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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.

KEYWORDS: *Vitis vinifera*; mineral deficiency; abiotic stress; symptom identification

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[1, 2] and abiotic stresses[3-5]. The plant adaptation to environmental conditions could also promote mechanisms able to modify plant interactions with the surrounding stresses[6]. 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[7]. Also, excessive radiation does not necessarily result in evident photo-oxidative sunburn symptoms: the berry susceptibility depends on the cultivar and the phenological phase[8]. 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.[9] 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.[10] 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.[9] studied potassium deficiency in grapevine by using hyperspectral reflectance. Martín et al.[11] and Meggio et al.[12] proposed the use of remote sensing indices for the spatialization of iron deficiency chlorosis. Taskos et al.[13] tested 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[14].

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 hours of light and 8 hours 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.).

2.3. Data elaboration and statistical analysis

Spectra were first elaborated by using the script reported in Rustioni et al.[4] by R software (R Core Team)[15]. 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.[16]. 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 (≈650nm) 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.

Figure 1-a 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

137 associated to the accumulation of yellow pigments, such as carotenoids, to compensate decrease in
138 chlorophyll absorption contribution at these wavelengths. Figure 1-b shows the variation in pigment
139 proportions with respect to chlorophyll *a*. Relative increase in yellow pigments appeared to be significant
140 for both N and Mg deficient plants. The variation of pigment absorption contribution in the blue-green
141 region is also demonstrated by the hypsochromic shift of the main band in N and Mg deficient plants. Also,
142 the proportion between chlorophyll *a* and *b* appeared to be modified by mineral deficiency. In particular, N
143 deficiency appeared to produce a lowest chlorophyll *b* ratio (≈650 nm).

144

145 *3.2. Symptom characterization*

146 Depending on mineral deficiency, the symptoms could prevalently show an acropetal or a basipetal
147 development. To support spectra interpretation, we remind that figures 2 (average spectra) and 3
148 (variations with respect to the control) differentiate basal, young and apical leaves, while figures 4 report
149 the spectral variations between distal and basal leaves within the same plant. Figures 6 show the spectral
150 variations within the same leaf, by comparing the reflectance properties of veins and leaf margins with
151 interveinal areas.

152

153 *3.3. Iron deficiency*

154 It is well known that iron deficiency affects plant apexes, and also our data indicate that this condition
155 clearly influence distal leaves pigmentation, while basal leaves are much less affected. In basal leaves,
156 pigment composition of iron deficient plants (fig 2-b1 and 3-b1) is equal to control plants. In basal leaves, a
157 slightly lower concentration in pigments (fig 2-a1 and 3-a1) with respect to the control was observed,
158 however these symptoms were much less evident than the one observed in distal leaves, where iron
159 deficiency clearly caused a decrease in pigmentation with respect to the control (fig 2 and 3). Considering
160 the pigment proportion, iron deficiency increased the chlorophyll *a*/*b* ratio in young leaves (fig. 3-b2).
161 Considering apical leaves, it is interesting to note the higher proportion of anthocyanins in iron deficient

plants (fig. 3-b3). Within the same plant, decrease in leaf pigmentation in distal leaves was observed also in control plants, nevertheless iron deficiency intensified this trend (figures 4-a). Considering young leaves, control plants did not produce significant modifications in pigment proportion, while iron deficiency caused a chlorophyll disequilibrium disfavoring chlorophyll b (figures 4-b1). In apical leaves, again, an increased proportion of red pigments were observed (fig. 5). Considering the variations within the same leaf, the incipient iron deficiency symptoms (fig. 6-a4, 6-b4) produced small modifications with respect to the control (fig 6-a1, 6-b1). In young leaves, veins appeared more pigmented than interveinal areas in both control and iron deficient plants. Nevertheless, veins of iron deficient young leaves showed a lower increase in absorbance in the green region (≈550 nm), resulting in a higher green contrast between veins and interveinal areas with respect to control plants, where the increased absorbance interest all the visible spectrum. Finally, in basal leaves, the lower pigmentation in veins with respect to interveinal areas appeared less marked in iron deficient plants than in control plants.

3.4. Magnesium deficiency

According to the expectations, magnesium deficiency produced chlorotic symptoms in the entire plant (figure 2a). The $1/N_{800}$ spectra in basal leaves were similar to that one observed in nitrogen deficient plants (fig. 2-a1 and 3-a1), while the $1/N_{800}$ spectra in the apex were similar to that one observed in iron deficient plants (fig. 2-a3 and 3-a3). In young leaves, magnesium deficiency produced the strongest depigmentation symptoms (fig. 2-a2 and 3-a2) among the tested conditions. Also, the pigment relative proportion was affected by magnesium deficiency. An increase in the chlorophyll a/b ratio was observed, counterbalanced by a relative increase in yellow pigments proportion (fig 2-b and 3-b) with respect to control leaves. This variation was particularly evident in young leaves. In basal and apical leaves, this effect is less evident, however the slight hypsochromic shift of the 495 band; the modification in the shoulder around 430 nm; as well as the lower chlorophyll b relative absorption around 650nm (which should, theoretically, results in a decrease in the blue absorption band of the same pigment) reveal the modification of the yellow pigments

