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Plant high-throughput phenotyping using photogrammetry and imaging techniques to measure leaf length and rosette area

Nan An [a,*], Christine M. Palmer [b], Robert L. Baker [c], R.J. Cody Markelz [b], James Ta [b], Michael F. Covington [b], Julin N. Maloof [b], Stephen M. Welch [a], Cynthia Weinig [c]

[a] Department of Agronomy, Kansas State University, Manhattan, KS 66506, USA
[b] Department of Plant Biology, University of California Davis, Davis, CA 95616, USA
[c] Department of Botany, University of Wyoming, Laramie, WY 82071, USA

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ABSTRACT

Plant phenotyping is central to understand causal effects of genotypes and environments on trait expression and is a critical factor in expediting plant breeding. Previously, plant phenotypic traits were quantified using invasive, time-consuming, labor-intensive, cost-inefficient, and often destructive manual sampling methods that were also prone to observer error. In recent years, photogrammetry and image processing techniques have been introduced to plant phenotyping, but cost efficiency issues remain when combining these two techniques within large-scale plant phenotyping studies. Using these high-throughput techniques in basic plant biology research and agriculture are still in the developmental stages but show great promise for rapid phenotyping, which will materially aid both science and crop improvement efforts.

In this study, we introduce an automated high-throughput phenotyping pipeline using affordable imaging systems and image processing algorithms to build 2D mosaicked orthophotos. Chamber-based and ground-level field implementations are used to measure phenotypic traits such as leaf length and rosette area in 2D images. Our automated pipeline has cross-platform capabilities and a degree of instrument independence, making it suitable for various situations.

1. Introduction

Global crop production and plant biology research are facing a tremendous challenge in that current production rates will be insufficient to meet the demands of the world's population by 2050 (Bongaarts, 2014). Previous studies (Furbank et al., 2009; Reynolds et al., 2009; Tester and Langridge, 2010) showed that traditional breeding programs cannot sufficiently increase annual crop production for the three major cereal crops: rice, maize, and wheat. In the past decade, advances in genetic technology, such as next generation DNA sequencing, have provided new methods to improve plant breeding techniques. With these new techniques, breeders can potentially increase the rate of genetic improvement by molecular breeding (Phillips, 2010).

Many molecular genetic studies have focused on *Arabidopsis thaliana*, an important model system that has been used for identifying plant genes and determining their functions (Arabidopsis Genome Initiative, 2000). These studies have elucidated plant developmental processes and pathways that may generally contribute to yield in diverse crop species. O'Malley and Ecker (2010) reported that homozygous genome-wide knockout lines were available in *A. thaliana*. Weigel and Mott (2009) generated a sequence database of 1001 *A. thaliana* accessions, enabling comparative genomic analyses of yield. Similarly, the genome sequences of many crops, such as rice, maize, wheat, sorghum, and barley, have also been obtained due to the dramatic reduction in sequencing costs in the past few years (Furbank and Tester, 2011). Because of high-throughput genotyping, it is possible to develop large mapping populations and diversity panels for plant breeding (McMullen et al., 2009).

Unfortunately, in contrast to high-throughput genotyping that offers rapid and inexpensive genomic information extraction, conventional plant phenotyping methods are still labor-intensive and cost-inefficient. This greatly limits our ability to quantitatively relate genes to plant growth, environmental adaptation, and yield. Plant phenotyping methods for smaller plants, such as *A. thaliana*, are mainly dependent on intensive manual work for sampling, handling, and measuring plants often invasively, if not fully destructively. Due to this time-consuming process, very few phenotypic measurements can be acquired during the entire growing period (Arvidsson et al., 2011).

* Corresponding author.
E-mail address: an_198317@hotmail.com (N. An).

