DEVELOPMENT OF UAV-BASED HIGH-THROUGHPUT PHENOTYPING
TECHNOLOGIES TOWARDS IMPROVING SOYBEAN BREEDING EFFICIENCY

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of the Requirements for the Degree
Doctor of Philosophy

by

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The undersigned, appointed by the dean of the Graduate School, have examined the dissertation entitled

DEVELOPMENT OF UAV-BASED HIGH-THROUGHPUT PHENOTYPING
TECHNOLOGIES TOWARDS IMPROVING SOYBEAN BREEDING EFFICIENCY

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DEDICATION

To all my families and friends who supported, encouraged, and inspired me in the past 30 years of my life.

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LIST OF ABBREVIATIONS

1D	One-Dimensional
2D	Two-Dimensional
adMD	Adjusted Maturity Date
AEN	Auto-Encoder Network
AGL	Above Ground Level
ANN	Artificial Neural Network
ANOVA	Analysis of Variance
API	Application Programming Interface
AYT	Advance Yield Trial
B	Blue
BNDVI	Blue Normalized Difference Vegetation Index
BWDRVI	Blue-Wide Dynamic Range Vegetation Index
CCCI	Canopy Chlorophyll Content Index
CDT	Central Daylight Time
CI	Coloration Index
CNN	Convolutional Neural Network
CRP	Calibration Reflectance Panel
CVI	Chlorophyll VI
DAP	Day After Planting

DEM	Digital Elevation Model
DL	Deep Learning
DN	Digital Number
DNN	Deep Neural Network
EMS	Electromagnetic Spectrum
ExG	Excess Green
EXIF	Exchangeable Image File Format
fAPAR	Fraction of Absorbed Photosynthetically Active Radiation
FN	False Negative
fps	Frame Per Second
fps	Frame Per Second
FWHM	Full Width at Half Maximum
G	Green
GCP	Ground Control Point
GDVI	Green Difference VI
GEBVs	Genomic-Estimated Breeding Values
GLI	Green leaf index
gNDVI	Green NDVI
GNSS	Global Navigation Satellite System
GRNDVI	Green-Red NDVI

GRVI	Green Ratio VI
GS	Genomic Selection
GSD	Ground Sampling Distance
G _y	Genetic Gain
h	Selection Accuracy
H	Hue
H ²	Broad-sense Heritability
HSD	Honest Significant Difference
HSV	Hue-Saturation-Value Color Space
HTP	High-Throughput Phenotyping
IF	Shape Index
IO	Iron Oxide
k	Selection Intensity
Lasso	Least Absolute Shrinkage and Selection Operator
LiDAR	Light Detection and Ranging
MANOVA	Multivariate ANOVA
MAS	Marker Assisted Selection
MAV	Manned Aerial Vehicle
MG	Maturity Group
ML	Machine Learning

MTVI2	Modified Triangular Vegetation Index
MU	University of Missouri
N	Dimension of the moving window for the grey level co-occurrence matrix
NDVI	Normalized Difference Vegetation Index
NDVIrededge	Normalized Difference Rededge/Red
NIL	Near Isogenic Line
NIR	Near-Infrared
NormG	Norm Green
NS	No Significance
PCA	Principal Component Analysis
PLSR	Partial Least Square Regression
PT	Progeny Trial
PYT	Preliminary Yield Trial
QTL	Quantitative Trait Locus
R	Red
r	Pearson Correlation
R ²	Correlation of Determination
R2	Reproductive growth Stage 2
R3	Reproductive growth Stage 3
R5	Reproductive growth Stage 5

R7	Reproductive growth Stage 7
R8	Reproductive growth Stage 8
RaDAR	Radio Detection and Ranging
Re	Red Edge
ReLU	Rectified Linear Unit
RGB	Red-Green-Blue
RGR	Red–Green Ratio
RIL	Recombinant Inbred Line
RM	Relative Maturity
RMSE	Root Mean Square Error
RNN	Recurrent Neural Network
S	Saturation
SfM	Structure from Motion
std	Standard Deviation
TBV _s	True Breeding Values
TGI	Triangular greenness index
TP	True Positive
UAV	Unmanned Aerial Vehicle
V	Value
V4	Vegetative growth Stage 4

