EVALUATION OF WINEGRAPE ANTHOCYANINS IN THE VINEYARD USING A PORTABLE FLUORIMETRIC SENSOR: SEASONAL AND WATER REGIME EFFECTS•

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1. INTRODUCTION

The content in anthocyanins (Anth) in coloured-skinned berries is a fundamental parameter of winegrape quality. It is largely affected by environmental factors, therefore, temporal variation in Anth can occur among seasons (Guidoni *et al.*, 2008). Even within the same year, vineyard variability in Anth can be large because of soil, plant vigor and vine canopy management (defoliation, spur pruning, cluster thinning) (Guidoni *et al.*, 2008). Water supply and its period of application affect also berry accumulation of Anth (Ojeda *et al*., 2002). The best way to monitor the large temporal and spatial heterogeneity in Anth content would require a rapid and non-destructive tool, such those provided by optical devices. Among these, a fluorescence-based sensor was recently developed and proposed for in-vineyard detection of Anth (Ben Ghozlen *et al*., 2010). In this work, we tested the potentiality of this new sensor on two different Italian winegrape cultivars, 'Aleatico' and 'Nero d'Avola', under different climate and water treatments.

2. MATERIALS AND METHODS

2.1. Grape cultivars and treatments

Vitis vinifera L. cv 'Aleatico' plants, trained to 2-3 vertical shoots, in North-South oriented rows and spaced 0.6 x 2.0 m at the Bulichella winery (Suvereto, Livorno, Italy) were studied during the 2008 and 2009 seasons. In 2008, water was supplied by a drip irrigation system in the amount of 46.9 and 23.4L per plant on day of the year (DOY) 210 and DOY 214, respectively. In 2009, two water regimes were applied: irrigated plants received 67.5 and 22.5 L of water per plant on DOY 201 and DOY 227, respectively. Soil at the non irrigated plants was covered by plastic transparent sheets to avoid rain penetration.

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A second experiment was conducted at the Villa Albius winery (Acate, Sicily) in 2009. The cv 'Nero d'Avola' vines were trained as an upward single spurred cordon with approximately 12 buds. Rows were East-West oriented and spaced 0.9 x 2.2 m (5,000 plants per hectare). The water treatments (WT) applied to 'Nero d'Avola' were: i) WT1, irrigated from 1 month before veraison to veraison with 25.2 L of water average amount per vine, supplied once a week; ii) WT2, irrigated between veraison and harvest, twice per week, at an average amount of 15.2 L of water per vine; iii) WT3, irrigated from 1 month before veraison to 20 days after veraison at a frequency of 1 per week (25.8 L of water per vine), and then until harvest twice per week (15.5 L of water per vine).

Midday stem water potential (MD Ψs) was measured once a week using a pressure chamber on non-transpiring leaves that had been bagged with aluminium foil for 1 hour before measurements.

2.2 Fluorescence-based sensor

The Multiplex (FORCE-A, Orsay, France) sensor detects fluorescence emitted by chlorophyll (Chl) under excitation with different LED sources in the UV (370 nm) and visible (blue at 460 nm, green at 516 nm and red at 637 nm). The sensor is insensitive to ambient light and then it can be used directly into the field. It measures an area of 50 cm^2 (80 mm diameter) which covers large part of a whole grape bunch. The intensity of the Chl fluorescence depends on the excitation light reaching the Chl layer inside the berry. It is therefore reduced by compounds localized into the outer skin layers which absorb part of the excitation light. The higher the concentration of these compounds the lower the Chl fluorescence signal. The attenuation is also depending on the excitation wavelength used, according to its overlapping with the absorption spectrum of Anth. This peaks at around 520 nm, therefore the attenuation of the green excitation by Anth will be much higher than that of the red excitation, where the Anth absorption is minimum. By comparing the Chl fluorescence intensity at the green and red excitation we can obtain an index of the Anth content, defined as ANTH = $\log(FRF_G/FRF_R)$ –constant +1. This formula was chosen in order to get positive and increasing values of the index during the last phases of ripening, corresponding to increasing values of Anth content (Cerovic *et al*., 2008).

3. RESULTS AND DISCUSSION

For all cultivars, the ANTH index measured by the Multiplex was found to be fairly correlated (r^2=0.815-0.863) to the Anth surface-based concentration (mg cm^{-2}), derived on the same samples by standard procedures of extraction and spectrophotometric or HPLC analysis (data not shown). Comparison of the time course of the ANTH index values for 'Aleatico' between 2008 and 2009, showed that in 2009 the accumulation of Anth was anticipated and reached higher values with respect to 2008. These results agree with the observation of the veraison onset (10 %) that occurred on July 31st in 2008 and on July 22nd in 2009. Furthermore, the time evolution of pH, sugar content and berry weight confirmed the delay in the berry ripening process in 2008. The higher Anth content at harvest in 2009 versus 2008 was confirmed by standard destructive spectrophotometric analysis.

