Scientia Horticulturae 226 (2017) 117–121

Contents lists available at ScienceDirect

Scientia Horticulturae

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

Short communication

Starch quantification in woody tissues by reflectance spectroscopy and on-solid iodine complexation

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ARTICLE INFO

Keywords:
On-solid reaction
Wood quality
Grapevine (*Vitis* spp.)
Plant reserves
Non-structural carbohydrates

ABSTRACT

Non-structural carbohydrates provide the carbon and energy for plant growth and survival, being starch one of the main compounds accumulated in woody organs of trees. However, starch quantification in woody tissues is difficult and time consuming. Therefore, we hypothesized that reflectance spectroscopy could provide rapid and low cost methods to quantify carbohydrates in woody tissues. With this aim we analyzed the spectra of trunk tissues from different grapevine species (*Vitis* spp.) and results were compared to standard analyses. PLS regression appeared particularly performant for the elaboration and analysis of the collected spectra. This statistical approach produced high correlations with the concentrations of both starch and soluble sugars. To focus on starch detection, a specific on-solid colorimetric reaction was also proposed. Starch-iodine complexation produced significant modifications in spectral features.

1. Introduction

Carbon reserves (also known as non-structural carbon storages) play an important role in perennial plants, in particular deciduous trees such as grapevines. Deciduousness is an adaptation that relies on the accumulation of carbohydrates during summer season to sustain plants through unfavorable winter conditions and for their regrowth in spring (Holzapfel et al., 2010). Carbon reserves supply the required energy for the emergence and growth of new plant organs at the beginning of the growing season (Naschitz et al., 2010). In grapevines, carbon assimilation remains low for several weeks after bud burst, thus early shoot growth during and after this stage is completely dependent on remobilized storage reserves (Keller, 2010; Zapata et al., 2004). $^{14}CO_2$ labeling experiments showed that sugars manufactured in leaves after harvest and converted to starch in the wood and roots are the first carbohydrates used by new shoots the following spring (Loescher et al., 1990). The whole tree may be considered a storage organ, and carbohydrates are commonly found in all the perennial parts of the tree. Sugars, which are soluble and important for osmotic regulation, and starch, which is insoluble, have functionally different roles in transport and storage (Dietze et al., 2014). Starch is ubiquitous and usually the main insoluble storage carbohydrate in most above-ground woody plant tissues (Loescher et al., 1990). Fluctuations of stored non-structural carbon compounds over time results from the impairment between carbon supply by photosynthesis and carbon demand for metabolic functions such as growth and respiration (Bansal and Germino, 2008; Sala et al., 2012).

Given the importance of non-structural carbon accumulation in plants, several methods for its quantification have been proposed, mainly for the quantification of soluble sugars and starch. Most of these methods are destructive and considers the liquid extraction of the analyte (i.e. starch) and a subsequent reaction, making them high time consuming (Dayer et al., 2013; Gomez et al., 2007; Richardson et al., 2013; Woodruff and Meinzer, 2011). Therefore on-solid spectroscopy analysis could represent an alternative method.

Optical properties are widely used in many research fields. During the last decades, spectroscopy techniques have been developed to support agricultural practices and plant researches. The approaches vary between remote sensing indexes (Curran, 1989; Lamb and Brown, 2001; Seelan et al., 2003) until microscopy image analyses (Biggs, 1985; Pilati et al., 2007; Piller, 2012). Recently, a new approach was proposed for compositional evaluation of grapevine woody tissues,

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http://dx.doi.org/10.1016/j.scienta.2017.08.036
Received 21 April 2017; Received in revised form 19 August 2017; Accepted 21 August 2017

based on on-solid colorimetric reactions, in a macro-scale, coupled to reflectance spectroscopy detections (Rustioni et al., 2016). This approach bypass the common limitations due to the optical properties of target compounds by the introduction of stains, but it keeps the advantages of proximal sensing concerning costs, rapidity and analytical scale.

A rapid assay could be of enormous interest for nurseries interested to check the quality of the wood reserves in future graftings. The aims of this work are: i) to explore the spectrum obtained by on-solid starch-iodine complexation in woody tissues; ii) to develop rapid assays (considering both chemical and statistical approaches) able to produce indexes representative of the starch content in woody tissues by reflectance spectroscopy.

2. Materials and methods

2.1. Plant material

Vines considered in this experiment were grown in the germplasm collection of the University of Udine, northeastern Italy (40°02′N. 13°13′E). Vines were trained as single Guyot within North-South oriented rows (2.5 m between rows × 0.8 m between vines). All the plant materials (cane internodes) were collected from different *Vitis* spp. (Table 1) during the winter dormancy, to minimize the tissue variability due to instable phenological phases. A total of 47 internodes were analyzed.