187 composition (fig. 2-b1 and 2-b3). Considering the symptoms distribution within the same plant (fig.4), the
188 chlorotic symptoms appeared to be particularly marked in young leaves (much less pigmented than basal
189 leaves), while the apex were quite similar to control plants. Distal leaves appeared to have a higher
190 chlorophyll a/b ratio, and a relative higher contribution of yellow pigments (maximum absorbance around
191 430 nm). Considering the pigment distribution within the same leaf, magnesium deficiency produced
192 evident modifications with respect to the control plant. In basal leaves, the veins are expected to be less
193 pigmented than interveinal areas (fig. 6-a1), but in magnesium deficient plants this difference was not
194 significant in mostly of the visible spectrum (fig. 6-a3), and veins appeared to be higher absorbance in the
195 yellow spectral region (≈600 nm) probably also due to lower chlorophyll a/b ratio (fig. 6-b3). Also, a
196 characteristic pigmentation of basal leaf margins (significantly different than interveinal areas only in
197 magnesium deficient plants – fig. 6-a) appeared. A slight reddish color resulted from the relative increase of
198 the absorbance in the green spectral region (fig. 6-b3). Concerning young leaves, an intensification of the
199 chlorophyll accumulation in veins, with respect to interveinal areas, was observed. Thus, the increased
200 relative reflectance in the green region of veins, resulted in the characteristic interveinal chlorotic
201 symptoms appearance (fig. 6-3a). In general, magnesium deficiency produced a strong variability in the
202 pigment proportions within the same leaf, in both basal and young leaves (fig. 6-b3).

203

204 *3.5. Nitrogen deficiency*

205 Nitrogen deficiency affects the leaf pigmentation of the entire plant. However, it is worth to notice that in
206 basal leaves nitrogen deficiency caused the strongest discoloration, while in distal leaves (less pigmented
207 also in control plants), the symptoms were more intense in magnesium and iron deficiency conditions (fig
208 2-a; 3-a). In fact, also the difference between distal and basal leaves of the same plant (figure 4-a) showed
209 small variabilities, resulting in the lowest variations between apical and basal leaves (figure 4-a2).
210 Furthermore, chlorophyll a variation between young and basal leaves was equal to the ones observed in
211 control plants (figure 4-a1, wavelength 678 nm). These results are coherent with the visual aspect of

212 nitrogen deficient plants: similar color among all the plant. Nitrogen deficiency generally produced a
213 disequilibrium among pigments localized in photosystems. In comparison to the control plants, our data
214 evidenced a relative increase of carotenoids and a relative decrease of chlorophyll b, with respect to
215 chlorophyll *a* concentration in all the leaves of nitrogen deficient plants (fig. 2-b and 3-b). Within the same
216 plant, the carotenoid ratio increase was particularly evident in basal leaves, while the disequilibrium
217 between chlorophyll a (which kept the same variation among basal and young leaves as the control plants –
218 figure 4-a1) and chlorophyll b was stronger in young leaves (figure 4-b1), resulting in a general decrease in
219 the absorption ratio in the green spectral region in distal leaves (fig 4-b). Considering the variability within
220 the same leaf (figure 6-a2; 6-b2), despite the increased variability among sample (thicker error bars), young
221 leaves did not show significant variations among veins, leaf margins and interveinal areas (observed in
222 control plants). In basal leaves, the lower pigmentation of veins was less remarked than the one recorded
223 in control plants. On the contrary, a decrease in chlorophyll contents in the leaf margins was observed.
224 Considering the pigment proportions, nitrogen deficiency produced an increase in the absorbance in the
225 green region in the leaf margins with respect to interveinal areas of the basal leaves, probably due to red
226 and brown pigment accumulation (fig. 6-b2). Contrariwise, veins of basal leaves had lower concentration of
227 the same pigments than interveinal areas (fig. 6-b2).

228

229 *3.6. Potassium deficiency*

230 Potassium deficiency symptoms were not particularly evident: deficient spectra nearly overlap control
231 spectra considering both pigment concentrations (fig.2-a) and proportions (fig.2-b). Nevertheless, the small
232 modifications in the basal leaf pigmentations appeared to be significant ($\Delta(1/N_{800}) \neq 0$ in figures 3-a1 and 3-
233 b1). The significant chlorotic symptoms in basal leaves appeared to be nearly imperceptible in young leaves
234 and become undetectable in apical leaves. It is interesting to note the increased proportion of anthocyanins
235 in young leaves (figure 3-b2), resulting in a faint reddish color. As observed for all the nutritional conditions,
236 distal leaves appeared less colored than basal leaves, however this trend was less intense in potassium

deficient plants, especially in the green spectral region (fig. 4-a). Considering the pigment proportions, potassium deficiency produced an intensification of the reddish color (anthocyanins relative increase) in the distal part of the plant (fig. 4-b). Considering the spectral variations within the same leaf, potassium deficiency slightly decreased the gap between veins and interveinal areas in basal leaves with respect to control plants (Figures 6-a5, 6-a1). Considering young leaves, iron deficiency overrides the higher pigmentation of leaf margins observed in control plants and intensified the veins pigmentation with respect to the interveinal areas (Figures 6-a5, 6-a1). A light intensification of the pigment proportion variability within the same leaf was observed with respect to the control leaves (Figures 6-b5, 6-b1).