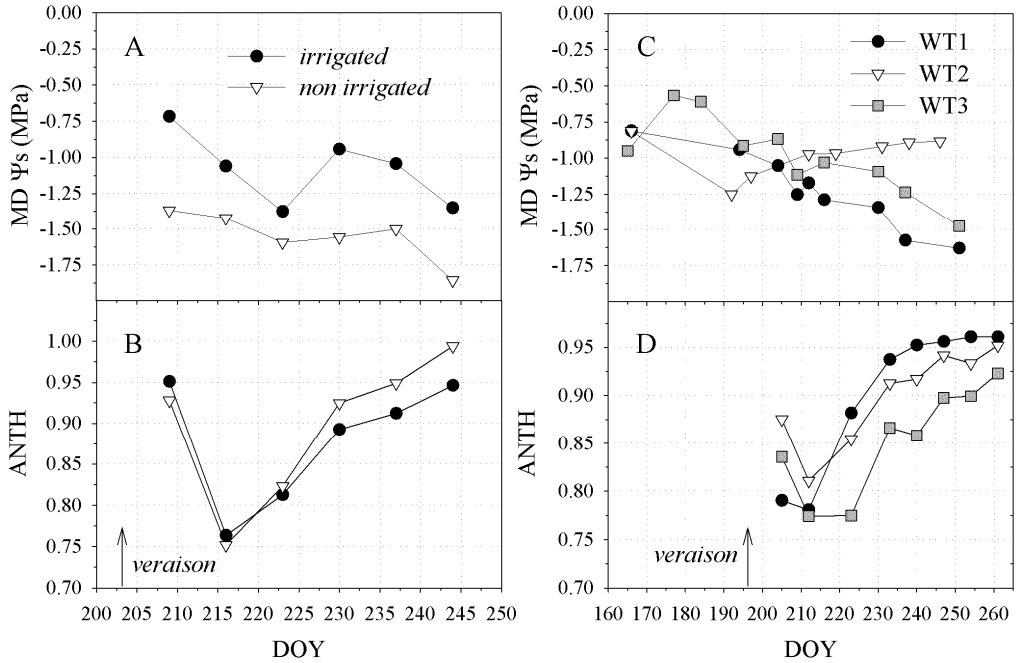

Fig. 1 - Time course of midday stem water potential (A,C) and the ANTH Multiplex index (B,D) for 'Aleatico' (A,B) and 'Nero d'Avola' (C,D) under different water regimes.

The water stress experienced by the 'Aleatico' vines is represented by the mid-day stem water potential (fig. 1A). In non irrigated plants it was already intense at veraison and increased thereafter, with stem water potential values lower than -1.4 MPa. Irrigated plants were also partially stressed but at a less severe level. The accumulation of Anth was determined by the ANTH index for the two water regimes (fig. 1 B). ANTH time evolution is similar for irrigated and non irrigated vines, but values for more stressed plants were significantly ($p<0.05$) higher with respect to those of less stressed ones, starting from DOY 230, indicating a higher accumulation of Anth under higher water stress condition. The larger content of Anth in bunches of non irrigated vines compared with irrigated ones was confirmed by standard destructive analyses (data not shown). The effect of the different water regimes on the 'Nero d'Avola' cultivar for the water potential and ANTH index, respectively was compared (fig. 1C and 1D). The most water stressed vines induced by the post-veraison water deficiency (WT1) accumulated Anth at the maximal rate and amount. The well watered vines (WT3) delayed Anth accumulation with respect to other treatments and reached the lowest values at harvest. Interesting to note is the slope change in the Anth accumulation curve under the WT2 regime (fig. 1D), probably due to the recovery of the water stress after veraison (fig. 1C). Our results indicate the utility of the Multiplex optical sensor as rapid and non-destructive tool for Anth content evaluation.

Abstract

Accumulation of anthocyanins (Anth) on whole winegrape (*Vitis vinifera* L.) bunches attached to the vine was monitored by a fluorescence-based sensor (Multiplex) on 'Aleatico' and 'Nero d'Avola'. Different water regimes were applied. The Anth index (ANTH) provided by the

Multiplex sensor was able to detect a shift in the berry ripening curve of 'Aleatico' between two consecutive seasons: it was at least one week anticipated in 2009 with respect to 2008, as also confirmed by the visual estimation of veraison onset and by the time evolution of technological maturity. For both cultivars under different water regimes, the ANTH index indicated that in plants under water stress the rate of Anth accumulation was increased with respect to those under moderate water deficit. The Multiplex sensor resulted to be an useful device for the rapid and non-invasive estimation of Anth in the vineyard.

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