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Rapid measurement of total non-structural carbohydrate concentration in grapevine trunk and leaf tissues using near infrared spectroscopy

R. De Bei [a], S. Fuentes [a,b], W. Sullivan [a], E.J. Edwards [c], S. Tyerman [a], D. Cozzolino [d,*]

[a] The University of Adelaide, School of Agriculture, Food and Wine, Waite Research Institute, PMB 1 Glen Osmond, 5064 South Australia, Australia
[b] The University of Melbourne, Faculty of Veterinary and Agricultural Sciences, Parkville, 3010 Victoria, Australia[1]
[c] CSIRO Agriculture, Waite Campus Laboratory, Private Bag 2, Glen Osmond, SA 5064, Australia
[d] Institute of Future Farming, School of Health, Medical and Applied Sciences, Central Queensland University, Rockhampton, Queensland, Australia

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ABSTRACT

Carbohydrate assays are commonly used in crops and plant research to understand the way in which carbohydrates are allocated within the vine and to assess its influence on the physiology and phenology of the plant. Total non-structural carbohydrate (TNC) concentration is normally assessed by wet chemistry methods which are time consuming and costly, especially when studying carbohydrate dynamics over seasons. Near infrared (NIR) spectroscopy is a fast and easy technique that has lately gained wide acceptance for the analysis of the chemical composition of grain, food, wine, pharmaceutical products, among others. Near infrared is the region of the electromagnetic spectrum between 750 nm and 2500 nm and it is used to gather information on the relative proportions of C—H, N—H and O—H bonds of the organic molecules. This study collected NIR spectra from grapevine trunk and leaf tissues, measured TNC concentration of the same samples using a wet chemical method and compared the results using multivariate data analysis to develop a rapid procedure for the estimation of TNC concentration in grapevine tissues. Results showed that NIR spectroscopy could be used to predict starch and TNC concentration in freeze-dried and ground grapevine trunk and leaf tissues. Moreover, it has been demonstrated that a robust universal model could be applied to the prediction of TNC in both leaves and trunks. Therefore, this method could be used as a practical tool for a rapid screening of TNC concentration for high temporal and spatial assessment of grapevine tissues at given phenological stages. The main advantages of this technique over traditional methods are the rapidity and the ease-of-use protocol in routine analysis, which allows a considerable reduction of costs and time.

1. Introduction

The ability of a vine to produce grapes and its resilience to biotic and abiotic stresses are highly dependent on the way in which carbohydrate is allocated for storage, root growth and fruit production (Holzapfel and Smith, 2012; Smith and Holzapfel, 2009). Changes in climate and decreased water supply over the past decade in Australia have prompted research into the short and long-term effects of environmental stresses on grapevines and their resilience capacity to those stresses (Edwards et al., 2011; Dayer et al., 2013). Moreover, climate change models predict an increase in the atmospheric CO_2 concentration and this is likely to have an effect on carbohydrate concentration in grapevines as already shown for other species (Ackerson et al., 1984; Bhattacharya et al., 1990; Downton et al., 1990; Havelka et al., 1984). Vineyard management factors such as irrigation, canopy management and fertilisation also have an influence on the carbohydrate reserves status (Bennett et al., 2005; Holzapfel et al., 2010; Schmidtke et al., 2012). Edwards et al. (2011) reported that there are over 160 published studies on carbohydrates content in grapevines; these discuss source-sink relations (Grechi et al., 2007), mobilization for early season growth (Greer and Sicard, 2009), control of fruitfulness (Sánchez and Dokoozlian, 2005), as well as reserves status (Smith and Holzapfel, 2009; Dayer et al., 2013; Zapata et al., 2004).

Total non-structural carbohydrate (TNC) concentration is normally assessed by wet chemistry methods such as simple colorimetric analyses or more sophisticated high performance liquid chromatography (HPLC) (Peris-Tortajada, 2000; Edwards et al., 2011) or gas chromatography mass spectrometry (GC–MS)

* Corresponding author.
E-mail addresses: roberta.debei@adelaide.edu.au (R. De Bei), sigfredo.fuentes@unimelb.edu.au (S. Fuentes), Everard.Edwards@csiro.au (E.J. Edwards), d.cozzolino@cqu.edu.au (D. Cozzolino).
[1] Current address.

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methods (Ibañez and Cifuentes, 2003; Edwards et al., 2011). Edwards et al. (2011) reviewed and reported a technical brief describing an adaptation of the anthrone method (Dreywood, 1946) to be applied to the determination of soluble carbohydrate concentration in grapevine tissues. However, even the latter method could be considered time consuming and costly when studying large numbers of samples, such as carbohydrate dynamics in grapevines within and over seasons. Therefore a method for analysing carbohydrate concentration that did not require any wet chemistry, particularly extraction protocols, would be advantageous where large numbers of sample analyses are required. Such a method might also be used in non-specialised laboratories, or even growers, to study carbohydrate dynamics or changes in management practices. Despite the large number of published methods for analysis of carbohydrates, until recently there has been a lack of a simple, rapid and low cost procedure to be applied to a large number of samples.