VI	Vegetation Index
VIF	Variance Inflation Factor
VIP	Variable Importance in Projection
y	Number of years required to complete a breeding cycle
YT	Yield Trial
σ_A	Genetic Variance

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Dr. Jianfeng Zhou, Dissertation Supervisor

ABSTRACT

Development of new crop varieties with improved traits through crop breeding programs is one of the promising solutions for the estimated food crisis in 2050 when agricultural production needs to double its current growth rate to feed 10 billion people in the world. Conventional crop breeding strategies largely rely on ‘trial-and-error’ and human input, limiting the trial capacity of breeding materials, the accuracy of measuring plant traits, and selection intensity of elite genotypes. These limitations have become a bottleneck to boost breeding efficiency as it is expected to meet the food demands.

The goal of this research was to develop an integrated and automated high-throughput phenotyping (HTP) framework leveraging advanced technologies in remote sensing and artificial intelligence for estimating key traits and selecting elite genotypes towards improving the selection intensity and accuracy of conventional soybean breeding. To achieve this goal, this research features three objectives: (1) develop an integrated and automated UAV HTP framework for measuring crop traits accurately and efficiently, (2) estimate soybean yield and maturity date of breeding materials using UAV image features and machine learning models., and (3) select elite soybean lines using UAV image features towards improving the selection intensity and accuracy.

A UAV-based HTP platform was developed to carry multispectral and high-

resolution digital cameras and geo-referencing units. The platform was able to cover a 9-acre field within 2 hours. An automated pipeline was developed to process the collected time-series images and generate labeled image features. It was shown that the developed methods can deliver accurate measurements on plant height (coefficient of determination R^2 up to 0.90 with average errors within 5 cm) and consistent spectral reflectance.

The UAV platform and image processing pipeline were applied to estimate two key agronomic traits for soybean breeding, i.e., the maturity date and yield. A group of image features was collected on 326 soybean progeny lines near their maturity stages (R7-8). Their maturity dates were estimated using a partial least square regression (PLSR) model with the image features as inputs and the visual maturity dates taken by breeders as outputs. The results showed that the image-based maturity dates highly agreed ($R^2 = 0.81$) with the visual ones with the root mean square error (*RMSE*) of 1.4 days.

For estimating the soybean yield, 972 soybean breeding plots in three maturity groups were planted under rainfed conditions. A mixed convolutional neural network (CNN) model was built to estimate soybean yield by taking seven image features (associated with plant height, canopy color, and canopy texture) and two categorical factors, i.e. maturity group and drought tolerance, as predictors. The prediction model could explain 78% of the measured yield with an *RMSE* of 391.0 kg·ha⁻¹ (33.8 % to average yield).

To model the breeder's selection criteria and select elite soybean genotypes, a soybean breeding program was traced for three years. The progeny trial (PT) had 11,473 rows, and 1,773 among them were selected for a preliminary yield trial (PYT) and 238 were further selected for an advance yield trial (AYT). Seven agronomic traits, including

yield, plant height, maturity data, flower and pubescence color, moisture and lodging were manually measured for soybeans in the two yield trials. The UAV imagery was collected every two weeks over the growing seasons, and a group of image features was extracted for each trial. Results show the progeny lines had the most variation among the three trials and the images collected at earlier stages (before R5) explained more variation than those at later stages. A Lasso model for selecting soybean lines with image features correctly identified 71% and 76% of the breeder's selection for the PT and PYT. The model selections in PT and PYT had respectively 4% and 5% higher yield, comparing the breeder's selection.

In summary, the developed UAV HTP platform is capable of collecting image features of soybean breeding materials efficiently and delivering estimations of agronomic traits accurately. The accurate and subjective estimations of plant traits decrease the phenotypic variations in breeding trials. By liberating human labor from the onerous field evaluation, the population size of soybean breeding lines could be increased a lot, leading to increased selection intensity. Moreover, the proposed variety selection model was able to narrow down the breeder selections, further increasing the selection accuracy and intensity. Therefore, it is be concluded that the developed UAV HTP platform has great potential in improving soybean breeding efficiency by decreasing the phenotypic variations, increasing the selection accuracy and intensity. This research could be scaled up to other crop breeding programs and offered a paradigm of improving the breeding efficiency using HTP technologies.

