



Contents lists available at ScienceDirect

ISPRS Journal of Photogrammetry and Remote Sensing

journal homepage: www.elsevier.com/locate/isprsjprs

Estimating rice yield related traits and quantitative trait loci analysis under different nitrogen treatments using a simple tower-based field phenotyping system with modified single-lens reflex cameras



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ARTICLE INFO

Article history:

Received 21 June 2016

Received in revised form 30 December 2016

Accepted 12 January 2017

Keywords:

Breeding

Field based high throughput phenotyping

Remote sensing

Rice

Single lens reflex camera

Vegetation index

Yield related traits

ABSTRACT

Application of field based high-throughput phenotyping (FB-HTP) methods for monitoring plant performance in real field conditions has a high potential to accelerate the breeding process. In this paper, we discuss the use of a simple tower based remote sensing platform using modified single-lens reflex cameras for phenotyping yield traits in rice under different nitrogen (N) treatments over three years. This tower based phenotyping platform has the advantages of simplicity, ease and stability in terms of introduction, maintenance and continual operation under field conditions. Out of six phenological stages of rice analyzed, the flowering stage was the most useful in the estimation of yield performance under field conditions. We found a high correlation between several vegetation indices (simple ratio (SR), normalized difference vegetation index (NDVI), transformed vegetation index (TVI), corrected transformed vegetation index (CTVI), soil-adjusted vegetation index (SAVI) and modified soil-adjusted vegetation index (MSAVI)) and multiple yield traits (panicle number, grain weight and shoot biomass) across a three trials. Among all of the indices studied, SR exhibited the best performance in regards to the estimation of grain weight ($R^2 = 0.80$). Under our tower-based field phenotyping system (TBFPS), we identified quantitative trait loci (QTL) for yield related traits using a mapping population of chromosome segment substitution lines (CSSLs) and a single nucleotide polymorphism data set. Our findings suggest the TBFPS can be useful for the estimation of yield performance during early crop development. This can be a major opportunity for rice breeders whom desire high throughput phenotypic selection for yield performance traits.

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1. Introduction

Achieving maximum grain yield with fewer input costs is the ultimate goal of intensive rice production. A key step in this process lies in identifying rice varieties that enable farmers to produce

higher yield with minimum use of water and fertilizer. The rapid development of such rice varieties needs an advanced breeding system to surpass the traditional approaches. To accelerate the breeding of novel agricultural traits to produce environmentally adapted varieties with which require fewer input costs, new

Abbreviations: AlaAT, alanine amino transferase; ANOVA, analysis of variance; BRDF, bidirectional reflectance distribution function; CC, canopy cover index; CCD, charge coupled device; CIAT, International Center for Tropical Agriculture; CRG, Curinga; CSSLs, chromosome segment substitution lines; CTVI, corrected transformed vegetation index; DAT, days after transplanting; DG, dough grain stage; DN, digital number; DVI, difference vegetation index; EV, early vegetative phase; F174, Fedearroz 174; FBP, field based phenotyping; FB-HTP, field based – high throughput phenotyping; FL, flowering stage; FM, Fedearroz Mokari; FP, Farmer's practice; GCP, ground control point; GS, grain sterility percentage; GW, grain weight; HTP, high throughput phenotyping; IR64, IR-64; LAI, leaf area index; LV, late vegetative phase; LVA, late vegetative phase-A; LVB, late vegetative phase-B; MKG, milk grain stage; MSAVI, modified soil-adjusted vegetation index; MTG, mature grain stage; N, nitrogen; N4, NERICA 4; NDVI, normalized difference vegetation index; NIR, near infrared; PH, plant height; PI, panicle initiation; PL, panicle length; PN, panicle number; PVI, perpendicular vegetation index; QTLs, quantitative trait locus; R^2 , coefficient of determination; RGB, red, green and blue; RPG, *O. rufipogon*; SAVI, soil-adjusted vegetation index; SB, shoot biomass; SLR, single-lens reflex; SPAD, soil and plant analyzer development; SR, simple ratio; T, transgenic line; TBFPS, tower-based field phenotyping system; TSAVI, transformed soil-adjusted vegetation index; TVI, transformed vegetation index; U. Tokyo, The University of Tokyo; VIs, vegetation indices; WdVI, weighted difference vegetation index; WT, wild type.

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<http://dx.doi.org/10.1016/j.isprsjprs.2017.01.010>

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technologies which bridge the gap between genotype and phenotype are essential for plant researchers (Furbank, 2009; Fiorani and Schurr, 2013; Pieruschka and Lawson, 2015). Now high throughput phenotyping is one of the key technological solutions being explored to fill this gap.

While efforts are being taken to achieve this goal, drawbacks related to the traditional phenotypic methodologies makes this process very slow. Traditional phenotyping methodologies are often time-consuming and labor intensive because of the abundance of manual operations. Since additionally, these methods are often destructive, which makes it impossible to do sequential measurements on the same plant. In order to speed up the varietal development process the development of a high-throughput phenotyping (HTP) platform that allows fully automated crop image acquisition and advanced image processing technology is necessary (Li et al., 2014a). Plant analysis via image based systems has been well studied since the 1980s (Omasa, 1990; Omasa et al., 2002), with recent works focused on a broad range of topics within agriculture.

