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Determination of rice panicle numbers during heading by multi-angle imaging

Lingfeng Duan[a,b], *Chenglong Huang*[a,b], *Guoxing Chen*[d], *Lizhong Xiong*[c], *Qian Liu*[b], *Wanneng Yang*[a,c,*]

[a]*College of Engineering, Huazhong Agricultural University, Wuhan 430070, China*
[b]*Britton Chance Center for Biomedical Photonics, Wuhan National Laboratory for Optoelectronics, Huazhong University of Science and Technology, Wuhan 430074, China*
[c]*National Key Laboratory of Crop Genetic Improvement and National Center of Plant Gene Research, Huazhong Agricultural University, Wuhan 430070, China*
[d]*MOA Key Laboratory of Crop Ecophysiology and Farming System in the Middle Reaches of the Yangtze River, Huazhong Agricultural University, Wuhan 430070, China*

ARTICLE INFO

Article history:
Received 12 October 2014
Received in revised form
1 March 2015
Accepted 10 March 2015
Available online 11 April 2015

Keywords:
Plant phenotyping
Rice panicle number
Multi-angle imaging
Image analysis

ABSTRACT

Plant phenomics has the potential to accelerate progress in understanding gene functions and environmental responses. Progress has been made in automating high-throughput plant phenotyping. However, few studies have investigated automated rice panicle counting. This paper describes a novel method for automatically and nonintrusively determining rice panicle numbers during the full heading stage by analyzing color images of rice plants taken from multiple angles. Pot-grown rice plants were transferred via an industrial conveyer to an imaging chamber. Color images from different angles were automatically acquired as a turntable rotated the plant. The images were then analyzed and the panicle number of each plant was determined. The image analysis pipeline consisted of extracting the i2 plane from the original color image, segmenting the image, discriminating the panicles from the rest of the plant using an artificial neural network, and calculating the panicle number in the current image. The panicle number of the plant was taken as the maximum of the panicle numbers extracted from all 12 multi-angle images. A total of 105 rice plants during the full heading stage were examined to test the performance of the method. The mean absolute error of the manual and automatic count was 0.5, with 95.3% of the plants yielding absolute errors within ±1. The method will be useful for evaluating rice panicles and will serve as an important supplementary method for high-throughput rice phenotyping.

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* *Corresponding author*. Tel.: +86 27 87282120; fax: +86 27 87287092.
E-mail address: ywn@mail.hzau.edu.cn (W. Yang).
Peer review under responsibility of Crop Science Society of China and Institute of Crop Science, CAAS.

http://dx.doi.org/10.1016/j.cj.2015.03.002
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1. Introduction

According to the recent Declaration of the World Summit on Food Security, 70% more food is needed by 2050 to meet the demands of the increasing population (www.fao.org/wsfs/world-summit/en/). Global climate change and demand for biofuel feedstocks have exacerbated this problem, resulting in growing pressure on crop breeding. Rapid screening for crops with high yield and increased tolerance to abiotic and biotic stresses could be an important tool to help meet these demands [1].

The genome sequencing of *Arabidopsis* and other crop varieties has resulted in the accumulation of terabytes of sequence information that need to be linked with function [2]. However, identifying links between genotype and phenotype is hampered by inefficient, destructive, and often subjective manual phenotyping [3,4]. High-throughput phenotyping has become the new bottleneck in plant biology and crop breeding [5].

Plant phenomics promises to accelerate progress in understanding gene function and environmental responses [6]. There has been progress in automating plant phenotyping, including automated counts of plant parts [7–9] and whole adult plants [10–12]. Efforts have also been made to develop automated growth and observation facilities, such as at the High Resolution Plant Phenomics Centre in Australia, the Jülich Plant Phenotyping Centre in Germany, the Leibniz Institute of Plant Genetics and Crop Plant Research in Germany, and the French National Institute for Agricultural Research.