Near infrared (NIR) is the region of the electromagnetic spectrum between 750 nm and 2500 nm and it is often used to gather information on the relative proportions of C—H, N—H and O—H bonds of the organic molecules (Murray, 1993). NIR spectroscopy has been applied in the grape and wine industry for a variety of analysis in the past, demonstrating its ability to determine: concentration of anthocyanins, soluble solids and pH in red grape homogenates Cozzolino et al., 2004, 2006; Dambergs et al., 2006; Gishen et al., 2005), wine composition (Cozzolino et al., 2007; Smyth et al., 2008) and, more recently, to estimate vine water potential (De Bei et al., 2011) and berry shrivel (Beghi et al., 2015), among others. Schmidtke et al. (2012) have developed a rapid method to monitor grapevine reserves (carbohydrate and nitrogen content in trunks and roots) using attenuated total reflectance (ATR) coupled with mid-infrared (MIR) spectroscopy. This technique allows for rapid estimation of starch and nitrogen content in dried and ground woody tissues. Differently, in this study we combined NIR spectra of ground grapevine trunk and leaf tissues, in comparison to the measure of carbohydrates concentration using the method of Edwards et al. (2011) as a reference, plus multivariate data analysis to develop a rapid procedure for the estimation of carbohydrates concentration in grapevine tissues. The main characteristics of NIR spectroscopy such as easy to use, portability and instrument availability were considered as an advantage compared with MIR. The main advantages of these techniques (NIR and MIR) over traditional methods are the rapidity and the ease of use in routine analysis, which allow a considerable reduction of costs and time (Cozzolino et al., 2009).

This study aimed to collect NIR spectra from grapevine trunk and leaf tissues, measure the TNC concentration of the same samples using a wet chemical method and compared the results using multivariate data analysis to develop a rapid procedure for the estimation of TNC concentration in grapevine tissues.

2. Materials and methods

2.1. Trunk and leaf samples

Grapevine trunk and leaf tissues used for the development of the calibration models were sourced from a commercial Chardonnay vineyard, located in Qualco, South Australia (34°6′0.76″S; 139°50′55.76″E), Australia. The vines were 5-year-old Chardonnay plants, grafted on Ramsey rootstock. The vines were subjected to irrigation treatments ranging from fully irrigated (control = 5 ML ha^{-1} $year^{-1}$) to 10% of water applied from control (0.5 ML ha^{-1} $year^{-1}$). Wood samples were obtained from the trunks at key phenological stages during the seasons 2008–2009 and 2009–2010 starting from budburst. The same plant was never sampled more than twice to avoid potential effects of sap flow disruption on carbohydrate mobilisation and reserve. The samples were obtained from the trunk using a drill with a 5 mm diameter drill bit. A slow drill speed was used to ensure the wood sample was never heated to more than approximately 30 °C. The drill was inserted perpendicular to the trunk and through the entire trunk diameter. The tailing was collected in a paper bag and immediately frozen in liquid nitrogen, stored in dry ice in the field and then at −80 °C until use in the laboratory. Leaf blades were sampled during the 2009–2010 season at bloom, fruit set, veraison and post-harvest. Leaves were sampled at the fifth position from the shoot tip, in order to have leaf samples at similar developmental stage at the time of samplings. After sampling, the leaves were placed in a paper bag and snap-frozen in liquid nitrogen. Samples were then stored in dry ice for transport and then at −80 °C until chemical analysis in the laboratory.

2.2. Chemical analysis

All samples (trunk and leaves) were freeze-dried using a Telstar LyoQuest freeze-drier (AVT Services Pty Ltd., Seven Hills, NSW, Australia) and then ground to a fine powder (particle size ~50 μm) using a Labtech Essa LM1-P mill (Labtech Essa Pty Ltd., Bassendea, WA, Australia). The freeze-dried and ground samples were analysed for starch and sugar concentration according to the adaptation of the anthrone method proposed by Edwards et al. (2011). In brief, a series of extractions from the ground material using deionised water were made, with the supernatant used for soluble carbohydrate analysis and the pellet for starch determination. The concentration of insoluble starch (expressed in mg g^{-1} of fructose equivalents) was analysed using a commercial enzyme assays (Total Starch Assay Procedure, K-TSTA-50A/K-TSTA-100A 08/16, Megazyme International, Bray, Ireland) and the concentration of soluble carbohydrates was measured using anthrone reagent. The samples were read in microplates a Labtech FLUOstar Optima microplate reader (BMG Labtech, Mornignton, VIC).

2.3. Visible and near infrared scanning

The freeze-dried and ground leaf and trunk samples were scanned with a NIRSystems 6500 (FOSS NIRSystems, Silver Spring, MD, USA) instrument, from 400–2500 nm, in reflectance mode in a rectangular cuvette with a 10 mm path-length (FOSS NIRSystems, Silver Spring, MD, USA). The spectrum of each sample was the average of 32 successive scans (1050 data points per scan). Samples were not rotated when spectra collection was made. Two pairs of lead sulphide detectors collected the reflectance spectra. Reflectance energy readings were referenced to corresponding readings from a ceramic disk. Spectral data was collected using Vision software (Version 1.0, FOSS NIRSystems, Silver Spring, MD, USA) and stored as the logarithm of the reciprocal of reflectance (R) (i.e. as absorbance = log (1/R) at 2 nm intervals.

2.4. Chemometrics and data analysis

Chemometric analysis was performed using The Unscrambler X software package (Version 10.2, CAMO ASA, Norway). The spectral region from 400 to 1099 nm (visible and short wavelength NIR) was not used for the analysis since it is mostly related to absorptions by pigments. Principal component analysis (PCA) was performed before partial least squares regression (PLS) models were developed. PCA was used to identify the dominant patterns in the spectral data and for detection of outliers (Cozzolino et al., 2009). The Unscrambler software detects outliers based on the Hotteling T2 test. However, for the purpose of this study a spectral outlier was defined as any samples falling outside the 95% of the