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Orignial papers

High-throughput field phenotyping in dry bean using small unmanned aerial vehicle based multispectral imagery

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ABSTRACT

Phenotyping traits in large field crop trials with numerous breeding lines is an arduous task. Unmanned aerial vehicle (UAV) based remote sensing is currently being investigated for high-throughput agricultural field phenotyping applications. The system is conducive for rapid assessment of crop response to the environment, at a desired spatio-temporal resolution. Therefore, objective of this study was to evaluate such technology towards monitoring responses of dry bean lines to drought and low nitrogen stress (i.e., two trials and two seasons) under field conditions. A semi-automated image processing protocol was developed to extract features such as: (i) average green normalized difference vegetation index (GNDVI); and (ii) canopy area (total number of plant pixels) from individual plots. The data were acquired at mid-pod fill and late-pod fill growth stages in 2014 season, and at flowering, mid-pod fill, and late-pod fill growth stages in 2015 season. The relationships between remotely sensed image features with that of crop response variables such as seed yield, days to flowering, days to harvest maturity, days to seed fill, and biomass rating (for drought trial only) were assessed temporally. Overall, in drought experiment, both average GNDVI and canopy area were significantly correlated with seed yield in all trials at 5% level of significance. The average GNDVI and canopy area at flowering growth stages and average GNDVI at mid-pod fill stage were consistently highly correlated ($r > 0.73$) with seed yield. The average GNDVI at flowering (r of -0.54 to -0.73) and mid-pod fill (r of -0.52 to -0.73) stages was highly correlated with biomass rating. Thus, average GNDVI could possibly be used as a viable phenotype for capturing biomass differences as well. A pilot thermal imaging of the sample breeding plots in drought trials also indicated its potential in capturing the temperature differences resulting from stress. For the nitrogen stress experiment, the correlations between remotely sensed image features and response variables were lower than in the drought experiment. The nitrogen from vegetative growth did not effeciently partition into seed production, which could have resulted in low correlations.

1. Introduction

Grain legumes provide an important source of vegetable protein in human diets. Moreover, they provide additional benefits as a rotational crop for weed and disease cycle disruption and nitrogen (N) fixation to improve soil health. The common bean (*Phaseolus vulgaris* L.) is the most consumed (12 million metric tons) food legume worldwide (Petry et al., 2015). It provides important vitamins and minerals, i.e., calcium, copper, iron, manganese, magnesium and zinc, protein, and fiber (Miklas et al., 2006). Much common bean production occurs on small farms in developing countries. In the U.S. alone, about 1.5 million metric ton of dry bean (USDA-NASS Crop Production, 2015) is produced annually. Beans are vulnerable to abiotic (drought, low soil fertility) and biotic (diseases) stress. In subsistence farming, low soil fertility is a limitation to bean production; while, in developed countries, there is an interest around improving nitrogen use efficiency. Thus, one of the primary goals of bean breeding programs globally is to improve stress tolerance through new cultivar development, while maintaining or improving the yield potential (Frahm et al., 2004; Miklas et al., 2006; Muñoz-Perea et al., 2006, 2007; Singh, 2007; Beebe et al., 2013).

Phenotyping is an important process in plant breeding that

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evaluates physiological and performance related traits of a specific genotype in a given environment. The expressed phenotypes, resulting from a complex interaction between the genotype and environment, require careful examination to select genotypes with a desirable trait (e.g. disease resistance, drought tolerance, high yield potential, etc.). The standard methods (e.g. visual rating of biomass/disease resistance) for phenotypic evaluation are limited by capacity, accuracy, and subjectivity. Thus, research efforts need to be focused towards developing high-throughput phenotyping capacity, where trait evaluation can be conducted at an accelerated pace without compromising precision and accuracy. Although gaining momentum, there is still a need to validate application of sensing technologies to phenotype specific crop traits under field conditions.

Unmanned aerial vehicles (UAVs) when integrated with suitable optical sensors can be a reliable tool for rapid phenotyping in field conditions. The on-board sensors can capture data in a high-throughput manner from multiple plots at a desired spatio-temporal resolution (Sankaran et al., 2015a; Haghighattalab et al., 2016; Shi et al., 2016). Moreover, such technology can be used to relate the aspects of field conditions (soil variability, wheel tracks, previous irrigation line, compaction, etc.) on phenotypic expressions (Sankaran et al., 2015a). Some of the prior studies on UAV based field phenotyping include assessment of: ground cover in sorghum (Chapman et al., 2014); emergence and spring stand (Sankaran et al., 2015b), grain yield (Haghighattalab et al., 2016; Shi et al., 2016), crop height (Holman et al., 2016), and crop lodging (Chapman et al., 2014) in wheat; senescence (Zaman-Allah et al., 2015) and crop height (Shi et al., 2016) in maize; and water stress in apples (Gómez-Candón et al., 2016). Many of the prior studies have related normalized difference vegetation index (NDVI, Rouse et al., 1974), an indicator of overall plant health (associated with chlorophyll reflectance), with traits such as crop emergence, yield potential, and senescence. Similar to NDVI, green-NDVI (GNDVI) is also a reliable index for indicating crop canopy health and vigor (Gitelson et al., 1996; Khot et al., 2016). GNDVI unlike NDVI, measures green spectrum instead of red spectrum and has been found to be sensitive to changes in plant chlorophyll concentrations (Gitelson and Merzlyak, 1998).