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In the past few years, there has been increased interest in high-throughput phenotyping approaches in controlled indoor environments (Fiorani and Schurr, 2013). These new approaches linking functional genomics, phenomics, and plant breeding are needed to improve both crop production and crop yield stability and also for efficient screening of high-yielding/stress-tolerant varieties (Bolon et al., 2011). Walter et al. (2007) and Jansen et al. (2009) used the GROWSCREEN/FLUORO system to measure chlorophyll and leaf counts. Granier et al. (2006) utilized the PHENOPSIS system to automate the soil water content control for screening soil water deficit responses. Many studies (Furbank and Tester, 2011; Dornbusch et al., 2012; Green et al., 2012; Chen et al., 2014; Dornbusch et al., 2014) have extracted certain phenotypes using LemnaTec Scanalyzer HTS systems (http://www.lemnatec.com) that scan plant surfaces with imaging or laser systems to acquire and analyze plant images, or 3D point clouds for extracting certain phenotypic traits. The main advantage of the Scanalyzer HTS is that it is a fully-automated processing pipeline containing image acquisition, storage, management, and processing components, along with some subsequent statistical analyses of the resulting data.

Some larger-scale, fully-automated high-throughput phenotyping facilities have also been deployed in the greenhouses or growth chambers of private sector firms such as Monsanto and Dupont Pioneer and a number of advanced national plant research institutions, such as the Australian Plant Phenomics Facility, the European Plant Phenotyping Network, and USDA. In these installations, robotics, precise environmental control, and remote sensing technologies are used to monitor and assess plant growth and development over time. However, such high-end facilities require budgets far beyond those of most research laboratories and may not be suitable for all situations, such as field environments.

To date, current field phenotyping approaches have mainly focused on automated solutions for data acquisition using platforms that integrate a vehicle, robotics, imaging systems, and sensors. Although this is changing, less work has been directed toward automating data storage, processing, and analysis. Due to these considerations and limitations, high-throughput phenotyping under field conditions has not yet reached its full potential.

Many previous indoor and field studies used imaging systems (cameras or scanners) and invasive sampling methods (excised plant parts) to extract phenotypic traits (Candela et al., 1999; Pérez-Pérez et al., 2002; Cookson et al., 2007; Bylesjö et al., 2008; Ali et al., 2012; Chitwood et al., 2012). These studies, however, did not take into account the optical distortion generated by imaging system lenses and the perspective distortion created by the angle of view. True distances and areas cannot be determined from a 2D image if either optical distortion or perspective distortion are present, and merely facing the imagers straight down does not fix this problem. In particular, when closely packed, the large number of plants more toward the corners of each frame will be distorted by the perspective viewing angle of the wide-angle lens (Fig. 1). The optical distortion and perspective distortion of the imaging system must therefore be removed before measuring any geometric quantities from a 2D image.

The objectives of this study are to (1) present a low-cost and fully-automated high-throughput imaging-based phenotyping pipeline suitable for both controlled environments and the field, (2) develop novel image processing algorithms for measuring time-series leaf length and 2D rosette area, and (3) model the relationship between rosette area and total leaf expansion.

2. Materials and methods

2.1. Imaging pipeline characteristics and design

The pipeline presented here has three advantages compared to other existing systems: (1) a low-cost imaging system, (2) elements of instrument independency, and (3) cross-platform capability. The first advantage is that off-the-shelf, low-cost digital cameras were used as imaging devices. This technique allows phenotypic traits (e.g., leaf length, rosette area, diurnal plant nastic movements, and plant vegetation conditions) to be extracted and measured directly from images.

The second advantage of this pipeline is a degree of instrument independency. For example, high-level scripts were used to interface with camera-manufacturer-supplied image processing software. Because many camera manufacturers provide similar tools, exchanging cameras becomes mainly a matter of altering those interface scripts. The image analysis algorithms can also be modified based on image features for different image sensors. For example, we successfully integrated a multispectral image sensor in our pipeline on a moving platform with proper modifications for computing vegetation indices.

Fig. 1. Original image with optical distortion and perspective distortion. An original image from indoor environment showing plants before optical distortion and perspective distortion correction. The plants from the corners were seriously distorted and true distances and areas cannot be measured directly from the image.