Scientia Horticulturae 185 (2015) 145–150

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

# Chlorophyll role in berry sunburn symptoms studied in different grape (*Vitis vinifera* L.) cultivars

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ARTICLE INFO

*Article history:*
Received 4 December 2014
Received in revised form 23 January 2015
Accepted 26 January 2015
Available online 17 February 2015

*Keywords:*
Abiotic stress
Grapevine
Optical properties
Pigments
Photo-oxidation
Reflectance spectroscopy

ABSTRACT

In grape berries, extreme oxidative stress results in skin tissue bleaching and brownish areas appearance. Thus, these damages could negatively affect the fruit quality and the commercial value. The paper focuses on the chlorophyll roles in sunburn symptoms appearance in white skinned grape berries. In the present work, 20 cultivars were studied in three phenological stages. Berries were kept under temperature and light controlled conditions. Chlorophyll content and symptom appearance were quantified by reflectance spectroscopy indexes. The central role of radiation (and, thus, photo-oxidative damages) in berry sunburn injuries was underlined. Cultivars were classified, based on their susceptible or tolerant responses to radiation excess.



## 1. Introduction

Chlorophylls are composed by a porphyrin ring and a lateral hydrocarburic chain with a magnesium atom in the center of the ring. The alternating system of single and double bounds of the chlorophyll ring is the typical structure of those molecules able to strongly absorb in the visible spectra region (Solomon et al., 2008). Two are the main chlorophyll molecules: *a* and *b*. They just differ for having a methilic or a carbonilic substituent. This difference shifts the wavelength of the adsorbed and reflected bands: chlorophyll *b* appears yellow–green, while chlorophyll *a* looks brilliant green (Solomon et al., 2008).

In nature, chlorophyll optical properties allow the conversion of luminous energy in chemical energy through the photosynthetic process, upon which all life ultimately depends. The primary event is a light-driven electron transfer (Allen et al., 1995). The photosynthetic redox reactions reveal their importance not only in the well-known energetic conversion, but also in their oxidative stress inductive and signaling roles (Allen et al., 1995; Kreslavski et al., 2012; Müller et al., 2001).

Stressful conditions shift the balance between oxidant and antioxidant compounds toward the former. This becomes the reason of the intracellular oxidative stress development (Kreslavski et al., 2012). Oxidative burst could be determined by various reasons, however, one of the main reactive oxygen species (ROS) sources in the cell is known to be the photosynthetic process. Unfavorable environmental conditions suppress the Calvin cycle function, resulting in an over-reduction of the electron transport chain components. The consequence is the production of ROS such as superoxide radical ($O_2\bullet-$) and hydroxyl radical ($OH\bullet$), singlet oxygen ($^1O_2$) and hydrogen peroxide ($H_2O_2$) (Kreslavski et al., 2012). In grape berries, extreme oxidative stress results in irreversible damages manifested as photosynthetic pigment bleaching, brownish areas appearance related to melanin-like pigments formation and, in severe cases, tissues death (Müller et al., 2001; Rustioni et al., 2014). These symptoms lead to fruit depreciation especially concerning table grapes.

The radiation quantity reaching berries is subject to enormous changes on a time scale that ranges from seconds to months. Under radiation excess conditions, plants need to minimize photo-oxidative damages regulating the light interception and removing the already absorbed excess light energy. This is the case of non-photochemical protective mechanisms able to quench singlet-excited chlorophylls and to dissipate excess excitation energy as heat (Müller et al., 2001). Due to environmental conditions, and physiological adaptations, radiative stress is often associated to

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http://dx.doi.org/10.1016/j.scienta.2015.01.029
0304-4238/

high temperatures. In the last decades, many studies were finalized to distinguish between the radiative and thermal damages in viticulture (Bergqvist et al., 2001; Ristic et al., 2007; Rustioni et al., 2011; Spayd et al., 2002), however the debate is still open and unequivocal answers are still missing.

Despite their potential for causing harmful oxidations, studies have recently considered the photosynthetic ROS generation to be part of the light-driven production of powerful signaling molecules, whose abundance provides essential information to the cell concerning imbalances between energy-generating and energy-utilizing processes (Foyer and Shigeoka, 2011).

From an analytical point of view, chlorophyll light absorption can also be used to develop non-invasive methods able to quantify the pigment contents by specific reflectance indexes (Merzlyak et al., 2003; Rocchi et al., 2015). In fact, visible reflectance spectra give information concerning the surface appearance in terms of colors, and, thus, of pigment composition. Absorption bands can clearly appear analyzing singular spectrum (Merzlyak et al., 2003; Rocchi et al., 2015) or they can be underlined by comparing tissues before and after compositional changes. It is the case of the browning intensity index (BII) developed by Rustioni et al. (2014) with the purpose of underlining the melanin-like pigment accumulation related to the sunburn symptom appearance.

The present work focuses on the chlorophyll roles in sunburn symptom appearance in white grape berries. It aims to: (i) evaluate the radiation effect excluding thermal incidence; (ii) characterize chlorophyll degradation in berry bleaching; (iii) develop cultivar classification indexes to sunburn susceptibility.

