la curva di accumulo dei flavonoidi totali (soprattutto flavanoli e antociani, determinati attraverso la misura dell'assorbanza a 280nm) ha una pendenza maggiore di quella delle antocianine totali. Queste osservazioni suggeriscono la possibilità che nell'ambiente semiarido siciliano, la sintesi delle proantocianidine e/o dei loro precursori flavanoli, potrebbe continuare nelle cellule della buccia anche dopo l'invaiatura.

INTRODUCTION

Grape berries flavonoids are important secondary metabolites which in most cultivars are synthesized in the skins and seeds of the developing fruit. In red grapes proanthocyanidins (tannins) and anthocyanins are quantitatively the most important flavonoids. These compounds play important roles in the ecology of the plant in that they protect against the attack of parasites, and prevent oxidation and UV damages; they can help seed dispersal at the completion of berry development. Proanthocyanidins are synthesized before véraison, while anthocyanins start to accumulate afterward. Their synthesis can depend on the genetic make-up of the plants as well as on environmental conditions (reviewed in (Kennedy, 2008)).

In wines these compounds contribute in different ways to color, taste and feel. Proanthocyanidins confer astringency properties and participate in color stabilization reactions together with anthocyanins whose synthesis can be affected by climatic conditions and viticulture practices (reviewed in (He et al., 2010)). As a consequence longevity of red wines can also depend on the peculiarity of the area in which they are produced.

Sicily, in Italy, has the largest amount of land dedicated to vineyards and it is the fourth wine producing region of the Country (www.istat.it). However little information is available on the flavonoid synthesis from grapes cultivated in this territory and on how its particular climatic conditions can affect it. Therefore in the last recent years we have started to investigate flavonoid synthesis in Sicilian grapes under different cultural conditions and in different areas of the island.

MATERIALS AND METHODS

Sampling

2011. "Nero d'Avola" *cv* berries were sampled from field-grown vines of the "Istituto Regionale del Vino e dell'Olio" vineyard, 37°47'12'' North; 12°33'43'' East, Biesina district, Marsala, Trapani, Italy. Four experimental conditions were established: irrigated sun exposed; not irrigated sun exposed; irrigated shaded; not irrigated shaded (one row was dedicated to each trial). Grape sampling began at the onset of berries coloration (July 15), continued through harvest (September 2nd) and ended 2 weeks afterward (September 16). Grapes temperatures were recorded starting August 5 by placing thermometer probes inside "irrigated" grapes. For "sun exposed" and "shaded" grapes respectively, average maximum temperatures were 43.5°C (±2.3°C) and 38.9°C (±2.9°C); average medium temperatures were 27.3°C (±2.5°C) and 29.6°C (±2.2°C). No differences in the average minimum temperatures were recorded between "sun-exposed" and "shaded" grapes: these were 15.7°C (±3.0°C). No rain was recorded during the whole time period. "Irrigated" grapes received one supplementary irrigation on July 20 (120 liters by drip irrigation in 24h). After the first sampling (July 15), leaves were moved away from the bunches on the "sun- exposed" grapes; shading of the grapes was achieved through canopy regulation. Sampling began at 9.2(±0.1) °Brix and ended at 27(±2)°Brix.

2012. Marsala samples. Nero d'Avola and Syrah grape berries (both *V. vinifera* L.) were sampled during summer of 2012, from field-grown vines of the "Istituto Regionale del Vino e dell'Olio" vineyard, 37°47'12'' North; 12°33'43'' East, Biesina district, Marsala, Trapani, Italy. Sampling began at 50% berries coloration (July 20 and July 24, Syrah and Nero d'Avola respectively) when the °Brix was 11.6±0.1 and ended when the °Brix was 20.8±0.7 (August 24th). Average maximum temperature was 45.3°C; average minimum temperature was 17.6 °C; average medium temperature was 29.03°C (data of sun exposed grapes; it was not possible to record temperatures of the shaded grapes).