For each internode considered, 10 woody slides were obtained by a penknife. Half of these slides were used for the reflectance spectroscopy methods development for both the statistical and chemical approaches. The other 5 slides/internode were used for the PLS model validation. Reflectance spectroscopy analyses were carried out considering the xylem region. The remaining wood of the same internode was used for the non-structural carbohydrate (starch and alcohol-soluble sugars) quantification by wet chemistry (method described below).

All solvents and reagents were purchased from Sigma–Alderich.

2.2. Reference protocol for analysis of non-structural carbohydrates

Non-structural carbohydrate analysis was carried out as described in Herrera et al. (2015). Briefly, dried wood samples were finely ground into a powder using a Mixer Mill MM 400 (Retsch, Haan, Germany) and an aliquot (75 mg) of fine powder per sample was used for carbohydrate analysis. Alcohol soluble sugars were extracted in 80% ethanol and determined by an anthrone-sulfuric acid based methodology (Fales, 1951; Mccready et al., 1950; Yemm and Willlis, 1954). Starch was extracted from the sugar free residue with hydrochloric acid and determined using the same anthrone-sulfuric acid procedure. In both cases, D-(+)-glucose was used for the calibration curve.

Table 1
List of *Vitis* spp. and varieties analyzed.

Specie	Cultivar
Vitis champini	
Vitis slavini	
Vitis armata	
Vitis doaniana	
Vitis vinifera	Cabernet Sauvignon
	Gruner vertliner
	Friulano
	Picolit
	Ribolla gialla
	Syrah
	Sultanina

2.3. Reflectance spectroscopy records

A total of 930 reflectance spectra were collected using a Jaz System spectrometer (Ocean Optics, B.V., Dunedin, USA) set up as described in Rustioni et al. (2016).

For the statistical approach (PLS model), the spectral range 400–800 nm was considered and each spectrum was normalized at 800 nm. Only reflectance spectra before treatment (t0) were considered for the statistical indexes development. To develop the PLS model 330 spectra were used and 270 spectra were spent for the validation.

For on-solid iodine complexation, a total of 660 reflectance spectra were measured: 330 before coloration and 330 after iodine reaction. The spectral range 450–950 nm is presented and discussed. Each spectrum was then normalized by the 900 nm reflectance intensity and the reciprocal values were considered as representative of the absorbance. Iodine-Starch complexes were obtained by treatment with Lugol solution (*Re*) [10 g/L potassium iodide and 2,5 g/L iodine in water]. In details, 5 woody slides/internodes were analyzed. Reflectance spectra (t0) were collected on raw material, before treatment. Then, a drop of the Lugol dye was placed on each sample and kept for 3 min to react. After that, woody slides were dried with paper towels to remove excesses of Lugol solution. Finally, reflectance spectra of treated samples were measured (*Re*).

2.4. Statistical analysis

Average reflectance spectra and the INDEX $_{\text{Starch-IodineComplex}}$ were elaborated using Microsoft Office Excel and SPSS statistical software (version PASW Statistics 21, SPSS, Inc. Chicago, IL). PLS regression models were carried out using R software (R Core Team, 2015). The PLS regression analysis was obtained by using default parameters. In model development the quality of the component was scored by leave-one-out ("LOO") cross-validation (function "pls:plsr") (Mevik and Wehrens, 2007). Concerning the validation, the component quality was attested using a second dataset.

3. Results and discussion

3.1. Statistical approach for non-structural carbon prediction

Although the PLS regression appeared to be significant also considering a few components, the best predictions and validations was obtained with 7 and 8 components for starch and soluble sugars respectively. In details, the starch-PLS model, based on the first 7 components, was able to predict the starch content with an r of 0.75 and 0.6 respectively in prediction and validation, and a significance higher than 99.9%. Concerning soluble sugars, the PLS model, based on the first 8 components, was able to predict the concentration with an r of 0.89 and 0.81 respectively in prediction and validation, and a significance above 99.9%. Therefore, the PLS approach produced highly satisfactory results for non-structural carbon prediction (additional statistical indexes for cross-validations are available in supplementary material). The PLS results and the models including the obtained scripts are presented in the supplementary material.

A number of studies are available in literature showing high performances of the PLS regression for reflectance spectroscopy data elaborations. Considering wood characterizations, Ona et al. (1998) obtained highly significant correlations between sugar content in woods and non-destructive determinations by Fourier transform Raman spectroscopy. The same spectroscopy technique, in combination with multivariate analyses (including PLS) was also used for lignin, cellulose and hemicellulose contents in wood samples by Chen et al. (2010). Kelley et al. (2004) adopted the PLS regression for the prediction of chemical and mechanical properties of woods by using visible and near infrared spectroscopy. Rustioni et al. (2016) obtained a good prediction of the expected drought tolerance of different *Vitis* species by PLS regression