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Nondestructive diagnostics of magnesium deficiency based on distribution features of chlorophyll concentrations map on cucumber leaf

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13 Abstract

14 A new and nondestructive method for diagnosing magnesium (Mg) deficiency based on
15 chlorophyll concentration distribution features of cucumber leaves was proposed. Mg
16 deficient cucumber plants and Control plants were grown under non-soil conditions
17 with special nutrient supply. Cucumber leaves were employed to collect hyperspectral
18 images using a visible and near infrared (VIS/NIR) hyperspectral imaging system (400-
19 900 nm) and determine reference chlorophyll concentrations using high performance
20 liquid chromatography (HPLC). An optimal chlorophyll concentration calibration
21 model (Rp = 0.9087) was constructed and used to detect chlorophyll distribution maps
22 of Mg deficient leaves and Control leaves. Results shown that chlorophyll content
23 distributed more unevenly on Mg deficient leaves than Control leaves. The Standard
24 Deviation (SD) value of the chlorophyll content at all the pixels on a chlorophyll
25 distribution map was calculated for Mg deficient diagnostics. An Mg deficiency
26 diagnostics model with satisfied performance (diagnostic rate 93.33%) was obtained.
27 The result indicated the SD value of chlorophyll concentrations on the whole cucumber
28 leaf could be employed to diagnose Mg deficiency nondestructively.

29 Keywords: diagnostics; magnesium; deficiency; distribution; chlorophyll; cucumber

30

31

1. Introduction

Magnesium (Mg) is an essential macronutrient element in plant growth and productivity, the shortage of Mg element causes imbalances in plant growth and drastically most importantly affect the quality and yield of agricultural products (Khaitov, 2018; Kwano et al., 2017; Ortas, 2018). Low level of Mg supplement often result in low chlorophyll production, which drastically impact the plant growth, flowering and fruit bearing (Farhat et al., 2016; Farzadfar et al., 2017; Saghaiesh et al., 2019). Moreover, Mg shortage also affects the production of chemical components that are connected with the product qualities, like appearance, aroma, taste and nutrition (Canizella et al., 2015; Gomez-Perez et al., 2018; Nikolic et al., 2014; Schurt et al., 2014). Although Mg deficiency diagnostics methods based on the Mg element content analysis with chemical methods, like inductively coupled plasma mass spectrometry, get good performance for diagnosing Mg deficient plants, the chemical analysis process is time-consuming, laborious and high cost (Guo et al., 2016; Nartvaranant, 2018). Therefore, more attention should be paid to the diagnostics methods that assess Mg status more efficiently.

Rapid and nondestructive diagnostics methods based on leaf chlorophyll analysis have been proposed to diagnose nutrients deficiencies in plants. It is well known that the shortage of Mg element results in low chlorophyll in plant leaves and low chlorophyll density regions appear on Mg deficient plant leaves, so chlorophyll density can be used reasonably as an indicator for Mg deficient diagnostics (Samborska et al.,

2018; Tatagiba et al., 2016). Many research demonstrated that reflectance in Vis/NIR spectrum is sensitive to the chlorophyll concentrations of leaf tissues (Cayuela et al., 2014; Kurenda et al., 2014; Schouten et al., 2014; Zhou et al., 2017). Therefore rapid and nondestructive diagnostics methods based on soil and plant analyzer development (SPAD) analysis, visible (Vis) spectral technology or near infrared (NIR) technology have been proposed to diagnose Mg deficiency (Marian et al., 2009; Shaahan et al., 1999). In these methods, one value was used to represent the chlorophyll level of the whole leaf sample. However, Mg deficiency causes a low chlorophyll density in small regions of a cucumber leaf at the early stage. The measurement of chlorophyll content on the whole cucumber leaf can improve the performance for diagnosis of Mg deficiency.

