Preliminary image-based appraisal of starch in one-year-old grapevine shoots

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***Abstract*—**

Determination of starch concentration in grapevine woody tissues is pivotal to optimize some vineyard management techniques. Analytical assays represent the most reliable approach but nevertheless they are time-consuming. This study reports preliminary results on using imaging to estimate starch concentration in woody tissues stained with the Lugol's solution indicator in *Vitis vinifera* L.. One-year-old shoots (cv 'Primitivo') were sampled in winter time and forced to sprout inducing a starch depletion. The measured starch ranged from approx. 0.1 to 14.4 % (DW). Parallel image-based and analytical starch concentrations data (n =42) revealed that R (red), G (green) and B (blue) color channels were highly predictive across three phenological stages (*r*= - 0.92), rising the imaging technique proposed as a promising tool to estimate the starch content.

Keywords— *Vitis vinifera* L., RGB images, nonstructural carbohydrates

I. Introduction

Photoassimilates are stored as starch in chloroplasts and then in woody tissues of the plant when photosynthetic rate exceeds metabolic demand [1], [2]. In grapevine, the accumulation of excessive photoassimilates is reported to begin soon after bloom, when starch concentration increases in the parenchyma cells of all perennial organs (*i.e*. root, trunk, cordon), reaching the maximum level around harvest [2][6]. The starch stored in these organs ensure winter survival through respiration maintenance, frost resistance, embolism refilling and membrane stabilization [7]. In spring, the starch is remobilized to support bud-break and growth of vegetative and reproductive organs [4][5]. Hence, the quantification of starch content into woody organs before the beginning of the growing season is of great interest for the source-sink regulation between vegetative and reproductive organs, especially early in the vegetative season, when young shoots organs (leaves inflorescences, and stem) depend on the stored reserve substances.

Starch concentration might be assessed by different chemical methods [8]. Recently, NIR spectroscopy, reflectance spectroscopy on iodine-starch complexes, and X-ray microCT imaging, were developed [9]-[11] as rapid procedures for the estimation of carbohydrates concentration in grapevine tissues.

Although imaging is increasingly employed as non-destructive method also for analytical determinations [12], none of these studies have considered the use of imaging approach for estimating the woody starch content in combination with a starch colorimetric indicator.

Lugol's iodine solution is a starch indicator widely used to determine, for example, the starch in apple fruit [13, 15]. Hence, this study examined whether imaging may be used as an affordable method to estimate the starch concentration in *Vitis vinifera* L one-year-old shoot samples stained with Lugol's solution.

II. Materials and Methods

The experiment was carried out in the 2022-2023 dormant season, on one-year-old shoots of 'Primitivo', an early ripening grapevine cultivar. About 42 one-year-old shoots, bearing 2 clusters per shoot, with similar diameter of the internode included between the 2 clusters and with at least 15 lignified internodes, were collected in a commercial vineyard (Basilicata, South Italy).

At the sampling time (half January 2023), three one-year-old shoots were immediately used for image capture, and chemical analysis. The remaining shoots were forced, at 25°C temperature, to produce rooted cuttings by immersing their base in tap water. Cuttings progressively and differentially sprouted out, generating new roots and growing shoots during the following months.

Once the buds/shoots were at 00, 11, and 14 BBCH phenological growth stages [14] (Figure 1) at least three one-year-old shoots were processed for chemical and image analysis.

From each one-year-old shoot, three to five internodes were selected starting from the basal internode until the top of the shoot. The basal internode was that bearing the cluster the previous season. Each internode was cut into two parts: one half was used for imaging, preparing a thin woody section (~ 4 cm^2 area) using a penknife. The other half was sealed in a plastic bag and stored in a -80°C refrigerator, freeze-drying and then stored under vacuum until chemical analysis.

Image acquisition, processing, and data extraction

Sections were manually stained for 2 minutes using a 0.3% I2/1% KI Lugol's iodine solution according to [2]. After the reaction, each sample was placed on the base of a stand holder covered with a red paper as background. A Nikon D5100 digital camera (16.9 megapixels) was hold to the stand to have the 40 mm lens (AF-P DX Nikkor 18-55 mm, f/3.5-5.6 G VR, Nikon, Tokyo, Japan) positioned 40 cm away from the sample. The stand holder was enclosed in a 0.8 × 0.8 × 0.8 m portable photo studio box (Ombar Photography Light Box) equipped with led 5500K, 100 LEDs. Images were captured in JPG format and a X-Rite ColorChecker Classic color card (Grand Rapids, MI, USA) was used to ensure white balance.