CITATION OF PUBLISHED WORK

Two chapters of this dissertation have been published as follows:

Chapter 2

Zhou, J., Yungbluth, D., Vong, C. N., Scaboo, A., & Zhou, J. (2019). Estimation of the maturity date of soybean breeding lines using UAV-based multispectral imagery. *Remote Sensing*, 11(18), 2075. <https://doi.org/10.3390/rs11182075>

Chapter 3

Zhou, J., Zhou, J., Ye, H., Ali, M. L., Chen, P., & Nguyen, H. T. (2021). Yield estimation of soybean breeding lines under drought stress using unmanned aerial vehicle-based imagery and convolutional neural network. *Biosystems Engineering*, 204, 90-103. <https://doi.org/10.1016/j.biosystemseng.2021.01.017>

CHAPTER ONE

INTRODUCTION

1.1 Problem statement

Phenotyping is essential to plant breeding (Fasoula et al., 2020). From 10,000 years ago, plants with preferable phenotypes have been selected and bred by human beings for higher productions and environmental adaptivity (Ahmar et al., 2020). Since the early 1900s, crop development has been systemized with the introduction of Mendelian laws, followed by cross-breeding and hybrid breeding, and was further revolutionized with the availability of high-throughput genome information. However, it requires the accurate quantification of plant traits (phenotyping) to evaluate plant performance, characterize germplasm and experimental populations to identify useful genes/QTLs (Mir et al., 2019), or to train a prediction model, no matter in conventional breeding or molecular breeding (Lorenz et al., 2011). Similarly, phenotyping is also required to evaluate the results of mutagenesis, genetically modified organisms (Blum, 2014), or even clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein-9 nuclease (CRISPR/Cas9) (Araus et al., 2018).

A fundamental concept in breeding is genetic gain (G_y) that is the amount of increase in performance of a population achieved in one year through artificial selection (Fehr, 1991) as defined in Eq.1-1.

$$G_y = \frac{kh\sigma_A}{y} \quad (1-1)$$

Where k is selection intensity, h is selection accuracy, σ_A is genetic variance and y is the number of years required to complete a breeding cycle. In the context of phenotypic

(conventional) selection, h equals the square root of the narrow-sense heritability, while in the context of genomic selection (GS), h is the correlation between true breeding values (TBVs) and genomic-estimated breeding values (GEBVs) (Xu et al., 2017). The equation of genetic gain translates the efficiency of selection in plant breeding programs into measurable parameters that breeders could manipulate in their pipelines. In other words, the genetic gain can be increased by increasing population size (or selecting fewer numbers of varieties) to increase selection intensity, shortening the breeding cycle, ensuring suitable genetic variation in the population, and obtaining accurate estimates of the genetic and phenotypic values (Araus et al., 2018; Moreira et al., 2019; Xu et al., 2017).

Figure 1-1. Soybean breeding timeline

In conventional breeding, given many phases of crossing, selection, and testing involved in the production of new plant varieties, it can take one or two decades to create a new cultivar (Ahmar et al., 2020). There are several procedures following the timeline (as shown in Figure 1-1) to combine desired crop traits into individual crops and select superior varieties eventually. Taking a soybean breeding program as an example, roughly it takes seven to eight years to develop new soybean varieties in conventional crop breeding programs (Krueger, 2019). After identifying a key problem in a specific program, breeders select from parent lines from given populations that have desired traits to potentially address the problem. Then the selected parent lines are combined to obtain crosses that probably would exhibit desired traits from both parents simultaneously. The crosses are planted and harvested individually, and their generations are advanced to produce enough seeds for planting progeny lines. Then the progeny lines are selected for a combination of the desired traits eliminating those inheriting undesired traits from parents. The selected progeny lines are kept for multi-location yield trials in which breeders could keep selecting lines that are adapted either widely across the regions or specifically for targeted regions, depending on the purpose of the program.

