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Iron, magnesium, nitrogen and potassium deficiency symptom discrimination by reflectance spectroscopy in grapevine leaves

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ABSTRACT

This work aims at the identification and discrimination of mineral deficiency symptoms by reflectance spectroscopy. *Vitis vinifera* L. plants were subjected to 5 different hydroponic mineral nutrition: control and iron, magnesium, nitrogen and potassium deficiencies. Basal, young and apical leaves were studied. Spectra were collected along veins, in interveinal areas and in leaf margins. Reflectance spectroscopy appeared to be able to discriminate the mineral deficiencies, producing characteristic pigmentations and symptom distribution. These results appeared to be coherent with the physiological role of each nutrient. The most promising target in terms of leaf position and wavelengths of interest were identified for each condition. Mineral deficiencies also produced specific pigment distribution within the same plant, suggesting the possibility of symptom identifications also without the availability of well-fed control plants in field conditions. The reflectance spectral feature of the leaves could support the identification of mineral deficiencies in field conditions. These results could support further researches, including index development for symptom intensity quantifications and definition of threshold values for fertilization management. Due to the rapidity and low cost of the technique, future applications could support both technical requests and scientific researches.

1. Introduction

Biotic and abiotic factors could become source of stress for plants. Depending on the responses to the stress conditions, some physiological dysfunctions could occur in the plant, affecting the quality and quantity of crop productions. Thus, the presence of stress conditions is not sufficient to quantify the eventual damage to the crops. For example, concerning grapevine, resistant/tolerant cultivars have been described for both biotic (Toffolatti et al., 2016; Grzegorczyk and Walker, 1998) and abiotic stresses (Ksouri et al., 2007; Rustioni et al., 2016; Padgett-Johnson et al., 2003). The plant adaptation to environmental conditions could also promote mechanisms able to modify plant interactions with the surrounding stresses (Rustioni et al., 2014; Rustioni, 2017). For example, soil moisture is not sufficient to quantify the plant water status. In fact, partial rootzone drying have been also proposed in vineyard management to induce drought signaling with positive impacts on the production (Dry and Loveys, 1998). Also, excessive radiation does not necessarily result in evident photo-oxidative sunburn symptoms: the berry susceptibility depends on the cultivar and the phenological phase (Rustioni et al., 2015). Thus, the presence of stress conditions does not necessarily represent a sufficient risk to require the human intervention in agriculture.

Considering mineral nutrition, the analysis of the soil composition is a useful support for winegrowers, nevertheless it is not always sufficient to predict the impact on the crop production, understanding the physiological responses of the plant. Neither the mineral leaf concentration is always indicative of the physiological disorders. For example, Smart et al. (2007) found that potassium concentrations in leaves were not significantly different among samples having different percentage of visible leaf K deficiency symptoms. Thus, the direct quantification of the symptom represents the best approach to detect physiological disorders and, thus, the possible implications on crop productions.

Visible reflectance spectroscopy is a non-invasive technique able to describe the modifications in leaf pigmentations. It produces objective and quantitative results and it can detect modifications in the surface reflectance properties earlier than the human eyes. Optical approaches have been already proposed for the quantification of mineral deficiencies in grapevines. Shaahan et al. (1999) considered the possibility to use a portable chlorophyll meter to quantify leaf chlorosis related to nitrogen, magnesium and iron deficiencies in fruit trees, including grapevine. Smart et al. (2007) studied potassium deficiency in grapevine by using hyperspectral reflectance. Martín et al. (2007) and Meggio et al. (2010) proposed the use of remote sensing indices for the spatialization of iron deficiency chlorosis. Taskos et al. (2015) tested

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different optical indexes and sensors for nitrogen status evaluation in Greek vineyards. The major limitation for the application of these techniques is due to the similarity of the detection target: mostly of the stresses in plants result in a chlorosis and, thus, in a decrease in chlorophyll concentrations in leaves (Carter and Knap, 2001).

The present work aims at investigating the possibility of discrimination among iron, magnesium, nitrogen and potassium deficiencies in *Vitis vinifera* L. plants by reflectance spectroscopy. Beside spectral variations, due to modified pigmentations related to specific mineral deficiencies, the distribution of the symptoms among leaves and within the same leaf will be investigated. The paper will also focus on the identification of the most informative bands and sites of detection for each mineral deficiency, independently on the cultivar analyzed.

2. Materials and methods

2.1. Plant material

One-year woody cuttings were obtained from canes collected in the ampelographic collection of the *Università degli Studi di Milano*. Different *Vitis vinifera* L. cultivars were tested, to focus on the modifications ascribable to mineral deficiency, independently on the genotype. In each experimental condition, a total of 9 plants were grown: 3 Sangiovese, 3 Cabernet Sauvignon, 1 Sagrantino, 1 Trebbiano Abruzzese and 1 Trebbiano Toscano. All cuttings, obtained the 14th of February 2017, were rooted in a heated mist bed with 2000 ppm IBA in agriperlite substrate in a greenhouse. The temperature of the greenhouse heated counter was fixed at 25 °C, and the plants were subjected to 16 h of light and 8 h of dark conditions each day. Rooting started around the 1st of March. After two weeks of acclimatization to the full strength aerated hydroponic conditions (started the 11th of April), plants were subjected to the different mineral nutrition conditions. The composition of the nutrient solutions adopted in the experiment are summarized in Table 1. All the solutions were prepared with distilled water, to avoid contaminant interferences on the composition of experimental solutions.. Plants were maintained in these conditions for one month, substituting nutrient solution weekly.