Mount Etna samples. Grape berries of the Nerello mascalese and Syrah cultivars (*V. vinifera* L.) were sampled during summer of the same year, from field-grown vines of the "Cottanera" winery in Catiglione di Sicilia, 37°53'10'' North; 15°1'49'' East; Catania, Italy. Sampling began August 6 (40-50% coloration for the "Nerello mascalese", 50-60% coloration for the Syrah) when the °Brix was 14.2±0.5 and ended September 5 when the °Brix was 19.85±0.95.

For each cultivar two experimental conditions were established: "shaded" and "sun exposed". In the first case the bunches were left under their natural leaf coverage, in the second case some of the larger, older leaves were removed to expose the bunches. Average maximum temperatures were 48.7°C and 32.2 °C; average minimum temperatures were 9.8 °C and 16.4 °C; average medium temperatures were 25.2 °C and 23.8 °C for the "sun exposed" and "shaded" grapes respectively.

Sample processing and analysis

About 200 berries were collected at each sampling: 20 berries were utilized for weight measurements; 20 berries were utilized for determination of phenolic compounds; remaining

grapes were crushed, the juice was centrifuged and the supernatant was analyzed with Wine Scanner Foss. All measurements were performed at least in duplicate. Sample preparation for spectrophotometric determination of total anthocyanins and total flavonoids was done according to (Squadrito et al., 2007).

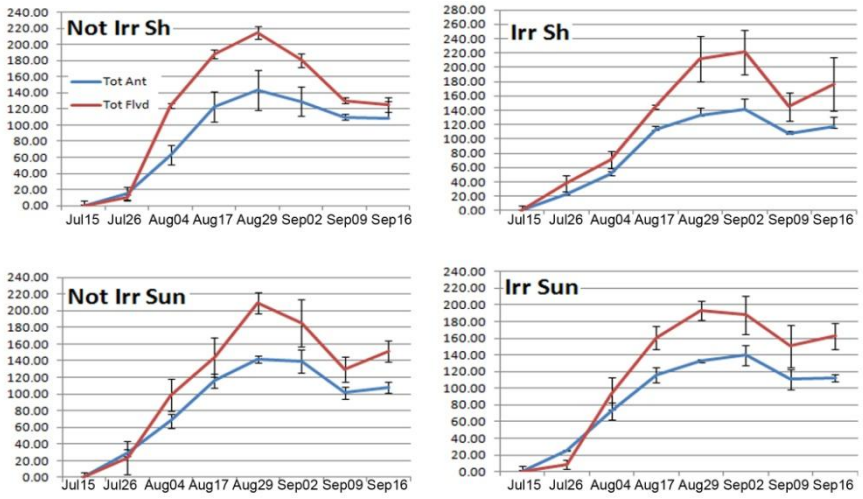
RESULTS AND DISCUSSION

Sicily has (on average) a Mediterranean climate wi_{th} mild wet winters and hot dry summers; this can however vary from one area to the other: on Mount Etna (in the East) a alpine climate can be found, while the Marsala area (west tip of the island) is considered semi-arid. In this work we collected berry samples from vines during the summer of two years: 2011 and 2012. In 2011 we were interested in understanding the effect of cultural conditions; therefore we concentrated on the Marsala area studying the effect of irrigation and sun exposure on grapes of the Nero d'Avola *cv* (previously reported in (Oliva et al., 2012).

In 2012 we compared the flavonoid accumulation profiles in three different cultivars (Nero d'Avola, Syrah and Nerello Mascalese) between the Marsala and the Etna area. This time grapes were sampled from vines as they are normally grown by viticulturists, leaving the natural leaf coverage over the bunches (these are refereed as "shaded grapes"). In addition to that, samples were also taken from vines whose bunches were exposed to sun by removing some of the largest oldest leaves ("sun exposed"). During both years we conducted our observations starting from véraison, when anthocyanins accumulate, and beyond full ripeness.