The conventional breeding cycle can be shortened by accelerating breeding procedures through integrated breeding approaches assisted with genetic markers (Xu et al., 2017), such as marker assisted selection (MAS) and GS. Genetic markers are the biological features that are determined by allelic forms of genes or genetic loci and can be transmitted from one generation to another. Thus they can be used as experimental probes or tags to keep track of an individual, a tissue, a cell, a nucleus, a chromosome or a gene (Jiang, 2013). MAS refers to a breeding procedure using genetic markers associated with

desirable traits as a substitute for or to assist conventional phenotypic screening (Fu et al., 2017). GS aims to use genome-wide markers to estimate the effects of all loci and thereby predict the genetic values of untested populations to achieve a comprehensive and reliable selection for accelerating genetic progress in crop breeding (Dwivedi et al., 2020). MAS and GS rely on marker-trait associations and they both have a prerequisite to use the markers in such selections that they should be closely linked to the precise phenotype or quantitative trait loci (QTLs) of interest and polymorphism for the markers between the parents (Shu & Wu, 2016).

Despite genomics and technical advances, identifying plants with desirable traits is the initial and most important step in breeding pipelines (Ahmar et al., 2020). It is well acknowledged in the breeding community that phenotyping efficiency still represents a bottleneck in crop breeding (Araus & Cairns, 2014; Araus et al., 2018; Furbank & Tester, 2011), as phenotypes are the bases to make selections in conventional breeding and a lack of suitable phenotyping data has led to poor results in gene/QTL discovery, limiting progress in genomics-assisted crop improvement programs (Mammadov et al., 2012).

Over the years, an explosion in state-of-the-art technologies, developed through collaborative efforts of multidisciplinary teams, to assist the process of high-throughput phenotyping (HTP) in crop breeding (Fasoula et al., 2020). In terms of improving genetic gain, HTP contributes directly to (1) increase selection accuracy by increasing the accuracy of phenotypic information; (2) increase selection intensity by increasing population size (or reducing the number of selected plants) with the increased phenotyping efficiency and (3) identify novel genetic variations. Extensive adaptations of HTP in breeding pipelines will indirectly impact (1) breeding circles with increase selection accuracy and intensity in

yearly trials, (2) budget allocation with reduced human input, (3) development of genetic selection with the availability of accurate phenotypic data in training populations.

Although the HTP advances have been proven capable of measuring a wide range of plant phenotypic traits, benefited by the rapid development of sensors and hardware platforms, the wide adoption of HTP in breeding routine procedures is hindered by a few limitations and challenges (Araus et al., 2018). There is an urgent need to ensure that these advances find practical application in breeding programs and contribute toward increased genetic gain.

1.2 Literature review

A phenotype is the result of interactions between plant genome and micro-and mega-environments encountered during its life span (Fasoula et al., 2020). Phenotyping is referred to as the set of methodologies (particularly noninvasive technologies) and protocols used to measure plant growth, architecture, and composition with certain accuracy and precision at different scales of organization, from organs to canopies (Fiorani & Schurr, 2013). Although there is not an official definition for the HTP, it is generally considered as an integrated system that can collect massive amounts of phenotypic data from hundreds (and more) of plants on a regular basis (days to months) with a high degree of automation (D. Li et al., 2021; Yang et al., 2017). With this broad definition, a diversified range of HTP systems has been developed at different scales of organization (from organs to canopies) of different types of crops under various conditions (from lab to field) for diverse purposes. The following contents summarize current HTP technologies applied in field conditions, HTP applications on crop breeding (conventional and molecular) as well as the limitations and challenges to be addressed before meeting the

needs for future breeding advances.

1.2.1 HTP technologies in field conditions

(1) Field HTP platforms

HTP can be implemented in field conditions with a variety of forms, mainly categorized into ground-based and aerial platforms. Ground-based platforms mainly include pole/tower-based systems, gantry-based systems, ground mobile systems, and cable-suspended systems. Ground-based platforms can provide data with higher spatial and temporal resolutions than the aerial ones, have the flexibilities to be equipped with multiple devices and usually do not require special operation skills, such as a remote pilot license. However, they have relatively low efficiency in terms of field coverage (D. Li et al., 2021).