Rice is the staple food for a large proportion of the world's population [13] and is an important model system for plant science research [14]. Pressure on rice supplies has increased significantly over the past decade. The rice panicle is closely associated with yield, given that it directly regulates the grain number [15]. Substantial effort has been expended on quantitative trait locus (QTL) analyses for rice panicle traits [16,17]. However, few contributions have been made in automating rice panicle counts. Liu et al. [18] applied hyperspectral reflectance and principal component analysis to discriminate fungal infection levels in rice panicles. Liu et al. [19] used hyperspectral reflectance data to discriminate the health conditions of rice panicles. Ikeda et al. [15] developed image analysis software to extract panicle traits, including values of the length and number of the various branches and grain numbers. However, in all of these studies, the rice panicles were cut from the rice plants, preventing the achievement of dynamic screening of rice panicles. To our knowledge, no publication has reported a noninvasive, in vivo determination of rice panicle numbers.

The panicle number is a key indicator of rice yield, and counting panicles at an early stage would provide useful information for estimating rice yield. Panicle identification is the first step in panicle assessments such as by panicle counting, panicle length calculation, maturity degree assessment, and biomass prediction. However, because the color of the panicle at early stages (for example, the heading stage) is similar to the rest of the plant (green), identifying green panicles is highly challenging. This paper presents a novel method for nonintrusive detection of panicle numbers of rice plants during the full heading stage by analyzing color images of rice plants taken from multiple angles. The specific goals were to: (1) differentiate rice panicles from other organs and (2) calculate rice panicle numbers.

2. Materials and methods

2.1. Automatic image acquisition platform

Because the panicles and leaves of rice plants usually overlap, visible light imaging from a single angle cannot detect all of the panicles. For this reason, multi-angle imaging was adopted in this study. Previously, our group developed a high-throughput rice phenotyping facility (HRPF) to measure 15 rice phenotypic traits, excluding panicle number [10]. The HRPF used an industrial conveyor to transfer pot-grown rice plants to an imaging area for image acquisition. A turntable was used to rotate the rice plants. A barcode scanner read the barcode of each pot for indexing. Plants were illuminated by fluorescent tubes from both the side and top. Images were taken at 30° intervals by a charge-coupled device (CCD) camera (Stingray F-504C, Applied Vision Technologies, Germany) as the turntable rotated. For each rice plant, 12 images (2452 × 2056 pixels) were taken from different angles. Lighting conditions were constant throughout the process. Image acquisition was performed by NI-IMAQ Virtual Instruments (VI) Library for LabVIEW (National Instruments Corporation, USA). More details about the HRPF system can be found in Yang et al. 2014 [10].

2.2. Automatic image analysis pipeline

An image analysis pipeline was developed to analyze images from each angle. One image at a time was analyzed. The image analysis software was complemented with NI Vision for LabVIEW 8.6 (National Instruments). The image analysis pipeline consisted of extracting i2 planes from the original color image, segmenting the image, discriminating the panicles from the rest of the plant using an artificial neural network (ANN), and then calculating panicle numbers in the current image (Fig. 1).

In the first step, the original images were preprocessed using an IMAQ (Image Acquisition System, National Instruments) low-pass filter to remove noise. After image filtering, the RGB image was converted into the i1i2i3 color space, a commonly used color space based on the Karhunen–Loeve transformation [20]. Philipp and Rath [21] compared discriminant analysis, canonical transformation, i1i2i3, HSI, HSV, and Lab color spaces to separate plants and background, and concluded that i1i2i3 represented the best method. The relationship between the i1i2i3 and RGB color spaces is shown in Eq. (1).

$$\begin{bmatrix} i1 \\ i2 \\ i3 \end{bmatrix} = \begin{bmatrix} 0.333 & 0.333 & 0.333 \\ 0.5 & 0 & -0.5 \\ -0.25 & 0.5 & -0.25 \end{bmatrix} \bullet \begin{bmatrix} R \\ G \\ B \end{bmatrix}. \quad (1)$$

After the tests on all of the images acquired from the rice samples used in this study, we found that the i2 plane was effective in segmenting panicles from the rest of the plant. We selected the hysteresis thresholding method [22] for

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