Hyperspectral imaging technology has been used to detect the chlorophyll concentration distribution map on cucumber leaves. Hyperspectral imaging acquire both spectral and spatial information from an object and produces a three dimensional data cube that includes the spectral data of each pixel. As the spectral data of pixels is sensitive to the quality compounds in biological products, hyperspectral imaging technology has been employed to determine the distribution of various quality indexes in food and agricultural products, like triterpene acids in loquat leaf (Shi et al., 2018), compounds (protein, carbohydrate, sialic acid) in edible bird's nest (Shi et al., 2017), chlorophyll in pepper leaves (Yu et al., 2016), tenderness in salmon (He et al., 2014), etc. These previous studies indicate that hyperspectral imaging technology could

74 determine the chlorophyll concentration distribution map on a cucumber leaf, and the
75 chlorophyll distribution map can be used for nutrient diagnostics.
76 In this paper, the distribution features of chlorophyll content were employed to
77 establish new diagnostics method for diagnosing Mg deficiency. Mg deficient leaf
78 samples were cultured under non-soil conditions and Mg status of the leaf samples were
79 confirmed by Mg element analysis. Cucumber leaves were employed to collect
80 hyperspectral images using a VIS/NIR hyperspectral imaging system (400-900 nm) and
81 determine reference chlorophyll concentrations using high performance liquid
82 chromatography (HPLC). The chlorophyll distribution maps of Mg deficient leaves and
83 Control leaves were detected nondestructively. The main objectives of this paper are to
84 (1) find differences in chlorophyll content distribution maps of Mg deficient and
85 Control leaf samples; (2) extract suitable chlorophyll distribution features for
86 diagnosing Mg deficiency based on chlorophyll content distribution maps.
87
88 2. Materials and methods
89 2.1 leaf samples
90 Fresh leaf of Mg deficient cucumber plants and Control cucumber plants (Cucumis
91 sativus, Biyu-3, FuNong seeds Co. Ltd., Shanghai, China) were used as analytical
92 samples. Cucumber seeds were germinated in plug plant seed tray with perlite rock and
93 distilled water. Seedlings were thinned to one plant/pot with a planting density of 20
94 cm × 20 cm. Complete nutrient solution with all necessary elements was applied to the

cucumber plants before the three-leaf stage, then all the cucumber plants were divided into two groups, Control group and Mg deficiency group. Control fertilization with all macronutrients was performed to plants in Control group, and no Mg fertilization (complete nutrient solution with Mg eliminated) was performed to plants in Mg deficiency group. The components of complete nutrient solution were NO_3 11.75 mmol L^{-1}, NH_4 1.0 mmol L^{-1}, H_2PO_4 1.25 mmol L^{-1}, K 6.5 mmol L^{-1}, Ca 2.75 mmol L^{-1}, Mg 1.0 mmol L^{-1}, SO_4 1.0 mmol L^{-1}, Fe 15 μmol L^{-1}, Mn 10 μmol L^{-1}, Zn 5 μmol L^{-1}, B 25 μmol L^{-1}, Cu 0.75 μmol L^{-1}, Mo 0.5 μmol L^{-1}. All the plants were grown under non-soil conditions (perlite rock) in green houses at Jiangsu University in China (32.11N, 119.27E). The amount of nutrient solution for each plant per day was 500ml for the first two weeks after transplanting and 800-1000ml until harvesting. The experiment was performed in three replications, and 60 Mg deficient plants and 60 Control plants were obtained.

2.2 Mg element determination

To detect the nutrient status of plants in Mg deficient group and Control group, Mg element concentrations in nutrient deficient groups and Control group were determined. 45 leaves at the first three nodes, the middle nodes, and the youngest nodes in Mg deficient and Control plants were collected for Mg elements determination. For each Mg element determination, 0.4g fresh leaf tissues without leaf veins was dried at 80℃ to a constant weight. The dry matter was dissolved into 25 mL nitric and perchloric acid mixture, placed on a electric hot plate and heated at 200 °C for 60 mins. Then the digest

was analyzed using atomic absorption spectrophotometer (AA300, PerkinElmer Inc, USA) (Sofi et al., 2017).