In each image, the area of the woody section corresponding to the parenchyma rays (see the yellow-edged part of the section in Figure 1B: Region of Interest) was segmented and processed to extract the red (R), green (G), and blue (B) mean values, using the open-source ImageJ software [16]. The R, G, and B values were used to calculate the grayscale (RGB) with the following equation: RGB = (R+G+B)/3.

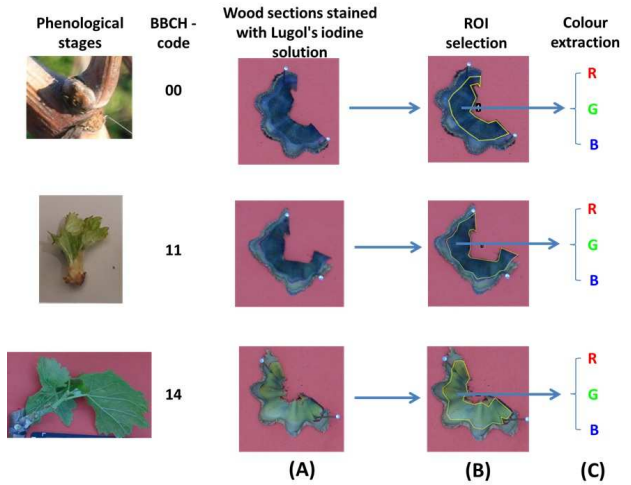

Figure 1: Phenological growth stages and BBCH code [15] of the buds/shoots when the thin woody sections, obtained with a penknife and stained for two minutes with Lugol's iodine solution, were used for image acquisition. Workflow of the image analysis: (A) imaging of the stained woody section, (B) segmentation of the woody Region of Interest (ROI) (edged in yellow), (C) extraction of the R, G, and B mean pixel values.

Enzymatic starch analysis

Starch analysis was performed by enzymatic hydrolysis which is the preferred methodology according to [17], using a commercial enzyme assay kit (K-TSTA, Megazyme International, Bray, Ireland). Starch concentration was determined according to the procedure outlined in the kit [18], with few adjustments according to other methods applied in *Vitis vinifera* L. lignified tissues (*e.g.*, cane, cordon and trunk [19] 20]).

The amount of starch per each sample was calculated as 'g/100g' of dry weight in woody stem. The glucose/GOPOD standard curve was calculated after the measurement of absorbance ('Multiskan GO' Spectrophotometer, Thermo Fisher), using the fitting linear equation ($R^2 = 0.9998$).

Statistical analysis

One-way ANOVA was used to compare data of starch concentration and R, G, B (RGB) pixel values of the three growing stages and the Duncan's test as a post-hoc test for multiple comparisons, p-values <0.05 were considered significant. The correlation between RGB pixel values and the paired starch concentrations were appraised through the Pearson's correlation test.

III. Results and Discussions

The presented study documents the correlation between the mean RGB pixel values and starch concentrations measured in woody shoots during different phenological growth stages.

At bud dormancy, BBCH 00 stage, the stained woody sections have shown the typical dark-blue colour reported also by [2]. Although eye-based differences were difficult to assess comparing sections at BBCH 00 and 11 stages after Lugol's iodine solution staining, the RGB analysis revealed they were significantly different (Figures 1, 2), also according to the starch analytical determination (Figure 3). Instead, differences in colour were visible among BBCH 14 stage and the two previous BBCH stages. Image segmentation shows significant increments in R, G and B components and mean RGB from BBCH stage 00 to 14 (Figure 2). The increment in R, G, and B components corresponded to a decrease in the blue-dark coloration of the Lugol-stained section predicting the decrease in starch concentration of the tissues, as already reported by [12] [2][11].