4. DISCUSSION

4.1. Iron deficiency

Iron deficiency produced evident chlorotic symptoms in the plants (fig. 1-a), due to the consequent chloroplast dysfunction. The deficiency of physiologically active iron decrease the number of thylakoid membranes in chloroplast as iron ions are constituents of the electron transport chains. Therefore, the dysfunction of chloroplasts results in a rapid inhibition of chlorophyll biosynthesis, leading to leaf yellowing[17]. Iron deficiency clearly showed a basipetal symptom development, affecting first of all the youngest leaves (figures 2-a; 3-a; 4-a). This is the consequence of difficulties in iron redistribution within a plant due to the poor phloem mobility and, in general, to the chelation requirement for iron transport[18, 19]. In a previous work, the exacerbation of this trend highlighted a compensation effect of the iron deficiency in basal leaves, which appeared more concentrated then control plants in photosynthetic pigments[16]. It is worth to notice the well-known higher susceptibility of American *Vitis* species to iron deficiency with respect to *Vitis vinifera* cultivars[20]. Anyway, the results here presented clearly confirmed the classical basipetal symptom distribution of iron deficiency (fig. 4-a). In accordance to the previous results[16], iron deficiency resulted in an increased relative green reflectance in the veins with respect to the interveinal areas (which appeared chlorotic). Interesting variations in the red pigmentation (≈ 550 nm) were

particularly evident in apical leaves (figures 3-b3 and 5). This evidence was already observed in *Vitis* spp. rootstocks[16]. Recently, it has been demonstrated that anthocyanin synthesis in apical leaves was stimulated by iron deficiency by Caramanico et al.[21].

4.2. Magnesium deficiency

Chlorosis resulting from magnesium deficiency (figure 1-a) is not only due to the role of this ion as central atom of the chlorophyll molecules[22]. Being an ATP cofactor, magnesium deficiency causes dysfunctions in cell osmotic pressure regulation (resulting in a leaves transpiration decline) and in phloem transport regulation (resulting in the accumulation of sugars and starch in the source organs and, thus, downregulating the photosynthetic systems)[22-24]. In the case of magnesium deficiency, chlorotic symptoms could appear due to lower synthesis of chlorophylls in deficient plants or to the magnesium recycle strategy dechelating magnesium from photosynthetic pigments and making the ions available for apical tissue growth[22]. In fact, together with nitrogen, phosphorus and potassium, magnesium is considered a mobile ion, being found in high concentrations in phloem[24]. In our experiment, magnesium deficiency affected the entire plant pigmentation, nevertheless the symptoms were particularly evident in young leaves (figures 4). It has been found that magnesium retranslocation from old mature leaves is not as vigorous as that from young mature leaves[24].

The intensification of the chlorophyll accumulation in veins with respect to interveinal areas (fig. 6-3a) is coherent with the characteristic leaf interveinal chlorosis of magnesium deficient plants[22]. Generally, magnesium deficiency produces a higher reduction of chlorophyll b than chlorophyll a in chlorotic leaves[22], in accordance with our results (fig 2-b and 3-b). This trend is indicative of a relative loss of PSII peripheral antenna (Chlorophyll b is mostly associated with the light harvesting complex of photosystem II), or, alternatively, of a change in photosystem stoichiometry in favor of photosystem I[22]. Finally, magnesium deficiency produces a leaf premature senescence and it includes ROS and metal accumulations[24]. In rice, it has been reported the synthesis of anthocyanins[23]. In our results, the incipient leaf senescence of

magnesium deficient plants was also evidenced by the general yellowing and by the slight reddish color of basal leaf margins (fig. 6-b3).

4.3. Nitrogen deficiency

Following hydrogen, carbon and oxygen, nitrogen is the fourth major constituent of plants. It is an essential constituent of amino acids and nucleobases, and, thus, it is mandatory for all the main plant metabolism functionality. Considering leaf symptoms, it is worth to notice the involvement of nitrogen atoms in the chlorophyll molecules. In our experimental conditions, nitrogen deficiency produced evident symptoms, easily detectable also by visual inspection: the plant slowed down its growth, and leaves appeared uniformly yellow.

The symptoms particularly affected basal leaves (figure 4-a2), as expected in the case of a mobile ion such as nitrogen[24]. Nevertheless, the growth lag with respect of the other experimental conditions and the incipient senescence of the entire canopy (increased proportion of yellow pigments) are in agreement with the expected nitrogen redirection to the root system (stimulation of the hypogeum organ growth, searching for limiting resources)[25].

In agreement with Tewari et al.[26], nitrogen deficiency caused a general decrease in the chlorophyll a/b ratio (figure 3-b). Despite the chlorophyll decrease, carotenoids did not decrease their concentrations with respect to the control (Figures 1; 2 and 3), probably due to the nitrogen participation in the chlorophyll structure. Furthermore, as H_2O_2 accumulation is expected as response to nitrogen deficiency[26], and carotenoids could play a role in the oxidative stress management.

Leaves of nitrogen deficient plant appeared quite uniform in color, especially in distal leaves (figure 6-a2). While the strong modifications in pigment proportions shown in figure 6-b2, should be ascribed to the presence of necrotic areas in the basal leaves, concentrated in the margins. The broad band highlighted in figure 6-b2 should be ascribed to the accumulation of both anthocyanins and oxidized phenolics[28], probably resulting from the H_2O_2 in nitrogen deficient leaves[26].

4.4. Potassium deficiency

Potassium is the most abundant cation in plant tissues. It plays major role in maintaining cell turgor, stomatal activity and membrane potential, mobilizing assimilates through membrane transport processes and activating enzymes[28, 29]. In our experimental conditions, potassium deficiency did not produce strong symptoms, probably due to the absence of fruits. In fact, grape berries accumulate high concentrations of potassium[30, 31], becoming a strong potassium sink particularly during ripening[28]. Furthermore, being a highly mobile ion, potassium stored in the cuttings could be re-translocated to cope the deficiency in our experimental conditions[24, 28]. The high mobility of potassium ions is also coherent with the acropetal symptom development evidenced by our results (Figure 3-a). In contrast to Mg, translocation of K to the young sink organs from old mature leaves was reported to be comparable to that one from young mature leaves under K-deficient conditions[24]. Potassium ions play a fundamental role in plant growth mainly in the water regulation and thus, growing tissues are strong sink for this element [28].Therefore, symptoms of potassium deficiency are concentrated on the basal leaves (Figure 3-a).

