Nondestructive measurement of total nitrogen in lettuce by integrating spectroscopy and computer vision

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A B S T R A C T

This study was conducted to develop and assess a method of estimating the nitrogen (N) content of lettuce (Lactuca sativa) canopy using a combination of spectroscopy and computer vision for nondestructive N detection. In the experiment, 90 lettuce samples with five N treatments were collected for data acquisition by two different techniques. On the spectroscopy side, canopy spectral reflectance was measured in the wavelength range of 350 to 2500 nm at 1-nm increments. Four spectral intervals (376 variables) were selected by synergy interval partial least squares and were further reduced to 73 wavelength variables, chosen using a genetic algorithm applied to first-order derivatives of the canopy reflectance. On the computer vision side, 11 plant features were extracted from images, including top projected canopy area as a morphological feature; red, green, blue, hue, saturation, and intensity values as color features; and contrast, entropy, energy, and homogeneity as textural features. Next, principal component analysis was implemented on the spectral variables and on the image features, and extreme learning machine modeling was used to fuse the two kinds of data and construct a model. For the optimum model achieved, the root-mean-square error of prediction = 0.323% and the correlation coefficient of prediction = 0.8864. This work demonstrates that integrating spectroscopy and computer vision with suitable efficient algorithms has high potential for use in the nondestructive measurement of N content in lettuce, considerably improving accuracy over that using a single sensor modality.

C O R R E S P O N D I N G   A U T H O R

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1. Introduction

Nitrogen (N) is a vital element in all organisms and is essential in various physiological processes of plants, where it is required continuously and in large amounts; it is a constituent of DNA, RNA, protein, chlorophyll, adenosine triphosphate, auxin (ATP), and cytokinins (Andrews et al., 2013; Zhang et al., 2013). A key concern for lettuce growers is to be able to verify plant N status early while N deficiency can still be remedied (Abdel-Rahman et al., 2010); thus, the early diagnosis of N deficiency plays a key role in the regulation of lettuce nutrition. However, the traditional process of N measurement has involved collecting samples at representative sites, pre-treating them, transporting them to the laboratory, analyzing them, and communicating the results back to the grower, which is a destructive, time-consuming, and inefficient procedure.

More recently, spectroscopy and computer vision, two commonly used nondestructive inspection techniques, have been widely employed to evaluate nutritional status expeditiously. Spectroscopy is an increasingly favored technique because of its rapidity, simplicity, and capacity to measure chemical characteristics, and it has been used to quantify the N content of plants by a number of researchers (Bajwa et al., 2010; Gislum et al., 2004; Mphokasap et al., 2012; Mitchell et al., 2012; Pacheco-Labrador et al., 2014; Padilla et al., 2014; Ramoelo et al., 2013; Ulissi et al., 2011). However, spectral data have high redundancy and collinearity and sometimes noise, which can reduce the estimation capability and computing efficiency of the model. Therefore, spectral transformation techniques are needed to enhance absorption features of biochemical constituents (Ramoelo et al., 2013), and wavelength selection methods are required to produce better prediction results with simpler models (Balabin and Smirnov, 2011). First-order derivative (FD) transformation can resolve problems such as multiple scattering of radiation due to sample geometry or surface roughness. By locating the positions of absorption features and inflection points on the spectra, it has proved to be a useful technique in estimating biochemical parameters such as...
chlorophyll and N content (Abdel-Rahman et al., 2010; Wang et al., 2013; Zhao et al., 2005). A continuum is a mathematical function used to isolate a particular absorption feature for analysis. With the continuum-removed (CR) approach, the reflectance spectra are normalized to enable comparison of individual absorption features of samples from a common baseline (Clark and Roush, 1984; Green and Craig, 1985; Kruse et al., 1985). Synergy interval partial least squares (siPLS) can help in searching all possible subinterval combinations to find the best model (Narggaard et al., 2000). A genetic algorithm (GA) is an adaptive heuristic search algorithm that can be applied to spectral variable selection in combination with partial least squares (PLS) (Leardi, 2000). The siPLS algorithm can also be combined with GA, in an approach called GA-siPLS.