Based on imaging distance, there are three types of aerial HTP platforms, namely unmanned aerial vehicle (UAV), manned aerial vehicle (MAV), and satellite platforms. As imaging distances increasing, the aerial platforms have decreased image pixel resolutions, but accordingly with increased ability to cover large-scale fields (from a few to hundreds of acres) within a short time period. The UAVs and MAVs require operations from at least two dedicated persons and devices to provide information from the global navigation satellite system (GNSS) together with ground control points (GCPs). With limited controls on environmental lights, calibration procedures are necessary to correct light variations in images collected by the aerial platforms. Satellite platforms can provide imagery in several spectral bands at meter-level resolutions as well as radio detection and ranging (RaDAR) data (Jin et al., 2021). Processing satellite images is at a relatively low cost due to the international standard protocols and the accessibility of commercial satellite platforms, such as the Landsat series and WorldView series (C. Zhang et al., 2020). Satellite imagery

is susceptible to weather conditions, such as cloud, rain, fog and haze (D. Li et al., 2021), which is gradually compensated by the application of RaDAR data on sensing crop traits.

(2) Sensors

Sensors that are commonly equipped in the field HTP platforms including red-green-blue (RGB), multispectral, hyperspectral, and thermal cameras, stereo cameras; and light detection and ranging (LiDAR) devices. These sensors occupy different ranges of wavelengths in the Electromagnetic spectrum (EMS), as shown in Figure 1-2. Although with some overlaps, these sensors varied in bandwidth, image resolution, image quality and price. They have been used in many studies to sense crop traits for different purposes.

Figure 1-2. Electromagnetic spectrum (EMS) and sensors corresponding to different regions of wavelength (Fahlgren et al., 2015).

RGB cameras record red (564-580 nm), green (534-545 nm), and blue (420-440 nm) spectral bands in the visible EMS regions. to produce digital images and mimic human visions (Jin et al., 2017). They are capable to obtain color information, 2D geometric information (e.g., canopy area) and depth information (e.g., plant height) from crop canopy. RGB cameras are the most commonly adopted sensors in the HTP platforms due to their advantages of low cost, high-resolution, user-friendly operation, light weight and adaptability to the working environment (Yang et al., 2017). However, three spectral bands and their broad bandwidth of RGB cameras limit their ability to receive crop traits other

than human perceptions, and their pixel digital numbers (DNs) are largely dependent on environmental radiations, thus hardly being used to estimate plant traits without pre-processing.

Multispectral and hyperspectral cameras extend the sensing range of RGB cameras with narrower and more bands along with the EMS. Multispectral cameras commonly include several spectral bands in the infrared spectral regions that have been shown sensitive to leaf pigments, for example, the red-edge region that is the transition from the visible to the near-infrared (NIR) spectral region (Geipel et al., 2016). While hyperspectral cameras have a higher spectral resolution, with continuous or discrete spectral bands (Jay et al., 2017), enable calculating more advanced vegetation indices (VIs) and unperceived crop phenotypes. The multi-and hyper-spectral cameras often require serial steps of imaging and pre-processing, including calibration radiometric calibration to reduce effects of light variations, image stitching and alignment (Zarco-Tejada et al., 2012).

Thermal cameras are used to capture the long-wave infrared spectral radiation emitted from crops and convert such reflectance to an electrical signal which highly correlates with the surface temperature of crops. Such surface temperature can be an indicator of its evaporation or transpiration rates. When a plant experiences abiotic or biotic stresses, these rates will change as well as its surface temperature. Hence, thermal cameras are widely used to detect such stresses and also evaluate the responses of plants with tolerant or susceptible abilities to the stresses (Gago et al., 2015; Zhou et al., 2020).

Stereo cameras and LiDAR devices are generally used to obtain depth information of targeted objects. Stereo cameras are very similar to the traditional RGB camera but with two or more lenses. Given two sets of two-dimensional (2D) images from both lenses,

three-dimensional information can be generated using stereoscopy techniques that take advantage of the parallax characteristics of human eyes (W. Li et al., 2016). A LiDAR device has a single-beam laser (narrowband in 600-1000 nm) that emits a light pulse and a receiver measures the propagation time of the light pulse from emission to reflection. The time is then converted into a distance given the known speed of light (Guo et al., 2018).