2.3 Hyperspectral image collection

A line-scan reflectance hyperspectral imaging system with a spectral resolution of 2.8 nm was employed to collect raw hyperspectral image of cucumber leaf samples. The imaging system includes a spectrograph (ImSpector, V10E, Spectra Imaging Ltd., Finland), a high-performance camera (Bci4-1300, C-Cam Technologies, Belgium), an illumination unit consisting two fibre-optic light-guiding branches connected to a DC illuminator (DC-950A, Fiber Lite Illuminator，USA), a transitional stage operated by a stepper motor (Zolix SC300-1A, Zolix. Corp., China), an enclosure (450×600×750 mm) and a computer. Cucumber leaf sample was placed on the conveyor belt to be scanned line by line using the hyperspectral imaging system. In order to improve the signal-to-noise ratio, the speed of sample movement, exposure time and the distance of a sample from the camera were optimized and set to 10 mm/scan, 45 ms and 18 cm. White reference image (a white ceramic tile considering to be ~99% reflective) and dark reference image (an image taken when the light is off and the lens is covered) were acquired to correct the raw images. The three dimensional data cube of a cucumber leaf hyperspectral image was shown in Fig. 1.

Insert Fig. 1 here.

2.4 Chlorophyll concentration distribution map determination

As described in 2.3, the hyperspectral imaging data cube contains a spectrum with a specific wavelength range for each pixel in a 2-dimensional image of the sample. At the same time, researchers demonstrated there are good relationship between leaf spectra and chlorophyll concentrations (Cayuela et al., 2014; Kurenda et al., 2014). Therefore, it possible to estimate the distribution of chlorophyll concentrations on the whole cucumber leaf based on the spectra data of each pixel and the chlorophyll concentration calibration model. Basically, chlorophyll concentration distribution map determination contains three steps, (1) building chlorophyll calibration models, (2) testing the calibration models, and (3) determining chlorophyll distribution map.

2.4.1 Building calibration models

A calibration set and a prediction set were composed of 120 fresh cucumber leaves. After hyperspectral image acquisition, spectral data of cucumber leaves in the calibration set and prediction set was extracted by defining an region of interest (ROI) (50×50pixels) in the center region of hyperspectral images. Partial least squares (PLS), interval partial least squares (iPLS), simulated annealing - Interval partial least squares (SA-iPLS) were employed to build chlorophyll calibration models based on the extracted spectral data and reference chlorophyll concentrations determined using

157 high performance liquid chromatography (HPLC) (Shi et al., 2016; Shi et al., 2012).
158 The root mean square error of cross-validation (RMSECV), the correlation coefficient
159 in the calibration set (Rc), the root mean square error of prediction (RMSEP) and the
160 correlation coefficient in the prediction set (Rp) (Shi et al., 2012) were calculated to
161 evaluate the established calibration models.
162
163 2.4.2 Testing the calibration models
164 An independent testing set was composed of 40 fresh cucumber leaves. After
165 hyperspectral image data acquisition, the spectral data (430-960nm) were extracted
166 and substituted in the established PLS, iPLS, SA-iPLS chlorophyll concentration
167 calibration models to predict the chlorophyll concentrations of the samples in the
168 testing set. Then the reference chlorophyll concentrations of the testing samples were
169 detected using HPLC. Finally, the root mean square error (RMSET) and the
170 correlation coefficient of the testing set (Rt) were calculated to test the established
171 calibration models.
172
173 2.4.3 Estimating chlorophyll distribution map
174 The flowchart of estimating a chlorophyll distribution map was shown in Fig. 2.
175 After hyperspectral image acquisition, data cube of all pixels were extracted and