Three leaves were selected in each plant, at the end of treatments: one basal leaf; one young leaf; and one apical leaf (length ≈ 2 cm). In basal and young leaves 9 points were analyzed: 3 near veins; 3 in the interveinal areas and 3 in the leaf margins. Apical leaves, due to the small dimensions, were analyzed only at 3 positions, independently to the veins distribution.

2.2. Reflectance spectroscopy analyses

A total of 945 reflectance spectra were collected using a Jaz System spectrometer (Ocean Optics, B.V., Dunedin, USA), completed with a channel with a DPU module and an ILX511b detector, an OFLV-3 filter, an L2 lens, and a 50 µm slit as installed options. A reflection probe QR600-7-VIS125 consisting of a tight bundle of seven optical fibers (600 µm in diameter), in a stainless-steel ferrule (six illumination fibers around one read fiber), was coupled to the spectrophotometer. A probe holder was included to ensure the analytical reproducibility: distance of 12 mm was fixed between sample surface and probe. The instrument was set up with a near infrared–visible (NIR–vis) light source (Ocean Optics) 4095 power setting and an integration time automatically corrected by the instrument. Collected spectra ranged between 341 and 1025 nm and had a spectral resolution of about 0.3 nm. Each spectrum was set up to be the average of 15 spectra, which were directly calculated by the instrument. Calibration with a blank was obtained using a polytetrafluoroethylene (PTFE) diffuse reflectance standard (Ocean Optics B.V.).

Table 1
Composition of the nutrient solutions. Concentrations are reported in mM.

	Control	Iron deficient	Magnesium deficient	Potassium deficient	Nitrogen deficient
$Ca(NO_3)_2$	2	2	2	2	/
$CaSO_4$	/	/	/	/	2
KNO_3	0.75	0.75	0.75	/	/
$MgSO_4$	0.65	0.65	/	0.65	0.65
$(NH_4)_3PO_4$	/	/	/	0.75	/
KH_2PO_4	0.5	0.5	1	/	1.65
H_3BO_3	0.005	0.005	0.005	0.005	0.005
$MnSO_4$	0.001	0.001	0.001	0.001	0.001
$CuSO_4$	0.0005	0.0005	0.0005	0.0005	0.0005
FeIII EDTA	0.08	/	0.08	0.08	0.08

2.3. Data elaboration and statistical analysis

Spectra were first elaborated by using the script reported in Rustioni et al. (2016) by R software (R Core Team, 2015). This first data processing allowed to transform the spectra (400–800 nm) as percentage with respect to the blank; spectra were then normalized at 800 nm (N_{800}) and at 678 nm (N_{678}). To approximate graphs at the absorption spectra, the reciprocal of N_{800} and N_{678} were calculated, obtaining the $1/N_{800}$ and $1/N_{678}$ spectra. In particular, the bands highlighted in $1/N_{800}$, are representative of the pigment concentrations. $1/N_{678}$ spectra were used to put in evidence relative compositional variations in terms of pigments with respect to the chlorophyll *a*. Then, to highlight variations between tissues (deficient vs control plants; distal vs basal leaves; veins/leaf margins vs interveinal areas), each spectrum of the considered tissues (deficient leaves; distal leaves and vein areas/ leaf margins) were subtracted by the average spectra of the respective control condition (control plants; basal leaves; interveinal areas) of the same genotype. A similar data elaboration is reported in Rustioni et al. (2017). Statistical variability of the spectra was evaluated by considering the error bars (95% CI). These analyses were obtained by using Microsoft Office Excel and SPSS statistical software (version PASW Statistics 24, SPSS, Inc. Chicago, IL).

3. Results

3.1. Chlorosis symptoms in the whole plant

This paper will be focused on three main absorption regions. The main pigments absorbing in the red region are chlorophylls. In particular, 678 nm is considered the reference wavelength for chlorophyll *a*, while lower red wavelengths (≈650 nm) are indicative of chlorophyll *b* absorption. The peak around 495 nm (blue-green region) indicate the presence of yellow pigments such as carotenoids, but it also includes absorption bands of chlorophylls (*b* and *a*). In the green region (≈550 nm) the radiation is absorbed by red pigments, mainly represented by anthocyanins.

Fig. 1a shows the average spectra of the 5 nutritional conditions. All mineral deficiencies produced chlorotic symptoms resulting in a decrease in chlorophylls (not significant for K deficiency), highlighted by the lower spectral values around 678 nm. The Mg deficiency determined the lowest spectra values at this wavelength. Despite the chlorophyll concentration similar to Fe deficient plants, N deficient plants had an absorption in the blue-green region similar to the control. Thus, decrease in chlorophylls should be associated to the accumulation of yellow pigments, such as carotenoids, to compensate decrease in chlorophyll absorption contribution at these wavelengths. Fig. 1b shows the variation in pigment proportions with respect to chlorophyll *a*. Relative increase in yellow pigments appeared to be significant for both N and Mg deficient plants. The variation of pigment absorption contribution in the blue-green region is also demonstrated by the hypsochromic shift of the main band in N and Mg deficient plants. Also, the proportion between chlorophyll *a* and *b* appeared to be modified by