Potassium deficiency modified the pigmentation distribution within the same leaf, especially in young leaves (figure 6-a5). This could be due to the decreased rate of assimilate export and consequent soluble sugar accumulation in leaf of potassium deficient plants[32], starting from leaf areas more distant from the main veins. In fact, young leaves of deficient plants showed a decreased pigmentation of margins and a relative absorbance intensification around veins with respect to interveinal areas (compared to control plants) (figure 6-a1 and 6-a5). In general, the proportion between chlorophyll *a* and *b* was not affected by potassium deficiency (figure 2-b). In agreement with the observation of Zhao et al.[32], potassium deficiency seems to mainly produce a reduction in the photosynthesis system, reducing synchronously both chlorophylls.

In accordance with our results (higher proportion of anthocyanin pigments in young leaves), Smart et al.[9] found an increased red coloration in potassium deficient plants. Potassium have a critical role in plant

stress responses. For example, stomatal dysfunctions resulting from potassium deficiency could disturb the balance between ROS production and antioxidant defense resulting in oxidative stresses[26, 29]. Nevertheless, low levels of ROS could be involved in the stress-signaling pathway for stress responses[29]. We can suppose that incipient potassium deficiency obtained in the framework of our experiment slightly stimulated the accumulation of flavonoids (such as anthocyanins) with respect to other pigments which could be involved in ROS detoxification in case of stress intensification, resulting in brown pigment accumulation[33].

5. CONCLUSIONS

All mineral deficiencies considered in this experiment produced chlorotic symptoms in the plant canopy. Nevertheless, the intensity and distribution of the symptoms among the plant, and variations in the pigment proportions were able to significantly discriminate the stress origins. These characteristics perfectly fit with the well-known physiological roles of each element, strengthening the representativeness of our data. Thus, reflectance spectroscopy could find applications in the symptom detections and identification beside its quantification. This knowledge could support the development of new tools able to support viticulture by the identification of mineral deficiencies in field conditions. In fact, mineral deficiencies caused specific relative pigment variations also within the same plants (or reference leaf), and, thus, they could be highlighted also without a well-fed control plant. Finally, the identification of the most interesting symptomatic target and of the most relevant reflectance bands could support further research, including index development for symptom intensity quantifications and definition of threshold values for fertilization management. Finally, due to rapidity and low cost of the technique, future applications could support both technical requests and scientific research (e.g.: screening of tolerant genotypes; sensors development; fertilization studies…).

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443

TABLE

Tab. 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

FIGURE CAPTIONS

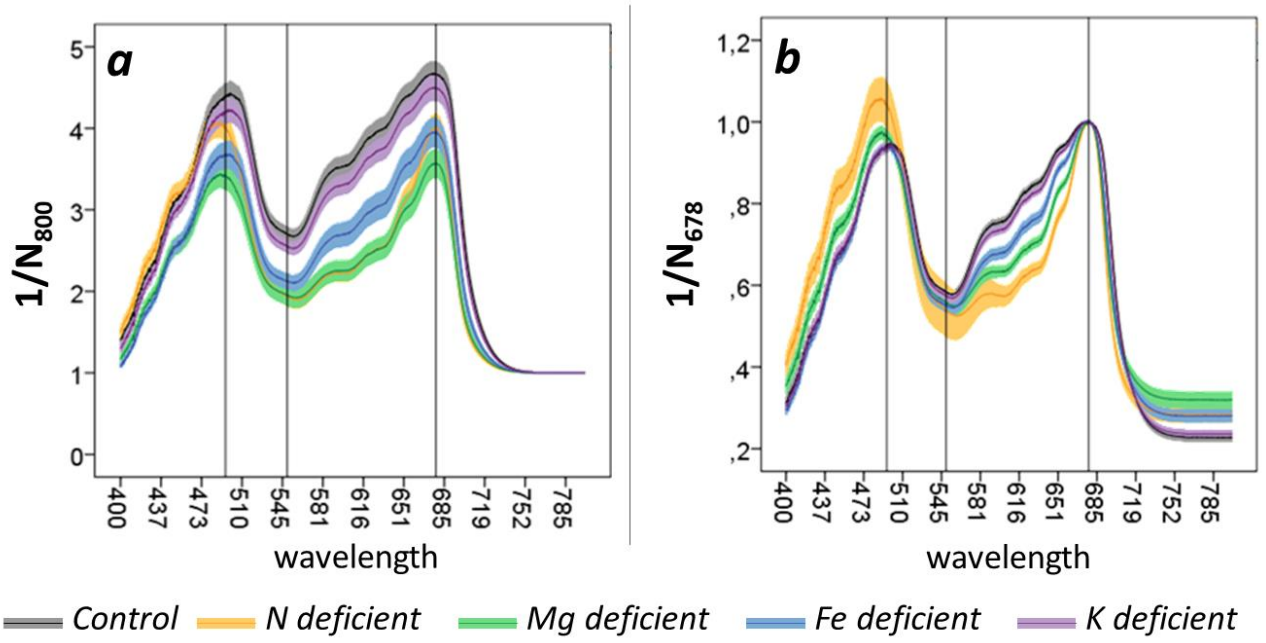


Fig. 1: average spectra of control, N, Mg, Fe and K deficient plants. Figure 1-a shows the $1/N_{800}$ spectra, and the positive bands represent pigment absorption. Figure 1-b shows the $1/N_{678}$ spectra, highlighting proportional variation among pigments with respect to chlorophyll a. Vertical lines are in correspondence

of the wavelengths: 495 nm; 550 nm and 678 nm. The line thickness is representative of the error bars (95% CI).