Computer vision has been applied in the visual evaluation of nutritional status by morphology, color, and texture (da Silva et al., 2014; Xu et al., 2011). Giacomelli et al. (1996, 1998) were able to determine nutrient stress in lettuce seedlings using a machine vision system that extracted the top projected leaf area. Ahmad and Reid (1996) compared red–green–blue (RGB), hue–saturation–intensity (HSI), and chromaticity coordinate color representations and their standards to evaluate the sensitivity of a machine vision system to detect color variations in stressed maize. Story et al. (2009, 2010) extracted the top projected canopy area, energy, entropy, and homogeneity and found them to be promising markers for the timely detection of calcium deficiency in lettuce; the methodology they developed was capable of identifying calcium deficiency one day prior to human visual detection.

However, it is difficult to fully assess N status using a single inspection technique such as spectroscopy or computer vision because one technique can gather only limited information. Clearly, spatial information cannot be extracted by spectroscopy, and chemical properties cannot be obtained using computer vision. In this study, we explore a new strategy for measurement of N content, one that integrates spectroscopy and computer vision. The intent is to take advantage of the strengths of the two techniques, spectroscopy performing a local measurement of inner chemical properties, computer vision performing a global assessment of external physical properties, and combine these two measurements in a way that improves N content assessment. The specific objectives of this study are (1) to identify the most suitable wavelengths for quantifying N; (2) to extract the morphology, color, and texture features from canopy images; and (3) to merge data from the two sensor modalities to assess the N content of lettuce and compare the performance of the combined model to that of the two individual models.

2. Materials and methods

2.1. Sample preparation

The experimental materials were lettuce (Lactuca sativa, from Woshu Seeds Co. Ltd., Nanjing, China). The experiment was performed in the greenhouse at Jiangsu University in China (32.11N, 119.27E). All the plants investigated were transplanted during the period of five true leaves and grown under non-soil conditions from May to June 2012. The Yamasaki lettuce recipe was used for lettuce growth. The composition was: Ca(NO₃)₂·4H₂O, 236 mg/L; KNO₃, 404 mg/L; NH₄H₂PO₄, 57 mg/L; MgSO₄·7H₂O, 123 mg/L; Fe-EDTA, 16 mg/L; MnCl₂·4H₂O, 1.2 mg/L; H₂BO₃, 0.72 mg/L; ZnSO₄·4H₂O, 0.09 mg/L; CuSO₄·5H₂O, 0.04 mg/L; and (NO₃)₂·2MgO·4H₂O, 0.01 mg/L. In this solution, NO₃⁻ concentration was 6 mmol/L and NH₄⁺ concentration was 0.5 mmol/L. For our study, we used five treatments having different levels of N, containing, respectively: (1) NO₃⁻ 1.5 mmol/L and NH₄⁺ 0.125 mmol/L, (2) NO₃⁻ 3 mmol/L and NH₄⁺ 0.25 mmol/L, (3) NO₃⁻ 4.5 mmol/L and NH₄⁺ 0.375 mmol/L, (4) NO₃⁻ 6 mmol/L and NH₄⁺ 0.5 mmol/L, and (5) NO₃⁻ 7.5 mmol/L and NH₄⁺ 0.625 mmol/L. Each group of lettuce roots was kept on its fixed nutrient solution content by a self-developed timed irrigation and collection system.

2.2. Spectral data acquisition and preprocessing

Canopy reflectance was acquired with a FieldSpec 3 spectroradiometer [Analytical Spectral Devices (ASD), Boulder, CO, USA] that provides measurements in the spectral range of 350–2500 nm. The built-in spectral resolution of data output from the ASD operating system is 1 nm along the whole spectrum. The spectra of all samples were measured in an opaque closed box with a 50-W halogen lamp as the light source. The diameter of the light spot was about 9 cm, from a fiber optic cable with a 25° field of view. Prior to the first scan, a calibration was performed by taking dark current and using a standard white Spectralon panel ( Labsphere Inc., USA) with nearly 100% reflectance. Five spectral measurements were taken for each sample in order to attain a reduction in noise by averaging the spectra.

