Development of an automated pigmentation phenotyping and low-cost multispectral imaging system

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ABSTRACT

Canopy imaging is a good phenotyping approach to non-invasively quantify parameters such as canopy size, stress symptoms, and pigment concentrations. Unlike destructive measurements, canopy imaging is fast and easy. However, analysis of the images can be time consuming. To facilitate large-scale use of imaging, the cost of imaging systems needs to be reduced and the analysis needs to be automated. We developed low-cost imaging systems using a Raspberry Pi microcomputer, equipped with a monochrome camera and filter, at a total hardware cost of ~$500. The latest version of our imaging system takes images under blue, green, red, and infra-red light, as well as images of chlorophyll fluorescence. Images taken under red, green, and blue light can be combined to generate color images. Other colors of light can be easily added, if desired. The imaging system is easily implemented in controlled environment agriculture and can be adapted for use in field settings. We will demonstrate examples of simple imaging techniques and automated image analysis using the Python programing language. The multi-spectral imaging system generates normalized difference vegetative index (NDVI) and anthocyanin content index (ACI) images and histograms, providing quantitative, spatially-resolved information.

Keywords: Multi-spectral imaging, Phenotyping, Automated image analysis, NDVI, ACI, Chlorophyll, Anthocyanins

1. INTRODUCTION

Image-based phenotyping has experienced an increase in interest due to its simplicity and efficacy[1, 2]. These methods allow for non-destructive characterization of anthocyanins and chlorophyll content, integrated across the whole plant area, eliminating sampling bias and allowing for a more representative measurement[3, 4]. Many commercial systems exist for making these measurements, with varying degrees of adaptability and quality. Some systems have a robust image capture system with limited post-processing capability, but rapid, automated analysis is important for widespread adoption of imaging. We describe a low-cost system that can be assembled and customized for specific desired outputs, with flexible post-processing. Alternatively, the post-processing can be done manually through a Python script.

2. Materials and Methods

For automated multi-spectral image analysis, we wrote a program in Python (3.8) using the OpenCV library (4.5.4). In summary, (1) the program reads multispectral images in a folder, (2) makes a mask image based on the chlorophyll fluorescence image, (3) executes image math calculations using the pixel intensity of the spectral images to calculate index values for each pixel, (4) generates a foreground index image and histogram, (5) exports average, standard deviation, and canopy size to a csv file, (6) moves on to next folder and repeats these steps. In the image math calculation, equations for normalized difference vegetative index [NDVI; $(R_{Near\ Infrared} - R_{Red}) / (R_{Near\ Infrared} + R_{Red})$][4] and anthocyanin content index [ACI; $(R_{Red} - R_{Green}) / (R_{Red} + R_{Green})$][4] were used, where R is intensity of the pixel, which is measure of reflectance.

To evaluate the program, indices from multispectral images of leafy vegetables, index readings from a portable pigment content meter, and pigment extract were compared. For evaluation of NDVI imaging, red lettuce (*Lactuca sativa*) 'Cherokee', green lettuce (*Lactuca sativa*) 'Little Gem', mizuna (*Brassica rapa* var. *japonica*), and spinach (*Spinacia oleracea*) 'Whale F1' were grown with different fertilizer levels. The fertilizer treatments induced a wide range of chlorophyll concentrations in the canopies. For anthocyanins, lettuce (*Lactuca sativa*) 'Rouxai', 'Rex', and 'Teodore' were used. Multispectral images of these leafy vegetables were taken periodically with the TopView system (ARIS, Eindhoven, Netherland), which utilizes seven colors of light emitting diodes (LED; 450, 521, 593, 625, 660, 730, and 870 nm) for acquiring multispectral images and a longpass filter (> 665 nm) to create mask image based on chlorophyll fluorescence. At the same time, chlorophyll and anthocyanin content indices were measured using a portable anthocyanin content meter (ACM-200plus, OPTI-SCIENCES, Hudson, NH, USA) and a portable chlorophyll content meter (CCM-200plus, OPTI-SCIENCES, Hudson, NH, USA). Concentrations of anthocyanins in leaf disks were measured by following a modified protocol of Lee, Durst and Wrolstad [5]. Regression analyses between indices from images, pigment extractions, and the portable meters were done using the R software. The automated program for image analysis has also been integrated into a low-cost imaging system prototype, using a Raspberry Pi (Raspberry Pi 4 model B, Sony, United Kingdom) to do both image capture and analysis, simplifying the user experience, with equivalent results to the above.