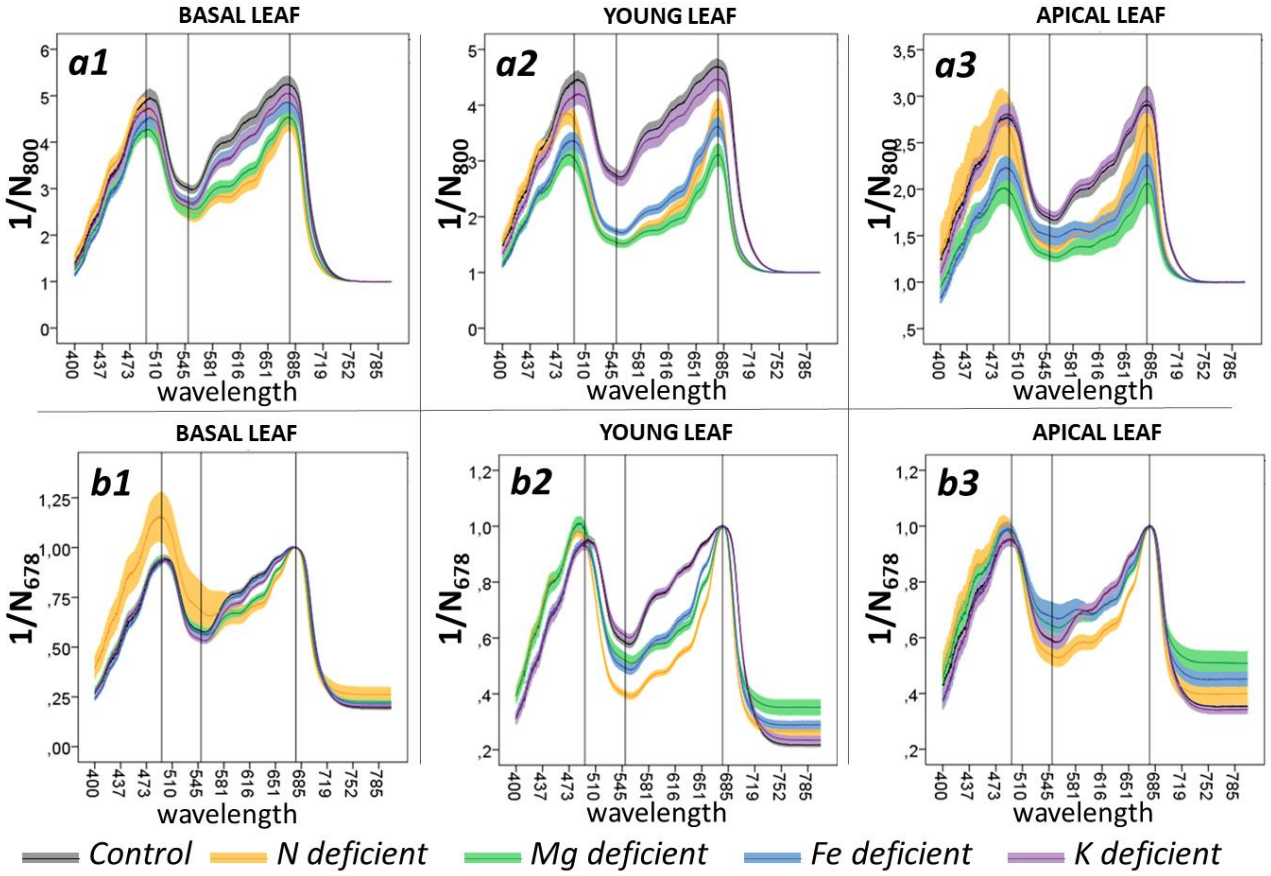
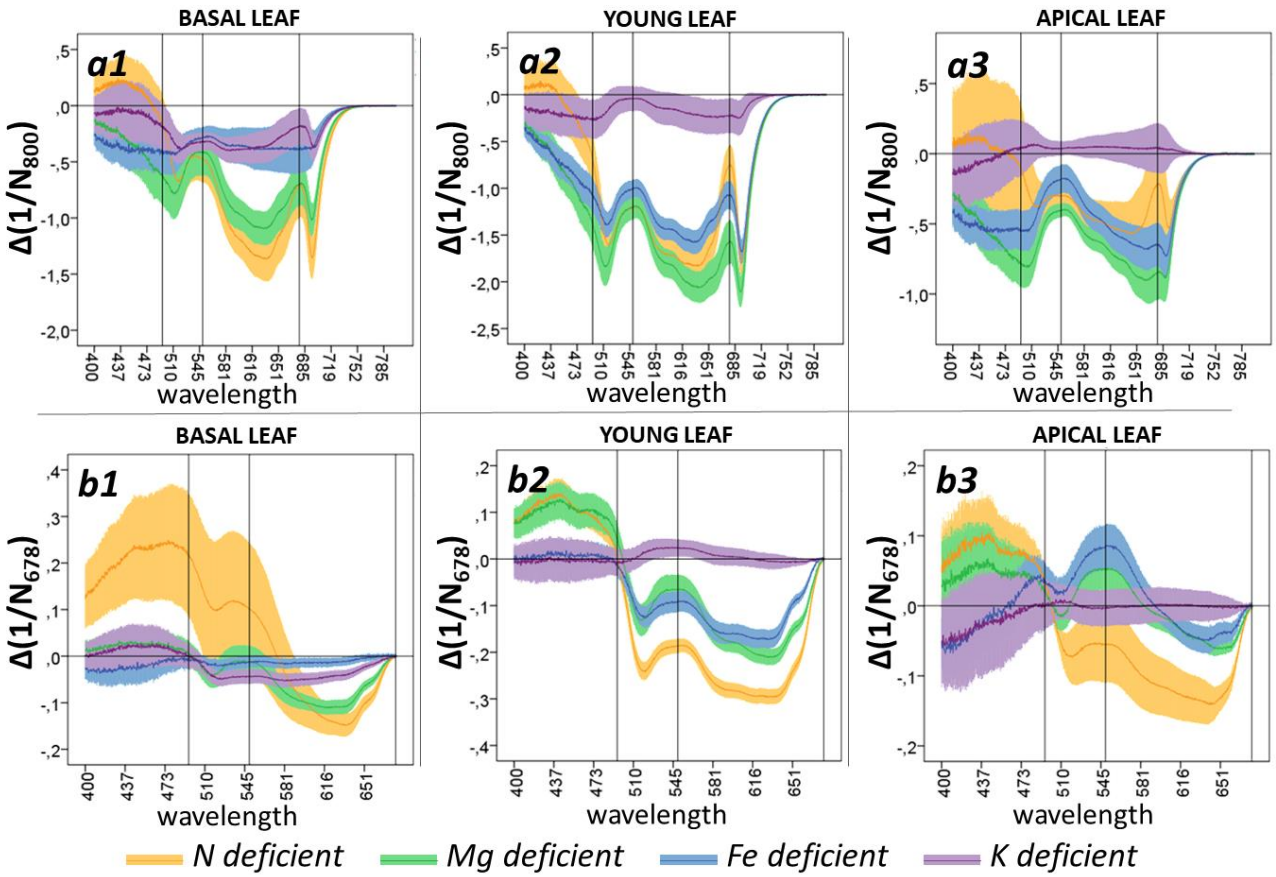