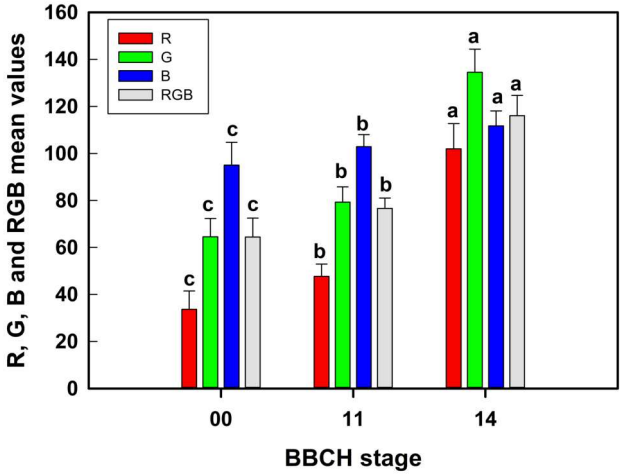

Figure 2. R, G and B single color channel values and mean RGB pixel values (±SD) of the ROI during the three phenological growth stages. Comparing the same color channel value or RGB mean value across the phenological stages, different letters indicate significant differences ($p<0.05$).

In this experiment, during bud-break and early shoot growth stages the starch localized in the parenchymatic rays rapidly declined from 12.89 ± 1.18% to 1.16 ± 0.57% (Figure 3). Comparisons of our results with others are difficult due to variable outcomes in starch concentration related to analytical methods used [8]. However, the starch concentration at dormant stage was similar to that found by [2] [3], but lower than that in [11]. Moreover, in our experimental conditions starch concentration in one-year-old

shoots decreased from dormant stage (BBCH = 00) to first leaf unfolded (BBCH = 11), while, in field-grown vines, the starch concentration would increase because of the mobilization of starch accumulated in trunk and roots [2].

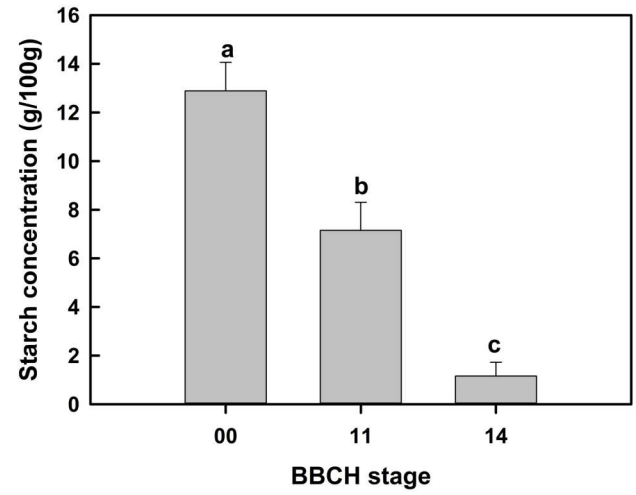

Figure 3. Starch concentration (±SD) in the internode of one-year old shoots of 'Primitivo' grapevine cultivar, in three phenological growth stages. Different letters indicate significant differences among phenological stages ($p<0.05$).

Data of starch concentration analytically determined versus RGB were significantly correlated (r= - 0.916, $p<0.05$) (Figure 4), pointing out the worth of the imaging approach in quantifying the starch in woody tissues as a suitable alternative to analytical assay. However, more efforts are required to achieve a reliable image-based model able to predict the starch concentrations.

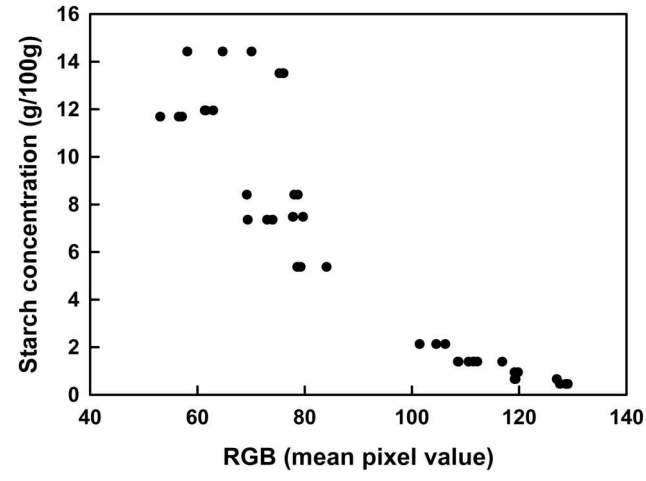

Figure 4. Scatterplot between RGB pixel values and starch concentration (g/100 g of dry weight) analytically quantified (r= - 0.916) in cv 'Primitivo' one-year old shoots.

In conclusion, this study preliminary documented the tight correlation between the starch concentration and RGB values in grapevine woody samples. Development of non-destructive starch determination methods would contribute to digital agriculture supporting vineyard management techniques such as pruning schedule, mineral nutrition and source-sink manipulation.

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