## 2. Materials and methods

### 2.1. Experimental design

Twenty white grape cultivars were selected to represent a wide range of phenotype variations and geographical origins. Following the alphabetic order of the cultivar "prime name" reported in the Vitis International Variety Catalogue (http://www.vivc.de/index.php), they were: Afuz Ali, Chardonnay Blanc, Cornichon Blanc, Cortese, Feteasca Alba, Fiano, Invernenga, Italia, Koenigin der Weingarten, Malvasia Istriana, Matilde, Moscato Giallo, Muscat blanc à petits grains, Muscat of Alexandria, Riesling Weiss, Rkatsiteli, Sauvignon Blanc, Trebbiano Toscano, Verdicchio, Verduzzo. Grapevines were all cultivated in the same germplasm collection located in Oltrepò Pavese (Lombardy region, northern Italy) already described in Rustioni et al. (2013). All samples were collected in 2014. Meteorological data of the experimental vineyard are reported in SI1. Treatments were performed in the laboratory adjacent the vineyard, within few minutes from the samples collection. For each cultivar, 3–6 shaded bunches (depending on the accession crop) were collected in three phenological stages: pre-veraison (BBCH: 79); post-veraison (BBCH: 84) and ripening (BBCH: 89) (Lorenz et al., 1995). For each sample, 60 berries were considered in the experimental plan, resulting in a total of 3600 analyzed fruits. Twenty berries were selected to represent the control blank and kept in dark conditions at 24 °C. Other 20 berries were exposed to artificial light after epicuticular wax removal (to maximize chlorophyll excitation avoiding light filtration according to Rustioni et al. (2012)) for a 4 h period. Two LED lighting systems (version LumiGrowTM Pro 325, LumiGrow, Novato, CA, USA) were used. Light intensity was set up to be similar to that one obtained at midday in a late spring sunny day in the local vineyards. Global solar radiation measurements at the exposed surface (spatialized in Fig. SI2), were carried on by means of a pyranometer (HOBO Silicon Pyranometer Smart Sensor, MicroDAQ.com, Contoocook, NH, USA) connected to a Hobo Datalogger HOBO S-LIB-M003. Photosynthetically active photon flux density (PPFD) had an average value of 1788 (±226 std. dev.) μm of photons $m^{-2}$ $s^{-1}$. Light source distance was fixed at 38 cm from the exposed surface. The system was kept in an air conditioned dark room (to avoid uncontrolled condition variations) at 24 °C. Due to the irradiation process, a berry temperature increase was expected and, thus, recorded by standard thermocouples with a copper-constantan sensor (American National Standards Institute (ANSI)) as shown in SI3. An average temperature of about 38 °C was reached by the berries, and, thus, this value was selected for the heat treatment, achieved on the rest of the collected bunches which were kept in a dark oven (Cavallo Com. Giuseppe S.N.C., MI, Italy) for 4 h. Afterward 20 berries were selected to represent the thermal stressed sample. All berries were wiped with laboratory papers to remove the epicuticular waxes before analysis.

A maximum of four samples/day were treated and analyzed, according to the phenological shifts between cultivars and the system set up limitations.

### 2.2. Reflectance analysis

Overall 3600 reflectance spectra were obtained by a Jaz System (Ocean Optics, B.V.) spectrometer, completed with a channel with a DPU module and ILX511b detector, OFLV-3 filter, L2 lens and 50 μm slit as installed options. A reflection probe QR600-7-VIS125 was coupled to the spectrophotometer. The instrument was set up with a NIR/vis light source 4095 power setting, and the integration time was automatically corrected by the instrument. Collected spectra ranged between 340 nm and 1025 nm with a stepwise of about 0.3 nm. The spectra were calculated as percentage of reflectance (*%R*) in comparison with a reference blank spectrum obtained by a PTFE diffuse reflectance standard (Ocean Optics, B.V.). A probe holder was included to ensure the analytical reproducibility: the distance between the sample surface and the probe was fixed at 12 mm. The probe holder ensured improvements in the spectra stability, however, the absorption bands appeared to be flattened. For this reason, with the purpose of underlining the reflectance variations among different wavelengths, each spectra value was raised to the fourth power.

For each spectrum, chlorophyll indexes were calculated by the algorithms proposed by Merzlyak et al. (2003) and Rocchi et al. (2015). In more details:

Chl*a* I = log [(R800/R675) − (R800/R660)];
Chl*b* I = log [(R800/R650) − (R800/R630)];
Chl I1 = Chl*a* I + Chl*b* I;
Chl I2 = (R800/R678).

where $R_x$ = reflectance at *x* wavelength; Chl*a* I = chlorophyll *a* index (Rocchi et al., 2015); Chl*b* I = chlorophyll *b* index (Rocchi et al., 2015); Chl I1 = total chlorophyll index proposed by Rocchi et al. (2015); Chl I2 = total chlorophyll index proposed by Merzlyak et al. (2003).

Sunburn damages were quantified by the formula proposed by Rustioni et al. (2014):$BII = \frac{100 \times Rt_{678}}{Rt_{490}} - \frac{100 \times Rc_{678}}{Rc_{490}}$

BII: browning intensity index; $Rt_{678}$: treated berry reflectance at 678 nm; $Rt_{490}$: treated berry reflectance at 490 nm; $Rc_{678}$: control berry reflectance at 678 nm; $Rc_{490}$: control berry reflectance at 490 nm.

### 2.3. Statistical analysis

Statistical analyses were performed by SPSS® statistical software (version PASW Statistics 21, SPSS Inc. Chicago, Illinois). Treatment effects were statistically tested by general linear model.