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Short communication

Case study: Estimation of sorghum biomass using digital image analysis with Canopeo

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ABSTRACT

High-throughput plant phenotyping has revealed its importance by illuminating complex questions about plant growth, development, and response to the environment. Recently emerging technologies including un-maned vehicle as an image acquiring technology and machine learning as image analyzing technology have enabled the use of high-throughput phenotyping of plants for basic and applied sciences. Digital image analysis is one of methods for high throughput phenotyping. However, those techniques are not accessible because of high costs in general and the lack of man power to efficiently operate them. Here, we propose an easily accessible high-throughput phenotyping tool for biomass for many researchers. Four sorghum cultivars were evaluated for their height, a variable well documented for its high correlation with biomass, with both hand-collection and image taking in order to determine if image analysis for biomass in sorghum could replace hand data collection of plant height. Vertical plant images were obtained using a digital camera at one-week interval for 5 weeks. Plant height and node height were hand-collected at the same time. Images were analyzed using a tool, called Canopeo (http://www.canopeoapp.com). Biomass was measured indirectly as percentage units by counting the green pixels in each image. Strong and significant correlations between the percentage of green color and plant height and between the percentage of green color and node height were found for each cultivar and measurement. The results of this proof-of-concept study strongly indicate that digital image analysis could replace the intense labor needed to collect data related to biomass.

1. Introduction

The labor-intensiveness and costly nature of conventional phenotyping led crop breeding programs to have a single measurement of final phenotypic traits for replicated plots in different environments over multiple seasons [1]. High throughput phenotyping can be the answer to overcome this issue [2–4]. Among many high throughput phenotyping technologies, visible light imaging technology has been widely utilized in plant science [5].

Among many traits in a variety of crops and vegetables, the biomass of sorghum (*Sorghum bicolor*), which is one of the most important biomass sources for ethanol production, was chosen for the case study using Canopeo for high throughput phenotyping. Plant height of sorghum is known to have a linear relationship with biomass [6]. Further, many researchers, including Godbharle et al. [7] and Makanda et al. [8], reported a positive correlation between plant height and biomass in sorghum; in consequence, it is possible to use plant height to estimate biomass. If biomass measured by digital image analysis is correlated with plant height, it can replace the hand-collected data to achieve same goal. Canopeo is an image analysis tool (available as an application for Android or iOS devices) developed in the Matlab programming language (Mathworks, Inc., Natick, MA) using color values in the red–green–blue (RGB) system [9,10]. It turns green color to white with an easy manual adjustment and produces the percentage of white colored pixels in a given picture frame. The measurement of green color out of the background in a picture by counting green pixels using digital image analysis could represent biomass. Thus, biomass could be estimated by green color.

Canopeo was originally developed to measure the percent green

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canopy cover for any type of crop on the ground by taking digital images against the soil by being held in the horizontal plane. In the current study, a modification was made to obtain images to the vertical plane instead of taking it in the horizontal plane. The objective of this study is to determine if the software, Canopeo, is suitable to evaluate sorghum biomass by comparing with biomass obtained with plant height (PH) and node height (NH).

2. Materials and methods

Four sorghum cultivars, which are Chuncheon Jerae (IT028133), Panweoldang (IT028135), Chosa (IT028134), and BTX623 (IT148333), were planted with a check, corn cultivar CNU154, to see if the growth rate could be separated by this method because corn has more biomass, in 20 cm pots in the greenhouse without artificial light, Chungnam National University, Daejeon, Korea (longitude 127.353700, latitude 36.368373) on June 8, 2016 with three replications (five plants per replication) in a complete randomized block design with subsampling. Each seed was planted in soil potting medium (Yongto, Tangsim-Bio, Sungju, Korea) and irrigated when soil surface was dry. PH was measured as the length of the top leaf extended vertically from the ground. NH was measured as the height of the node located right below the top leaf of each plant. PH and NH were measured manually using a ruler (mm) at the same time as when digital images were taken using a Canon CoolPix S3300 camera. The camera was placed at a 150 cm distance from each plot. The line was marked in the row for plant pot and a stake was punched on the ground for camera installation to have a consistent distance between camera and plants. Images were taken from the 3rd week, when 3–4 leaves emerged, at one week intervals for 5 weeks before the reproductive stage began. Each image file size was about 6 mega-bites. Biomass was measured as percentages by counting green pixels in each image using Canopeo with default parameters. Examples of original sorghum images and images converted by Canopeo are shown in Fig. 1. Spearman's rank correlation was used to study the association between plant height, node height, and percentage of green color (PGC) [11]. A Bayesian multivariate mixed model was fitted to the dataset with the objective to model the relationship between these measured variables with remaining variables of the complete randomized block