As we measured the accumulation of total flavonoids and total anthocyanins, we noticed that the slope of the accumulation curve of the formers was steeper than that of the latter. In figure 1 for each trial of 2011, we show the curves of total flavonoids together with that of total anthocyanins in the same graph. To shift the curves (so that the difference in slope could be easily visualized), the values measured at the start of sampling, of total anthocyanins and of total flavonoids have been subtracted from each point of the corresponding curve (values on the y axis are in mg/100 berries; initial values of total anthocyanins and total flavonoids are given in table 1).

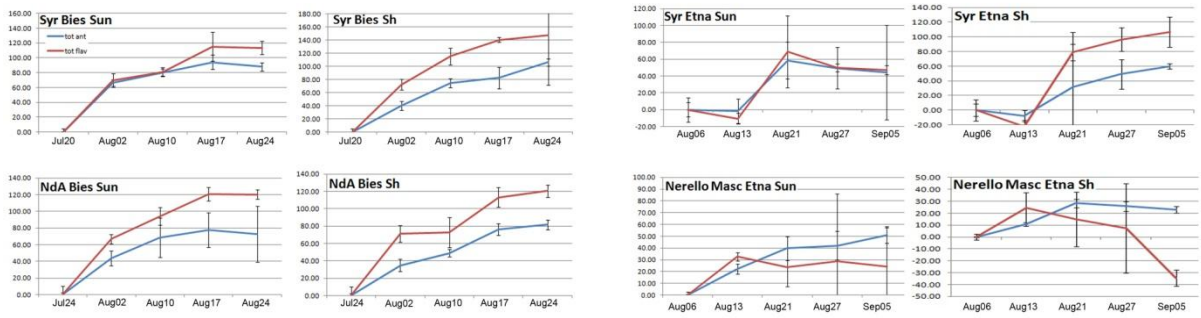
Figure 1



Summer 2011Total anthocyanins (blue curves) and total flavonoid (red curves) accumulation profiles for the Nero d'Avola samples. "Irr", irrigated; "Sh", shaded grapes; "Sun", sun exposed grapes.

As it can be noted the accumulation profile of the flavonoids always describes a steeper curve than that of anthocyanins. If there would have not been any synthesis of flavanols during the observation period, one should expect that the curve of total flavonoid and total anthocyanins would have had a similar slope. Since that was not the case (and since anthocyanins and flavanols are the most abundant classes of flavonoids in grape skins) this might indicate that some synthesis of flavanols might have occurred after véraison in the 2011 samples.
A similar situation was observed in the Nero d'Avola and Syrah samples collected in Biesina during summer 2012 (figure 2, curves were shifted similarly to what done in figure 1; initial values for total anthocyanins and total flavonoids are given in table 1).
However a different behavior was displayed by the mount Etna samples, except for the Syrah shaded grapes behaved in a way that was reminiscent of the Syrah samples from Biesina-Marsala.

Figure 2



Summer 2012. Total anthocyanins (blue curves) and total flavonoid (red curves) accumulation profiles for the Nero d'Avola and Syrah samples from Marsala Biesina (left four panels); and for the Syrah and Nerello Mascalese samples from mount Etna (right four panels). "Syr", Syrah; "NdA", Nero d'Avola; "Nerello Masc", Nerello Mascalese; "Sh" shaded grapes; "Sun", sun exposed grapes.

Table 1
Total anthocyanins and total flavonoids values (at véraison), in the 2011 and 2012 samples

	Nero d'Avola Biesina 2011	**Syrah Biesina 2012**	**Nero d'Avola Biesina 2012**	**Syrah Etna 2012**	**Nerello Mascalese Etna 2012**
Tot Anthocyanins	7.17 (1.65)	4.15 (0.50)	9.38 (2.86)	71.15 (8.40)	7.68 (0.52)
Tot Flavonoids	134.86 (6.4)	80.30 (4.76)	93.66 (10.43)	220.81 (14.29)	197.72 (2.59)

CONCLUSIONS

We have presented some observation on the total flavonoid and total anthocyanins accumulation profiles of grapes cultivated in Sicily, carried out during the summer of 2011 and 2012. The data we have collected from both years suggest that at least in some cultivar the synthesis of proanthocyanidins may continue after véraison in certain areas of the Island.

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