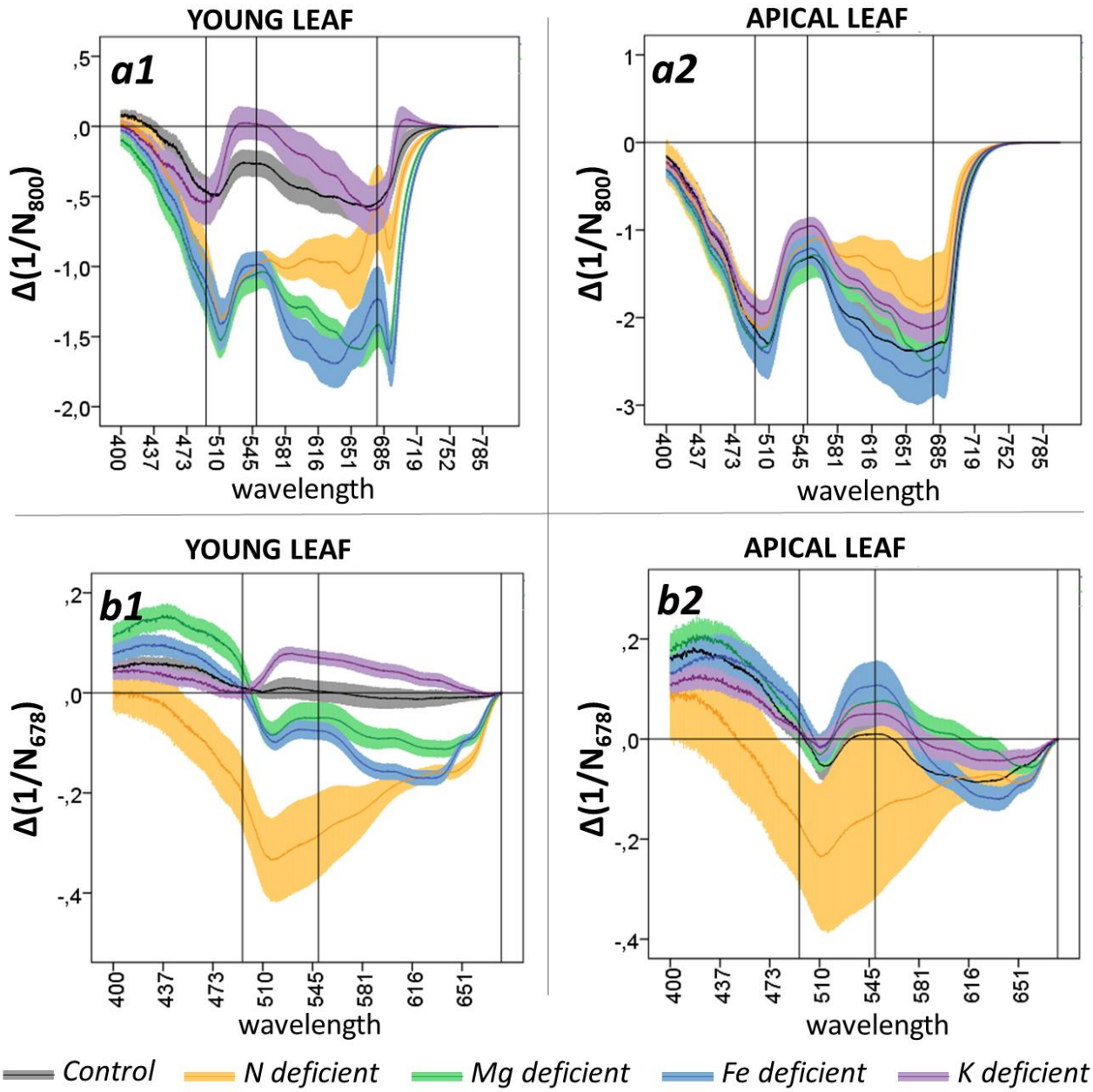