176 converted to spectra curves. The spectra data of every pixel was substituted in the
177 established chlorophyll concentration calibration model to predict chlorophyll
178 concentrations for each pixel. Then chlorophyll concentrations for each pixel were
179 ranked into a 2-D matrix according to the coordinates of the pixels. Finally,
180 chlorophyll distribution map can be obtained by converting the 2-D matrix to a 2-D
181 figure, as shown in Fig. 2.
182
183 Insert Fig. 2 here.
184
185 2.5 Diagnostics of Mg deficiency based on chlorophyll distribution map
186 Leaf samples in Mg deficiency group and Control group were used to detect
187 chlorophyll concentration distribution maps using the methods described in above
188 sections. The chlorophyll distribution maps of Mg deficient leaves were compared with
189 those of Control leaves. Based on the results of comparison, chlorophyll distribution
190 features were extracted and used to develop new Mg deficiency diagnostic methods.
191
192 3. Results and discussion
193 3.1 Mg nutrient status of cucumber plants
194 Mg content in Control plants growing with complete nutrient solution and Mg
195 deficiency group growing with no Mg nutrient solution were analyzed using the method

196 described in section 2.1. 15 cucumber leaves at the first three nodes, 15 cucumber leaves
197 at the middle nodes and 15 cucumber leaves at the highest nodes in plants of Mg
198 deficiency group and Control group were collected for Mg analysis. The results of Mg
199 element analysis for Mg deficiency group and Control group were shown in Fig. 3. In
200 Fig. 3, it shows that the averages of Mg element at the first three nodes, middle nodes
201 and the highest nodes in plants of Mg deficiency group were 0.81 mg/g, 1.15 mg/g and
202 1.39 mg/g, respectively. While in the plants of Control group, the averages of Mg
203 element at the first three nodes, middle nodes and the highest nodes were 1.04 mg/g,
204 1.26 mg/g and 1.43 mg/g, respectively. It could be found that the averages of Mg
205 elements increase from the lowest nodes to the highest nodes. It could also be found
206 that the average of Mg element at the first three nodes in Mg deficiency group is
207 obviously lower than the averages of the other nodes. This results show that leaves at
208 the first three nodes in Mg deficiency group have entered into Mg deficient status.
209
210 Insert Fig. 3 here.
211
212 3.2 Chlorophyll concentration distribution maps of Mg deficient leaves
213 3.2.1 Building calibration models
214 As described in section 2.4.1, a calibration set and a prediction set contained 120
215 fresh cucumber leaves were used to build chlorophyll content calibration models.
216 After hyperspectral image collection and HPLC analysis, the hyperspectral cucumber

images and reference chlorophyll content were obtained. Chemometrics methods PLS, iPLS, SA-iPLS were employed to build chlorophyll content calibration models using the hyperspectral image data and reference chlorophyll content. The performance of the PLS, iPLS, SA-iPLS models was shown in table 1. The Rc for PLS, iPLS and SA-iPLS calibration models were 0.8149, 0.8472 and 0.9165, respectively. The Rp for PLS, iPLS and SA-iPLS calibration models were 0.7928, 0.8294 and 0.9087, respectively. The results shown that the SA-iPLS calibration model got better performance than the rest two calibration models.

Insert Table 1 here.

3.2.2 Testing the calibration models

As described in section 2.4.2, an independent testing set contained 40 fresh cucumber leaves was used to test the established chlorophyll content calibration models. After hyperspectral image data acquisition, spectra data of the samples in testing set was extracted and substituted in the established PLS, iPLS, SA-iPLS models, so the predicted chlorophyll content of the samples in testing set was calculated. Then the samples in the testing set were used to determine reference chlorophyll content. Finally, the root mean square error of testing (RMSET) and the