It is suggested that high throughput phenotyping is key to the data mining of the huge sample sets present under field conditions (Araus and Cairns, 2014). Phenotypic traits are important to archive in order to clarify the black box interactions between the genotype and the environment. This can be accomplished by considering their interaction (George et al., 2014) or through linking the phenotypic data with different -omics data like genomes, transcriptomes, proteomes, metabolomes and metagenomes in field trials (Alexandersson et al., 2014). Thus, there is a need for simple and robust FB-HTP system for estimating phenotypic traits at the field level. Recently, there is a boom of constructing FB-HTP systems worldwide. Some studies propose several types of unique FB-HTP systems with land vehicle platforms (Svensgaard et al., 2014; White and Conley, 2013; Andrade-Sanchez et al., 2014; Barmeier and Schmidhalter, 2016), aerial platforms like blimps, helicopters, and fixed wing planes (Chapman et al., 2014; Liebisch et al., 2015; Zaman-Allah et al., 2015; Gonzalez-Dugo et al., 2015). In recent year, the use of multicopter has been increasing in field phenotyping studies (Sankaran et al., 2015; Haghghattalab et al., 2016; Inostroza et al., 2016; Tattaris et al., 2016; Yu et al., 2016). The history and applications of unmanned aerial system in the field of remote sensing are well reviewed by Colomina and Molina (2014). However, many of these systems come with considerable costs and often require substantial skill to operate. In this paper, we will describe an alternative low-cost, Tower-based field phenotyping system (TBFPS). This system includes low cost RGB and NIR cameras mounted on stable, yet movable, low maintenance towers. Tower based phenotyping platforms have the additional advantages of unmanned continual operation and repeatability (Deery et al., 2014).

To estimate rice yield related traits from towers, the conventional remote sensing approach using vegetation indices (VIs) is an efficient method. VIs have a long history within cereal crops (Jones and Vaughan, 2010) and have been used within rice to estimate phenotypic traits such as rice yield (Mosleh et al., 2015). In the area of field based phenotyping (FBP), VIs are considered powerful tools in the estimation of important growth traits like chlorophyll concentration, nitrogen (N), leaf area index (LAI), leaf number, plant biomass, and yield (White et al., 2012). In regards to rice research, VIs and near infrared (NIR) imaging approaches have been widely applied to record crop phenology (Sakamoto et al., 2011), estimate LAI (Shibayama et al., 2011), leaf greenness (Shibayama et al., 2012), N uptake (Shibayama et al., 2009) and yield (Harrell et al., 2011; Tubaña et al., 2011; Mosleh et al., 2015). RGB derived parameters without NIR are also available for estimating chlorophyll content and leaf N concentration of rice (Wang et al., 2014). Moreover, hyperspectral imagery is used for

crop phenotyping like estimating nitrogen content (Moharana and Dutta, 2016; He et al., 2016), chlorophyll fluorescence (Zarco-Tejada et al., 2016), or constructing hyperspectral 3D plant models (Behmann et al., 2015) in recent years.

Quantitative trait loci (QTL) analysis has become a powerful tool for identifying the genetic factors influencing quantitative traits like yield and other related traits. In recent years, QTL mapping studies have used rapid phenotyping methods like VIs together with conventional and manual phenotyping approaches. Under field conditions, QTLs of several crops (wheat (Pinto et al., 2010; Edae et al., 2014; Graziani et al., 2014; Li et al., 2014b; Li et al., 2015; Gao et al., 2015; Kumar et al., 2016), barley (Obsa et al., 2016), corn (Lu et al., 2012; Trachsel et al., 2016), potato (Khan et al., 2015), cotton (Pauli et al., 2016), and forage grass (Merewitz et al., 2012, 2014)) were successfully detected through the use of VIs. As for rice, Henry et al. (2015) evaluated QTL effects of NILs derived from IR64 rice under drought conditions by using the Normalized Difference Vegetation Index (NDVI). In the previous studies, VIs of plots were obtained by hand-held spectroradiometers like GreenSeeker (Trimble Navigation Ltd., California, USA) or FieldScout series (Spectrum Technologies, Inc., Illinois, USA). In terms of the throughput, image-based remote sensing technologies can cover a large number of plots in a short time. Trapp et al. (2016) identified dry bean's QTLs for image-based NDVI obtained from a multicopter. However, QTL analysis using VIs have not been studied in rice.

In this paper, we discuss the use of a simple TBFPS for estimating yield related traits in rice. The main objective of the present study is (1) to develop a TBFPS to estimate yield related traits in rice (2) to confirm the specific phenological stages at which the traits are highly correlated with spectral VIs to enhance early estimation efficiency and (3) to determine whether our FBP platform improves our ability to identify yield related QTLs using chromosome segment substitution lines (CSSLs).

2. Materials and methods

2.1. Plant materials and characteristics

Table 1 shows the genotypes and homozygous transgenic lines of rice used in this study. In the 2012 experiment, six genotypes of rice and six homozygous NERICA4 transgenic events (T1 to T6) carrying the pOsAnt1/AlaAT construct from Arcadia Biosciences at Brawley, California, USA were used. In the 2013 experiment, five genotypes of rice and three selected CSSLs (Ogawa et al., 2014a) derived from a cross between 'Curinga' and *Oryza rufipogon* (IRGC 105491) were used. While in the 2014 experiment, 48 CSSLs and their parents were used.

2.2. Experimental plot design of N omission field

Paddy field experiments were carried out at the confined N omission field facility, International Center for Tropical Agriculture (CIAT), Palmira, Colombia (3°30'N, 76°21'W). The site receives 1000 mm annual rainfall, is 965 m above sea level, and has an annual average temperature of 26 °C. Field experiments were conducted over three years (Fig. 1); one during a dry season (August to December 2012), one during a rainy season (January to June 2013), and again during a dry season (August to December 2014). Residual N was depleted from the field trial site prior to planting by cultivating maize over two consecutive seasons with no fertilizer application (Ogawa et al., 2014b). The 2012 and 2013 experiments focused on the development of the tower based FBP platform and the confirmation of the best growth stages to estimate phenotypic

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