Fig. 2: average spectra of control, N, Mg, Fe and K deficient plants in basal, young and apical leaves. Figures 1-a show the $1/N_{800}$ spectra, and the positive bands represent pigment absorption. Figures 1-b show the $1/N_{678}$ spectra, highlighting proportional variation among pigments with respect to chlorophyll a. Vertical lines are in correspondence of the wavelengths: 495 nm; 550 nm and 678 nm. The line thickness is representative of the error bars (95% CI).



460

461 **Fig. 3**: spectral variation between N, Mg, Fe and K deficient plants and the average control spectra for each
462 cultivar and leaf position in $1/N_{800}$ (a) and $1/N_{678}$ (b) spectra. Positive values indicate increase in pigment
463 concentrations (a) or relative increase of pigments in relation to chlorophyll a concentration (b). Negative
464 values indicate decrease in pigment concentrations (a) or relative decrease of pigments in relation to
465 chlorophyll a concentration (b). No significant variations are indicated by spectra overlapping the 0 value.
466 Vertical lines are in correspondence of the wavelengths: 495 nm; 550 nm and 678 nm. The line thickness is
467 representative of the error bars (95% CI).



468

469 **Fig. 4**: spectral variation $1/N_{800}$ (a) and $1/N_{678}$ (b) between distal (young and apical) leaves and basal leaves.

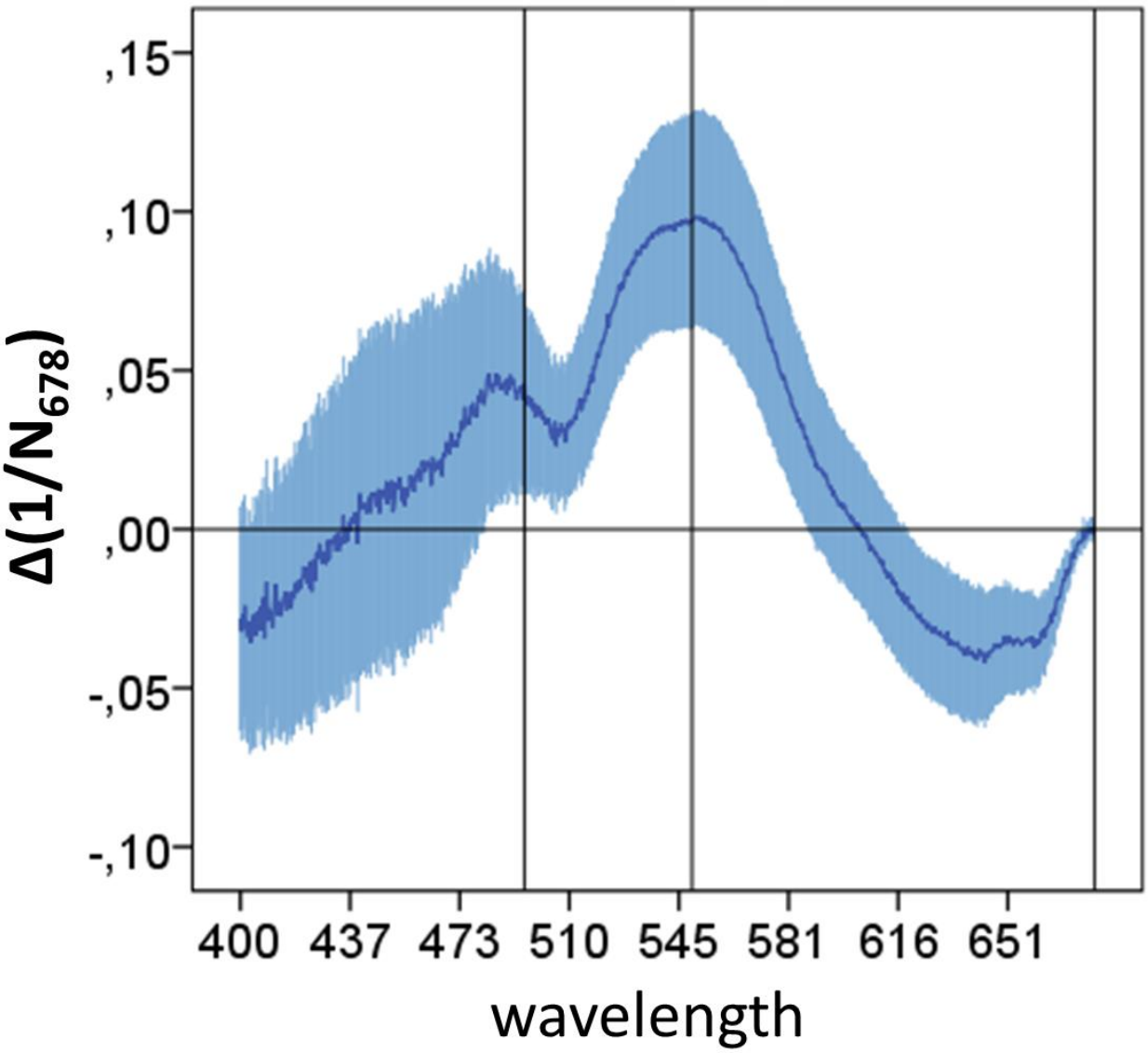
470 Negative values indicate decrease in pigment concentrations (a1; a2) or relative decrease of pigments