To eliminate multiple scattering, high-frequency noise, and other interferences that can reduce the performance of the model, it is necessary to apply an appropriate mathematical technique. In our analysis, the first step was to smooth the data using a nine-point weighted moving average to remove noise. Then, FD spectra (Fig. 1) and CR spectra (Fig. 2) were calculated from the smoothed reflectance. Both FD and CR preprocessing methods were...
performed in order to compare them and select the one that would best enhance the performance of the model.

2.3. Computer vision data acquisition

Top-view images of the lettuce canopy were taken using a digital camera (EOS 400D, Canon Inc.) from within the opaque closed box. In order to ensure the images were acquired in a totally sealed environment, a time-lapse photography system was used. To facilitate the subsequent image processing, the lettuce was put onto a white plate in the box. The conversion factor between the image size and the actual size was calibrated (110.25 pixel/mm²) before image acquisition. Aperture priority mode was selected, in which aperture and ISO were set independently at F8 and 100, respectively. White balance was calibrated by a standard white panel before images were collected. The images were stored in high-definition JPEG format with a resolution of 3888 × 2592.

2.4. Chemical analysis

After removal of the roots, the fresh samples were oven-dried at 70 °C for 24 h and then ground up and oven-dried again at 70 °C to a constant weight, resulting in powder. The powder was digested by 98% sulfuric acid (w/v) until the solution was transparent. The total N content was measured using the Kjeldahl method by a continuous-flow analyzer [AutoAnalyzer 3 (AA3), SEAL Analytical Co., UK] and expressed as a percentage of dry weight.

2.5. Software

FD and CR were performed using the ENVI (Environment for Visualizing Images) software application (Research Systems Inc., Boulder, CO, USA) version 4.5. The siPLS algorithm was applied using the package “Toolbox” provided by Nørgaard et al. (2000), and GA-PLS was performed using the package “The PLS-Genetic Algorithm Toolbox for MATLAB” provided by Leardi (2000). Images were processed and existing learning machine (ELM) algorithms were implemented in MATLAB R2009a (The MathWorks Inc., Natick, MA, USA) under Microsoft Windows 7.

3. Results

3.1. Creation and validation of calibration and prediction sample sets

Ninety samples from the five N treatments were randomly selected for reflectance measurement and image acquisition 20 d after transplantation. These were randomly divided into two sets, a calibration set to construct the model and a prediction set to verify the robustness of the model. Of the 90 samples, 60 samples (12 from each N-level group) formed the calibration set, and the remaining 30 made up the prediction set. Table 1 shows the number of samples used and the statistics associated with the N content as measured in the laboratory. The mean value and the standard deviation of the reference N measurements show no apparent difference between the calibration and prediction sets, thus demonstrating that the allocation of the samples into the calibration and prediction sets was appropriate and that the data sets are suitable for their intended use in calibration and prediction.

3.2. Determination of optimum spectral variables

The optimum spectral intervals were selected by siPLS, an algorithm developed by Nørgaard et al. (2000). The principle of this algorithm is to split the full-spectrum region (350–2500 nm) into n (n = 10, 11, . . . , 25) intervals, resulting in 16 cases; PLS regression models are then constructed on m (m = 2, 3, 4) intervals for each case. Thus, a total of Cm n PLS models were developed for predicting N content. The optimal model was selected by cross-validation, as the one having the lowest root-mean-square error of cross-validation (RMSECV) and the highest correlation coefficient of calibration (R²) (Nørgaard et al., 2000).

In this study, we found that the siPLS model developed from the FD reflectance of the lettuce canopy showed better performances than those developed from the CR, a result in line with the findings of Abdel-Rahman et al. (2010). Therefore, spectral data from the FD transformation processing were used for further analysis. The spectrum was divided into 23 equidistant intervals, because the utilization of more than this number did not improve the results, as can be seen in Fig. 3. The best siPLS model, with seven principal components, had an RMSECV = 0.3576 and an R² = 0.8268. It was achieved by combining four intervals (2, 3, 4, and 6), corresponding to 444–537 nm, 538–631 nm, 632–725 nm, and 820–913 nm, shown by the shaded areas in Fig. 4. The precision predicted by this optimum model was evaluated using the prediction set of samples and was found to have a root-mean-square error of prediction (RMSEP) = 0.3735% and a correlation coefficient of prediction (R²) = 0.8007.

