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The relationship between water status and chlorophyll *a* fluorescence in grapes (*Vitis* spp.)

Harrison Wright[a,*], John DeLong[a], Rajasekaran Lada[b], Robert Prange[a]

[a] *Atlantic Food and Horticulture Research Centre, Agriculture and Agri-Food Canada, 32 Main Street, Kentville, NS B4N 1J5, Canada*
[b] *Nova Scotia Agricultural College, Truro, NS B2N 5E3, Canada*

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ABSTRACT

The purpose of this study was to explore the utility of chlorophyll fluorescence to non-destructively monitor water status in plant tissue, specifically water loss in grapes (*Vitis* spp.) destined for wine production. An automated remote-sensing (ARS), pulse amplitude modulation (PAM) fluorometer prototype, capable of scanning a large surface area, was used to monitor chlorophyll fluorescence from 'L'Acadie' (LAc) and 'Thompson Seedless'-type (TS) grape clusters during postharvest dehydration. Increasing mass loss (%) in grapes correlated with increasing soluble solids (SS) content and decreasing osmotic potential (Ψ_s) ($p < 0.001$). All of the primary fluorescence parameters monitored (F_0, F_m, F_v and F_v/F_m) had a strong curvilinear relationship ($p < 0.001$) with grape mass loss. In both cultivars, F_0 increased during the later stages of dehydration, likely as a result of increased disorder within the thylakoid membranes and/or a reduction in energy transfer between LHCII and PSII. F_m, F_v and F_v/F_m declined, likely due to several factors that are known to inhibit photosynthesis and the primary charge recombination during osmotic stress. Chlorophyll degradation during dehydration was a major factor influencing cultivar differences in the fluorescence relationships. An inflection point in the F_0 value at ≈20–25% mass loss appeared to correspond with an inflection point in the decreasing glucose:fructose ratio. The relationship between chlorophyll fluorescence and water loss, SS, Ψ_s and potentially other indicators of metabolic change, could lead to practical applications of this technology in the slow dehydration of grapes and other fruits used to make high value wines.

1. Introduction

Postharvest water loss is a major factor affecting fruit and vegetable quality. Non-destructive monitoring of water loss has consisted mainly of repeat mass measurements which can be time-consuming and expensive. This study examines the possibility of using chlorophyll *a* fluorescence to non-destructively monitor postharvest water loss.

Water stress is known to inhibit photosynthesis in plants (Björkman and Powles, 1984; Ögren and Öquist, 1985; Da Matta et al., 1997) and has been linked to fluorescence changes (Prange, 1986; Flexas et al., 2000; Degl'Innocenti et al., 2008). Chlorophyll fluorescence could potentially be a useful tool for approximating water loss in dehydrating fruit. One possible commercial application would be monitoring water loss in grapes (*Vitis* spp.) used to make appassimento-style dessert wines; this procedure is one of the oldest methods for making dessert wines and is still used throughout southern Europe (Jackson, 2000).

As grapes dehydrate, their cell contents, including sugars, concentrate which increases cellular osmotic stress. Work performed by Matthews et al. (1987) demonstrated that there is a well-correlated, linear relationship between grape berry (*Vitis vinifera*) soluble solids content (SS) and osmotic potential (Ψ_s). There does not appear to be a single photosynthetic process sensitive to osmotic stress, but many that are simultaneously affected (Kaiser et al., 1981). Hypertonic conditions in plants have been found to inhibit enzymes important to photosynthesis in the stroma (Kaiser et al., 1983), induce osmoregulation of the chloroplast (Kaiser and Heber, 1981; Downton, 1983; Sen Gupta and Berkowitz, 1988) and inhibit the function of the oxygen evolution complex (OEC) (Wiltens et al., 1978; Govindjee et al., 1981). Unlike that which is found in most plant leaf-based studies, the 'stomatal' control of