471 composition with respect to chlorophyll a concentration (b1, b2). Positive values indicate increase in

472 pigment concentrations (a1; a2) or relative increase of pigments in relation to chlorophyll a concentration

473 (b1, b2). No significant variations are indicated by spectra overlapping the 0 value. The line thickness is

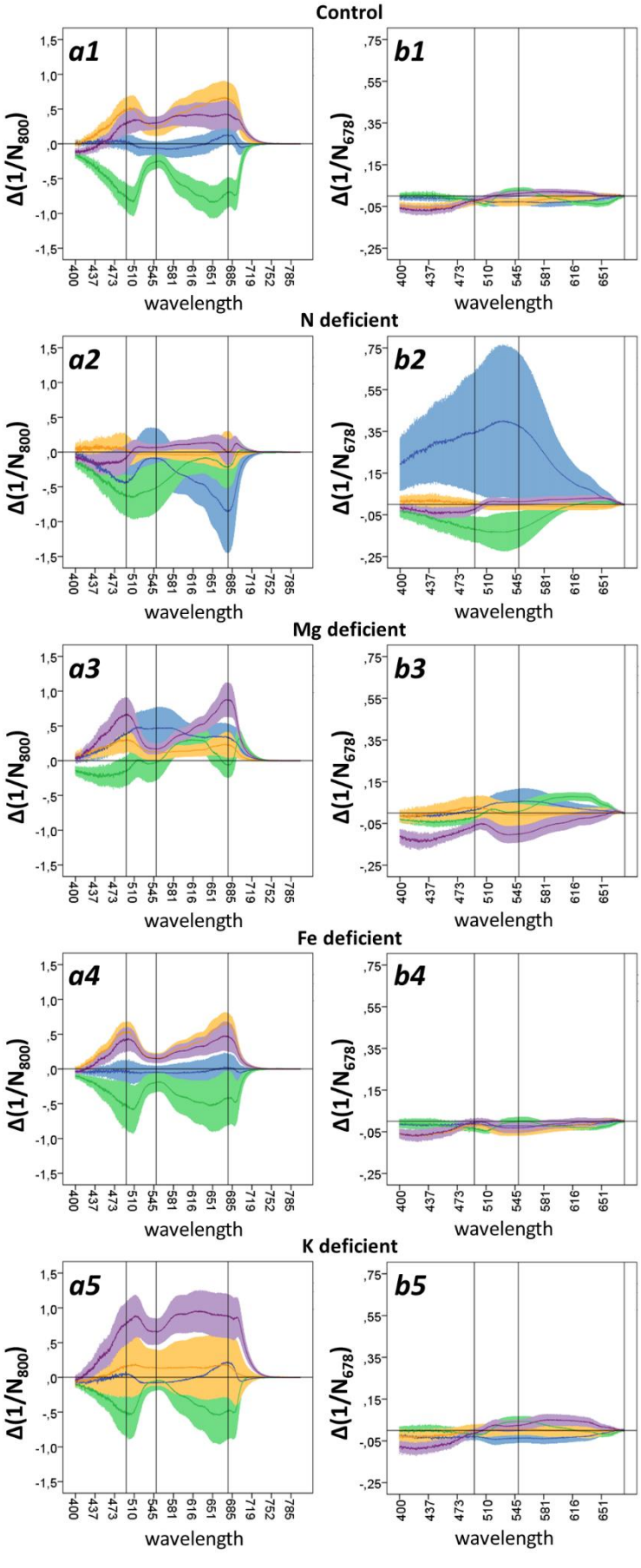
474 representative of the error bars (95% CI).



475

476 **Fig. 5**: detail of the iron deficiency effect on the increased relative proportion of anthocyanins in apical

477 leaves with respect to basal leaves. The line thickness is representative of the error bars (95% CI).


Control
a1
b1
N deficient
a2
b2
Mg deficient
a3
b3
Fe deficient
a4
b4
K deficient
a5
b5
Δ(1/N800)
Δ(1/N678)
wavelength
Basal leaves – Veins
Basal leaves – Leaf margins
Young leaves – Veins
Young leaves – Leaf margins

478

Fig. 6: spectral variation $1/N_{800}$ (a) and $1/N_{678}$ (b) between veins or leaf margins and interveinal leaf areas young and basal leaves in each nutritional condition. Negative values indicate decrease in pigment concentrations (a) or relative decrease of pigments composition with respect to chlorophyll a concentration (b). Positive values indicate increase in pigment concentrations (a) or relative increase of pigments in relation to chlorophyll a concentration (b). No significant variations are indicated by spectra overlapping the 0 value. The line thickness is representative of the error bars (95% CI).