The siPLS algorithm removed the spectral region that was irrelevant to the N content of lettuce and achieved a substantial reduction of data, the number of variables decreasing from 2151 to 376. However, there still existed high correlation between two adjacent wavelengths in the selected spectral intervals. To further eliminate redundant variables, GA-PLS was implemented on the 376 spectral variables that resulted from siPLS. In implementing GA-PLS, the control values were set to: probability of crossover, 0.5; probability of mutation, 0.01; and termination condition, 100 generations per iteration. Fig. 5 shows the calibration spectra with red lines corresponding to the regions selected, which included 73

![Fig. 3. Number of intervals optimized according to root-mean-square error of cross-validation (RMSECV) and correlation coefficient of calibration (R²) for synergy interval partial least squares (siPLS) model.](image-url)

3.3. Extraction of optimum image features

Fig. 6 illustrates the sequence of segmentation process, color space transformation, and feature extraction. To reduce computation burden, each lettuce image was first cropped (Fig. 6a) to include only the region of interest (the lettuce canopy), which was extracted using a threshold determined by experimentation and applying the “2G – R – B” index (Woebbecke et al., 1995). Then, a binary image was obtained, as shown in Fig. 6b, in which the lettuce canopy becomes the white foreground. Dividing the number of white pixels by the conversion factor previously determined (110.25 pixel/mm²), the top projected canopy area (TPCA) of each plant was obtained; this represents the morphological feature (MF).

When the white pixels of the binary image are converted into transparent, the background is then covered by all black pixels, as shown in Fig. 6c. The color features (CFs) of R, G, B based on the RGB color mode were extracted and then transformed into the H, S, I variables of the HSI color mode (Fig. 6d), resulting in a total of six CFs.

3.4. Combination of spectral variables and image features using extreme learning machine (ELM) modeling

N is directly related to the photosynthetic process and is used throughout plant growth and development (Andrews et al., 2013). When fertilization of lettuce results in a deficiency or an over-abundance of N, a complex dynamic process occurs that causes changes in both internal attributes (chemical components) and external attributes (morphology, color, and texture). The relationship between the changes in chemical components, morphology, color, and texture on the one hand and N content on the other is complex and difficult to model.

So far, our study has given us 73 spectral variables and 11 image features for establishing a prediction model, but a model with 84 variables is quite complex. There exist various techniques for dimensionality reduction; principal component analysis (PCA) is often used for diminishing computational burden (Cuzzolino et al., 2011; van der Maaten et al., 2009). PCA was implemented on the 73 spectral variables and on the 11 image features, with the result that three principal components (PCs) from spectra and two PCs from images were identified. In order to achieve the best performance for predicting N content in lettuce, the extreme learning machine (ELM) algorithm was adopted to develop a prediction model. The arrangement leading up to the ELM modeling is shown in Fig. 7.

ELM is a new fast learning algorithm proposed by Huang et al. (2006, 2011), one that not only learns much faster with higher generalization performance than the traditional gradient-based learning algorithms but also avoids many difficulties faced by gradient-based learning methods. In our ELM model, three layers were used: an input layer with the three spectral PCs and the two image PCs as the input, an output layer with the N content as the output, and a hidden layer in which the conjunction weights of the input and hidden layers and the biases of neurons in the hidden layer were randomly generated. The activation function was set as the “sig” function g(x) = 1/(1 + exp(−χ)) (Huang et al., 2006). The number of hidden nodes was attempted from 5 to 60 (60 being the number of samples in the calibration set) at intervals of 5. The optimal ELM model for N content was obtained with 30 hidden nodes, with a root-mean-square error of calibration (RMSEC) = 0.3015%, \( R^2_C = 0.9134 \), RMSEP = 0.3231%, and \( R^2_P = 0.8864 \). A scatter plot showing the correlation between predicted and measured N content in the prediction set is shown in Fig. 8.