3. Results and Discussion

3.1 Automated pigmentation phenotyping using multispectral images

The NDVI estimates derived from the image analysis showed a asymptotic relationship with chlorophyll content index (CCI) ($R^2 = 0.87$, $p < 0.0001$, n = 193). Our analysis program isolates plants from background based on chlorophyll fluorescence images[6]. This differentiation between background and plant material strengthens the correlation between averaged NDVI estimates and CCI measurements, compared to NDVI measurements that do not separate canopy from background. Differences chlorophyll levels between low and sufficient fertilizer treatments were evident from the average NDVI value of the plants and easily visualized in false color NDVI images (Figure 1). In the lowest fertilizer treatment, divergence of peaks was observed due to senescence of an older leaf, which did not occur in plants grown with high fertilizer levels.

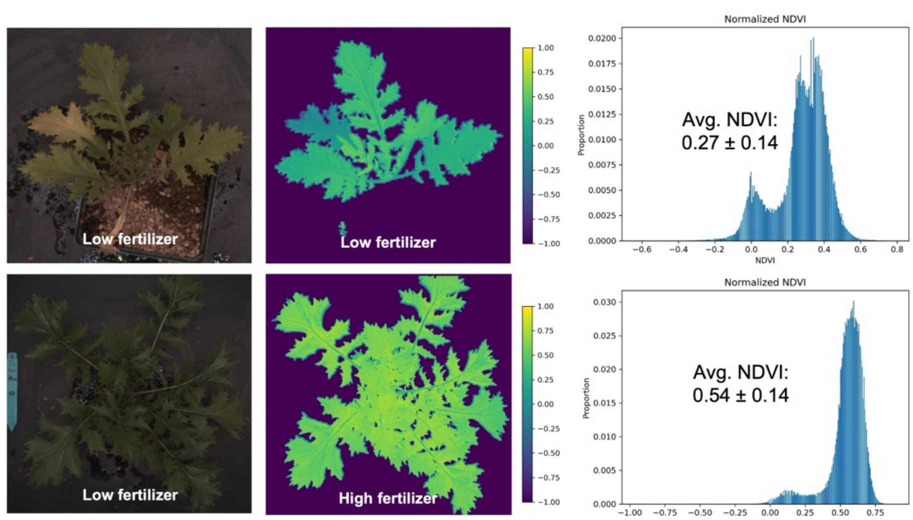

Figure 1. RGB (left) and Normalized difference vegetative index (NDVI) (middle) images of Mizuna under different fertilizer treatments and its corresponding histograms (right).

Averaged values of ACI from the image analysis system and anthocyanin content index measured by portable meter showed a significant positive correlation ($R^2 = 0.47$, $p < 0.0001$, n = 108). In different study, the ACI values from image analysis were strongly correlated with anthocyanin concentrations in leaf extracts ($R^2 = 0.83$, $p < 0.0001$ and n = 50). The greatest contrast in the optical properties of leaves due to the presence of anthocyanins is in the green part of the spectrum[7], but chlorophyll absorbs in the green part of the spectrum as well. Thus, the ACI also uses images taken under red light, where chlorophyll does and anthocyanins do not absorb, to normalize for the chlorophyll concentration. This approach is similar to NDVI imaging, where images taken under red light are indicative of chlorophyll levels, while infra-red images are used for normalization. ACI often is non-uniform throughout the canopy (Fig. 2), therefore, portable ACI measurements may not represent distribution of anthocyanin throughout the canopy. However, the ACI imaging can visualize the heterogeneous anthocyanin distribution in the canopy.

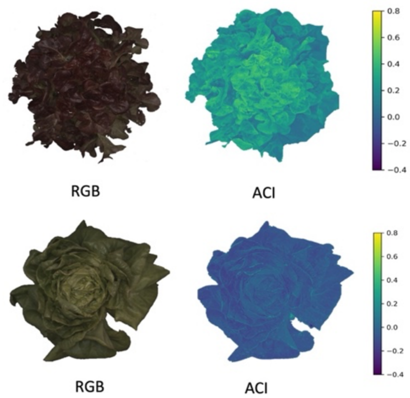

Figure 2. RGB and ACI images of 'Rouxai' (top) and 'Rex' (bottom). False color scale bar next to the ACI images is corresponding to ACI value on the canopy.

Some limitations of this image-based phenotyping are primarily due to the imaging from above. The images largely capture properties of cell layers near the top of the canopy. If anthocyanins are present on the abaxial side of leaves, the ACI imaging may not detect them. In addition, the camera can only see one layer of leaves and thus provides no information regarding lower leaves if they are obscured by upper leaves.