In our previous work (Trapp et al., 2016), UAV-based multispectral imaging was performed in multiple environments at mid- and late-pod fill growth stages for the 20 most and 20 least drought tolerant recombinant inbred lines (RILs) selected from an inbred dry bean population derived from a cross between 'Buster' pinto bean (susceptible) × 'Roza' pink bean (tolerant). A significant correlation was observed between mid-pod fill growth stage extracted GNDVI data with that of seed yield (Pearson's correlation coefficient, $r = 0.76$) and days to flowering ($r = 0.43$). The late-pod fill growth stage extracted GNDVI data was highly correlated with harvest maturity ($r = 0.63$) under drought stress. The biomass was also correlated with GNDVI at both growth stages ($r > -0.60$) in non-stress and drought stress trials. However, to adopt remote sensing techniques for high-throughput phenotyping in breeding programs, it is necessary to conduct studies with larger datasets across diverse environments.

Therefore, primary objective of this study was to validate the potential of UAV-based multispectral imaging for phenotyping large-scale dry bean breeding trials across multiple environments and multiple seasons. If reliable data indicative of crop performances (yield potential, biomass) can be acquired using remote sensing technology, significant resources (time, money) can be conserved during breeding process. Using such data, breeders can (i) increase the number of genotypes evaluated in a given field season, (ii) capture crop performances in early breeding cycle (single row plots), and (iii) acquire useful agronomic and physiological data (e.g. photosynthetic efficiency) at a higher frequency throughout the season.

2. Materials and methods

2.1. Field plots and response variables

Field trials from the United States Department of Agriculture (USDA) – Agricultural Research Service (ARS) Dry Bean Breeding Program were evaluated in this study. Terminal drought stress trials conducted at the Washington State University (WSU)'s Research station in Othello, WA, USA, consisted of 160 and 192 genotypes of the Durango Diversity Panel (DDP) tested in 2014 and 2015 season, respectively and 64 genotypes of the Andean Diversity Panel (ADP) tested in 2015 season. The experimental design was a randomized complete block design (RCBD) with two replications for DDP trials and three replications for the ADP trial, and two treatments, terminal drought stress and non-stress, planted side-by-side. The ADP trial included Buster pinto (drought susceptible) and Roza pink (drought tolerance) beans as checks (Trapp et al., 2016). The respective total number of plots for WSU-Othello research trials were 640 in 2014 and 1152 in 2015 season. The plot sizes were 4-rows of 3 m length and 0.55 m spacing between the rows. Seeding rate was 285,000 seeds ha^{-1}. The distance between ranges was 1.52 m. The irrigation was applied through furrows/corrugates. Both treatments (non-stress and terminal drought) received water to field capacity (pre-planting). The terminal drought treatment received one more irrigation at about 18 days after planting (DAP), after which the plots did not receive any more irrigations for the rest of the season. The irrigated treatment received irrigation applications every 7–10 days throughout the season to manage the crop for optimum yields.

Low nitrogen stress trials (~6.8 kg of residual N and no fertilizer applications throughout the season) were conducted at the USDA-ARS Research Farm, in Paterson, WA, USA. The DDP (192 genotypes) and ADP (96 genotypes) were in adjacent, separate low N trials with plot size and experimental designs same as described above except each trial had three replications (total of 864 plots). The nitrogen trial was grown under center pivot irrigation with irrigation applications made 3–5 times per week at an amount of 9.7 mm for optimum plant growth. Applications coincided with soil probes showing low moisture content. The response variables used for comparing the features extracted from aerial imagery were seed yield (kg ha^{-1}), days to flowering (FD), days to harvest maturity (DHM), days to seed fill (DSF), and biomass rating (for drought trial only). Biomass rating (visual scores) of 1 indicated complete row closure and densest canopy with minimal porosity, 3 indicated 75% closed rows and dense canopy allowing 25% light penetration, 5 indicated 50% closed rows and 50% light penetration, 7 indicated 25% closed rows and 75% light penetration, and 9 indicated no canopy to estimate biomass at mid-pod fill. Images of representative plot visual biomass rating (3, 5, and 7) and more details can be found in Trapp et al. (2016). For the scope of this study, all comparisons were performed on a plot-to-plot basis to evaluate crop performances in the form of above mentioned phenotypes/traits to validate the potential of utilizing aerial imaging for field phenotyping (including check plots). The genotype and genotype × environment interactions for these response variables will be reported separately.

As a ground reference data, leaf tissue nitrogen (%) was measured at late flowering/early-pod fill growth stages (about 7–10 days after flowering, between 60 and 75 days after planting) from ADP (96 genotypes × 3 replicates) plots of low nitrogen trial. From each plot, samples from two plants were collected randomly. These samples were oven dried for 48 h at 75 °C, ground in a mill, and separated with 1 mm sieve. The N concentration of the leaves were determined using a subsample of ground tissue (known weight) that was packed in a capsule. Finally, the data were acquired using a CN analyzer in tandem with a continuous-flow isotope ratio mass spectrometer (Europa ANCA-GSL, PDZ Europa Ltd, Sand Bach, UK) at the WSU Stable Isotope Facility.