236 correlation coefficient in the testing set (Rt) were calculated to test the established
237 calibration models, as shown in Table 1. It could be found that the RMSET and Rt
238 based on SA-iPLS were was 2.12 mg/g and 0.8938, respectively. The results mean
239 that chlorophyll calibration model based on SA-iPLS gets better performance for
240 predicting chlorophyll content of an unknown leaf sample.
241 3.2.3 Estimating distribution map
242 Mg deficient cucumber leaves and Control group cucumber leaves were used to
243 collect hyperspectral images, then data cube of all pixels were extracted to estimate
244 chlorophyll distribution maps, as described in Section 2.4.3. The chlorophyll
245 distribution maps of cucumber leaves in Control group and Mg deficiency group are
246 shown in Fig.4. The pixels with high chlorophyll content appeared along the main leaf
247 veins on the distribution maps of Control group and Mg deficiency group. However,
248 the pixels between the main leaf veins got lower chlorophyll content on the chlorophyll
249 distribution maps of Mg deficiency group than those of Control group. This result made
250 that chlorophyll content distributed less evenly on Mg deficient leaves than that on
251 Control leaves. Results indicated that the Standard Deviation (SD) value of the
252 chlorophyll content at all the pixels on a chlorophyll distribution map could be used as
253 indicator for diagnosing Mg deficiency.
254
255 Insert Fig. 4 here.
256

3.3 Diagnostics of Mg deficiency based on chlorophyll distribution map

A calibration set and a prediction set containing 120 fresh Mg deficient leaves and Control leaves picked from the first nodes of cucumber plants were used to develop an Mg deficiency diagnostic method. After hyperspectral image acquisition, chlorophyll distribution maps of the 120 cucumber leaves were detected using the procedures described in section 3.2. The SD of chlorophyll content of all pixels in a chlorophyll distribution map was calculated for diagnostics of Mg deficiency, as the results in section 3.2.3 indicated that the homogeneity of chlorophyll content at the pixels of Mg deficient leaves was worse than that of Control leaves. The SD values of the Mg deficient and Control leaves in the calibration set were shown in Fig. 5.

Fig. 5 shows the SD values of the chlorophyll content at all pixels of the Mg deficient cucumber leaves and Control cucumber leaves. The SD values of chlorophyll concentrations in Mg deficient leaves were higher than 2.25 mg/g, whereas most SD values of Control leaves were lower than 2.25 mg/g. Therefore, 2.25 mg/g was defined as a threshold for detecting Mg deficiency, as show in Fig. 5. According to this threshold, only one sample from Control group was misclassified into Mg deficiency group, as shown in Fig. 5 (a). After applying this threshold to an independent set (30 Mg deficiency leaves and 30 Control leaves), a diagnostic rates of 93.33% was achieved, as shown in Fig. 5 (b). Control leaves at the first node enter into aging status, in which chlorophyll in some small regions decreases slowly, early than the leaves at higher nodes. Chlorophyll decreasing in these leaves can increase their SD values and result

in the misclassification. Mg deficient leaves at the third nodes enter into Mg deficiency, which decreases chlorophyll in some small regions, lately than the leaves at lower nodes. Chlorophyll decreasing in these leaves can decrease their SD values and result in the misclassification.

Insert Fig. 5 here.

4. Conclusion

An optimal chlorophyll content calibration model based on the hyperspectral images of cucumber leaves and the reference chlorophyll content of cucumber leaves was established. Chlorophyll distribution maps of Mg deficient leaves and Control leaves were obtained through calculating the chlorophyll content at each pixel by substituting its hyperspectral signal to the optimal chlorophyll content calibration model. Compared with the chlorophyll concentration distribution map, the main feature of Mg deficient chlorophyll distribution map is that chlorophyll content decreases in the regions between main leaf veins. The SD value of the chlorophyll content at all the pixels in Mg deficient and Control leaves were extracted and used as the indicator for diagnosing Mg deficiency. Result shown that a diagnostics model with good performance (diagnostic rate 93.33%) were established for measurement of Mg deficiency in cucumber plants. The result indicated that the extracted chlorophyll distribution feature could be employed to diagnose Mg deficiency.

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