3.2 A low-cost imaging system using Raspberry Pi

Commercially-available multispectral imaging systems are expensive and often do not have easily customizable post-processing capability. Given these factors, a low-cost multispectral imaging system was built using Raspberry Pi microcomputer (Raspberry Pi 4 model B) and utilizes the image analysis program we developed previously, which the Raspberry Pi can run natively in conjunction with the automated multi-spectral image capture (Figure 3 and 4).

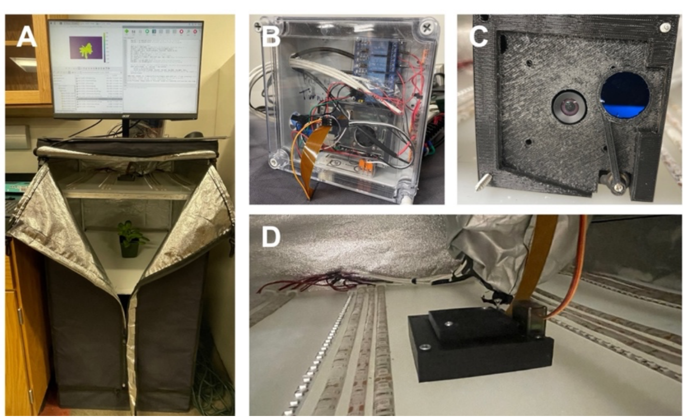

Figure 3. Overview of low-cost multispectral imaging system based on Raspberry Pi (A), Raspberry enclosure of wiring and relay array (B), detailed view of chlorophyll fluorescence filter system and bottom view of the camera module (C), and diffusion plate with LED strips and the camera module (D).

The imaging station was installed in a light-proof grow tent, which was modified by adding a diffusing acrylic shelf near the top. LED strips (blue, red, green and near-infrared) were mounted on top of this shelf, pointed upward to create a diffuse light environment. A monochrome camera (UC-599 Rev.B, ArduCam, China) (Figure 3 D) was added, with the lens going through the acrylic diffuser. The camera module was enclosed in a 3D printed case, which includes a movable long-pass filter (> 665 nm) that can be placed in front of the lens. Exposing the plant to blue light induces chlorophyll fluorescence, which passes through the filter. This is a simple method to generate a mask image to separate plant from background (Figure 3 C). To collect this mask image, the program triggers a servo motor to place the filter in front of the camera. The filter is removed to take the other images. The Raspberry Pi uses relays to trigger the different colors of LED strips with the collection of monochrome image under each color of LEDs. The image collection and analysis can be scheduled using a native bash script and the results can be uploaded to the cloud.

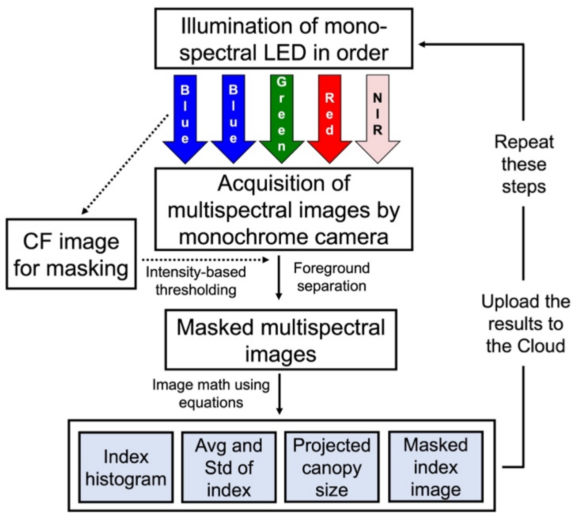

Figure 4. Schematic representation of programing logic in the low-cost multispectral imaging system.

The cost of the imaging system is ~ $500, including $60 for the Raspberry Pi, $50 for the camera, $50 for the LED strips, $240 of miscellaneous items (longpass filter, relays, monitor, keyboard, power supply, cases, wires, SD card, etc.), and a $100 growth tent. The software is freely available. The strength of this system is that it can be customized to user needs. For example, facilities in controlled environment agriculture can set up this imaging system without the grow tent, in a preexisting growth chamber.

4. Conclusions

The usability of imaging systems can be greatly improved by automated post-processing. Using relatively simple and rapid analyses, images can go from merely being data to information that can aid in decision making. Raspberry Pi microcomputers are an excellent tool for development of low-cost customizable imaging systems, since they have more than adequate processing for the required image analysis.

ACKNOWLEDGMENTS

This project was funded by USDA-NIFA-SCRI Award Number #: 2018-51181-28365, project Lighting Approaches to Maximize Profits.

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