Abbreviations: ANOVA, analysis of variance; ARS, automated remote-sensing; Chl *a*, chlorophyll *a*; Chl *b*, chlorophyll *b*; DMF, *N*,*N*-dimethylformamide; ETC, electron transport chain; F_0, minimum fluorescence; F_m, maximum fluorescence; F_v, variable fluorescence; F_v/F_m, quantum efficiency; HPLC, high-pressure liquid chromatography; LAc, 'L'Acadie'; LHCII, light harvesting complex II; MPC, manual point-and-click; OEC, oxygen evolution complex; PAM, pulse amplitude modulation; Pchl, pheophytin; PMF, proton motif force; Ψ_s, osmotic potential; PSII, photosystem II; P680, reaction centre (with absorption spectrum peak of 680 nm) of photosystem II; SS, soluble solids content; TS, 'Thompson Seedless'.

* Corresponding author. Tel.: +1 902 679 5514; fax: +1 902 679 2311.
E-mail address: wrighth@agr.gc.ca (H. Wright).

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photosynthesis, and its potential influence on fluorescence, will not be included in this study since grape berries contain few stomata compared with leaves and the few stomata that are located on the berry become clogged with wax and are non-functional after veraison (Rogiers et al., 2004).

The metabolism as well as the physiology of grapes changes over the course of dehydration (Bellincontro et al., 2004). The relationship between fluorescence and water status in grapes will be affected by many factors, particularly the changing chlorophyll content over time. The minimum fluorescence (F_0) parameter, for instance, has been positively correlated with the chlorophyll concentration in plant materials (Smillie et al., 1987; Toivonen and DeEll, 1998).

The main objective of this study was to determine the effect of grape mass loss via dehydration on berry chlorophyll fluorescence and to examine how these changes may be affected by SS, Ψ_s, fructose:glucose ratios and chlorophyll concentration.

2. Materials and methods

2.1. Water loss and chlorophyll content

Two different cultivars were used: 'L'Acadie' (LAc) (hybrid) (product of Nova Scotia) and a 'Thompson Seedless'-type (TS) (*V. vinifera*) cultivar, of South American origin, obtained from a local supermarket.

Forty LAc grapes were removed from the rachis, with the peduncles left intact, to be used in a single-factor, randomized experiment. Each grape had its initial mass (g) measured using a digital scale (Sartorius, CP4202S, Gottingen, Germany) and all grapes were held in a controlled storage room under normal room conditions (temperature = 23 ± 1 °C; RH = 35 ± 5%). The grapes were stored in the dark to avoid light-induced chlorophyll degradation. On day 1, 11, 18 and 25 each grape from a sample of 10 was weighed and had its pigment content (chlorophyll a (Chl *a*), chlorophyll *b* (Chl *b*) and pheophytin (Pchl)) destructively assessed. Grapes were finely diced and individually placed in small jars along with 15 mL of the solvent *N*,*N*-dimethylformamide (DMF). The jars were placed on an Orbit Environ-Shaker (Lab-Line Instruments Inc., Melrose Park, IL, USA) for 24 h at 80 rpm. A 3-mL sample was then removed from each jar and placed in a quartz cuvette for spectrophotometric analysis (Ultrospec 3100 pro UV/vis spectrophotometer, Biochrom Ltd., Cambridge, England) of Chl *a*, Chl *b* and Pchl pigment concentrations (mg L^{-1}) derived for each individual grape by means of the following equations (Moran, 1982):

$$\text{Chl } a = 12.65A_{664} - 2.99A_{647} - 0.04A_{625}$$
$$\text{Chl } b = -5.48A_{664} + 23.44 - 0.97A_{625}$$
$$\text{Pchl} = -3.49A_{664} - 5.25A_{647} + 28.3A_{625}$$

where *A* is the absorption measured at a particular wavelength (nm). For each grape the absolute pigment content of the solution (mg) was calculated by multiplying the pigment concentration by the 15 mL of DMF the grape was immersed in, plus the approximate water content of the grape (L) at the time of immersion:

$$\underset{\text{solution}}{\text{absolute pigment content}} = C_x \frac{15 + \text{mass}_i(100 - \text{dry mass}/100) - (\text{mass}_i - \text{mass}_f)}{1000}$$

where 'C_x' is the pigment concentration (mg L^{-1}), 'mass_i' is the initial grape mass (g), 'mass_f' is the final grape mass (g) and 'dry mass' was an approximation, based on a preliminary experiment (data not shown), of the cultivar's percent dry matter. The formula assumes that the mass lost during dehydration is mainly water and that 1 g of water has a volume of 1 mL. The absolute pigment content (mg kg^{-1}, mass_i) of individual grapes was calculated by dividing the absolute pigment content of the solution by the initial mass of the grape. The concentrated pigment content (mg kg^{-1}, mass_f) of individual grapes was calculated by dividing the absolute pigment content of the solution by the final mass of the grape.

The experiment above was repeated using TS grapes. The TS is a larger berry than LAc and therefore, was expected to dehydrate at a slower rate (Dreier et al., 2000). Because of this, the four measurement periods were adjusted to day 1, 14, 21 and 28 in order to achieve similar levels of dehydration as the LAc grapes. Due to the TS grapes' greater size, each berry was quartered and one of the sections immersed in 15 mL of DMF. The mass (g) of the excised tissue (exc. tiss.) was measured before immersion in DMF and the 'mass loss' (%) was expressed as a percent of the initial. Lower pigment content in the TS cultivar necessitated the use of a fourth wavelength (A_{603}) and equations designed for use with lower pigment concentrations (Moran, 1982):

$$\text{Chl } a = 12.81A_{664} - 2.16A_{647} + 1.44A_{625} - 4.91A_{603}$$
$$\text{Chl } b = -4.93A_{664} + 26.01A_{647} + 3.74A_{625} - 15.55A_{603}$$
$$\text{Pchl} = -2.52A_{664} - 0.79A_{647} + 36.55A_{625} - 27.08A_{603}$$

The new equation for the absolute pigment content (mg) of the solution was

$$\underset{\text{solution}}{\text{absolute pigment content}} = C_x \frac{15+((100-\text{dry mass}/100)(\text{exc. tiss.}/1 - (\text{mass loss}/100))) - (\text{exc. tiss.}/1 - (\text{mass loss}/100) - \text{exc. tiss.})}{1000}.$$

For the experiments described above, a single-factor balanced analysis of variance (ANOVA) (Minitab® Release 15) model was used to determine if there was a significant difference in the pigments (Chl *a*, Chl *b* and Pchl) treatment averages over time (days). Individual treatment averages were compared using a least-squares means (LSmeans) comparison (SAS Release 8.0). In addition, regression analysis was performed on an individual grape mass loss versus absolute Chl *a* plot for each cultivar. Significance was defined as $p < 0.05$.

2.2. Water loss, SS and Ψ_s

The term 'water status' will be used to collectively refer to the grapes' increasing mass loss, SS and decreasing Ψ_s during dehydration. The well-correlated, linear relationship between grape berry SS and Ψ_s, demonstrated by Matthews et al. (1987), was verified by performing concurrent SS (digital refractometer, Pocket Pal-1, Atago, Tokyo, Japan) and Ψ_s (C-52 psychrometer, Wescor® Inc., Logan, UT, USA) measurements on the expressed sap from both LAc and TS grapes of varying sugar content (data not shown). Because the refractometer used was sucrose-based, a correction factor of 0.022 per percent SS was added to the readings, as recommended by the Association of Official Agricultural Chemists (1960), in order to account for the high berry juice glucose and fructose content.

The relationship between mass loss and SS in dehydrating grape berries was determined using the LAc cultivar. Three groups of three grape clusters dehydrated under normal room conditions (temperature = 23 ± 1 °C; RH = 30 ± 5%). Daily SS measurements were performed on each group of grape clusters; each SS measurement was based on the sap expressed from a 10-grape sample and measured using a digital refractometer. The mass of eight other untouched LAc clusters placed in the room at the same time