4. Discussion

As can be seen in Table 2, while the model based on spectral data alone is superior to that based on image data alone, the model integrating both types of data is superior to either of the other two models. These results can be explained by the following reasons.

First, canopy light reflectance properties based mainly on the absorption of light at a specific wavelength are associated with specific plant characteristics (Özyiğit and Bilgen, 2013). Chlorophyll
PCA—principal component analysis; GA-PLS—genetic algorithm-partial least squares; PCA—principal component analysis; PCs—principal components; ELM—extreme learning machine.

Fig. 6. Extraction of image feature variables. TPCA—top projected canopy area.

Fig. 7. Arrangement for extreme learning machine (ELM) modeling. siPLS—synergy interval partial least squares; GA-PLS—genetic algorithm-partial least squares; PCA—principal component analysis; PCs—principal components; ELM—extreme learning machine.

Fig. 8. Measured and predicted nitrogen content values in prediction set by extreme learning machine (ELM) model.

α, Chlorophyll β, and protein exhibit characteristic absorption in the visible region and in the short-wave near-infrared region arising from functional groups of conjugated C–C single and double bonds of the porphyrin ring; C–H bonds in the phytol tail of the molecule, C–N bonds, and N–H bonds (Min et al., 2006; Mitchell et al., 2012; Ramoele et al., 2013). This demonstrates why spectroscopy has significant potential in estimating N content.

Second, plant N deficiency results in changes of morphology, color, and texture (Ahmad and Reid, 1996; Giacomelli et al., 1998). There is some evidence that N may affect the endogenous levels of phytohormones such as cytokinins (Walch-Liu et al., 2000), which are involved in the regulation of both cell division and cell elongation, known to be implicated in the regulation of plant morphogenesis (Kende and Zeevaart, 1997). Plants deficient in N are recognized by changes in foliar color (Romualdo et al., 2014; Xu et al., 2011); one identifying symptom of N deficiency is the yellowing of leaves due to the loss of chlorophyll (Kim and Reid, 2006; Özyiğit and Bilgen, 2013). Changes in texture (contrast, entropy, energy, and homogeneity) may occur because of changes in surface structure and yellowish appearance in the leaves (Story et al., 2010). However, these changes in morphology, color, and texture are inconspicuous in the early stages of N deficiency and only indirectly reflect the degree of N deficiency. Hence, the model based on image data is inferior to that based on spectral data.

Thirdly, lettuce display complex dynamic changes under N stress, including both internal attributes (e.g. chlorophyll, protein) and external attributes (morphology, color, texture). Moreover, plants under different stress conditions may nevertheless show similar external symptoms (Story et al., 2009). A single inspection technique can provide information about only one of these aspects; computer vision cannot identify or detect a plant’s chemical...
properties, and spectroscopy cannot observe a plant’s spatial information (Chen et al., 2013). Therefore, a fusion of spectroscopy and computer vision data can more fully estimate and provide more robust prediction results than can data from a single inspection technique.

5. Conclusions

In this study, the total nitrogen content of lettuce was measured using spectroscopy and computer vision individually and in combination, and three prediction models were established. In summary:

(1) Seventy-three optimal spectrum variables were selected from 2151 wavelengths using the GA-siPLS method.

(2) Six CFs (R, C, B, A, S, I), one MF (TPCA), and four TFs (contrast, entropy, energy, and homogeneity) were extracted from images of lettuce canopies using computer vision and image processing.

(3) Three ELM models based on single and multiple sensor modalities were established, and analysis of their respective results indicates that the model based on spectra is superior to the one based on image data and that the model based on a fusion of both is superior to either model based on a single sensor modality.

From our comparison and analysis, we conclude that multiple sensors integrating spectra and images can considerably improve the accuracy of nitrogen content prediction over that of a single sensor. As a nondestructive measurement tool, this approach has excellent potential for improved estimation of the N content of lettuce canopy. Improved assessment of N content from a crop canopy can help growers use N fertilizer more effectively and efficiently, thereby improving crop health and increasing productivity.

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