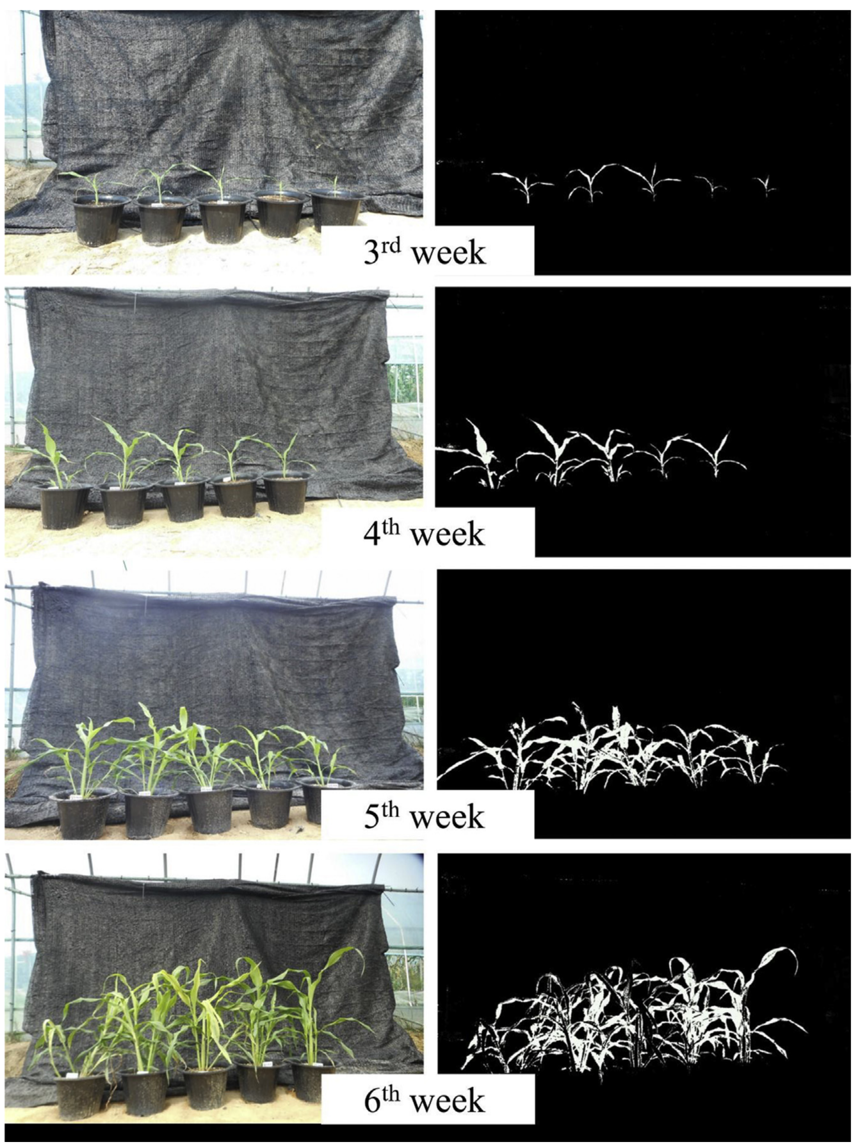

Fig. 1. Original images and their converted images for analysis.