(3) Analytical methods and models

The HTP platforms and sensors provide multi-dimensional data to characterize crop phenotypic traits across a spatial and temporal scale on hundreds or thousands of breeding materials. Those data for different research purposes require dedicated methods to remove irrelevant or redundant information and tune model arguments to eventually reach acceptable results. Although with excellent interpretability, traditional regression-based models are limited by their model complexity to explain multivariate relationships between the predictors and responses (Parmley et al., 2019). Over the past few years, with access to high-performing computers and Application Programming Interfaces (APIs, for example, Keras in Python), machine learning (ML) and deep learning (DL) models have been extensively applied in crop breeding problems with HTP inputs.

Classic supervised ML methods, such as support vector machine, tree-based models and Linear/Quadratic Discriminant Analysis are mostly fit to estimate continuous crop traits (e.g., yield and plant height) and classify categorical traits (e.g., stress injury scores and maturity groups). Selecting an appropriate set of input features to these models is critical to the classification or prediction performance (Chandrashekar & Sahin, 2014). Therefore, an unsupervised ML method, Principal Component Analysis (PCA), is often used to reduce feature dimensions by creating principal components (linear combinations

of relevant features) that explain the most variations in the dataset. Others supervised ML model, i.e., the Lasso (least absolute shrinkage and selection operator) and ridge regression, could also help to shrink the dataset by assigning extremely small coefficients (even 0) to the variables that had little contributions to the model performance (James et al., 2013).

DL is the most important branch of ML allowing hierarchical data learning. One of the advantages of DL is that it does not require an extra step for feature selection, instead, important features will be discriminated during the training process by adjusting a set of parameters associated with the input features. There are a few classic DL models widely applied in HTP, including artificial neural networks (ANNs) taking tabulated input similar to ML models, convolutional neural networks (CNNs) for image analysis, recurrent neural networks (RNNs) for sequence or time data processing, and auto-encoder networks (AENs) (Goodfellow et al., 2016). In terms of disadvantages, however, DL models require a large dataset for training, testing, and validation. They are also computationally expansive and have poor interpretability (L. Zhang et al., 2016).

1.2.2 The applications of UAV-based HTP in crop breeding

In the application of HTP for crop breeding, UAV platforms have been applied in many studies due to its proper balance between data collection efficiency and quality, easy operation and flexibility to carry various types of devices (e.g., sensors, power sources, GNSS units, data storages and processing units). It has been witnessed to become an essential method for quickly obtaining crop traits for various purposes (Näsi et al., 2018). The section summarizes the applications of UAV-based HTP in obtaining crop traits that are commonly considered as primary and secondary traits for breeding selections, including plant height, lodging, flower, maturity, stress and yield.

(1) Plant height and lodging

Plant height is one of the most important secondary traits for the breeders. Plant height usually had a significantly positive correlation with yield in many types of crops, thus it is often used as an indicator of yield potential (Feng et al., 2019; W. Li et al., 2016; Wan et al., 2019; Yue et al., 2017). Besides, plant height can be used to detect crop lodging that is observed as the permanent deviation of crop plants from the upright position (Pinthus, 1974). Lodging reduces harvest efficiency and increases yield losses (Fischer & Stapper, 1987).

Various sensors and methods have been developed to rapidly and accurately measure plant height, as the conventionally manual measurements are very time-consuming and labor-intensive. There are a few applications of crop phenotyping using LiDAR systems to estimate plant height and biomass due to high point density, high spatial resolution, lighter weight, and good performance in low-altitude detection. Walter et al. (2018) reported an R^2 of 0.94 with an *RMSE* of 11.5 cm between the LiDAR height measurements on 173 maize hybrid lines and manual measurements.

One of the most popular stereovision techniques, Structure from Motion (SfM) enables the reconstruction of digital elevation models (DEMs) from a series of overlapping 2D RGB images. Similar to LiDAR, the DEM is in a format of dense point cloud with each point represented by a position in a local coordinate (x, y, and z axis) or WGS84 (World Geodetic System 1984, i.e., latitude, longitude, and altitude) and a color (red, green, and blue). Zhou et al. (2021) reported an *RMSE* of 2.9 cm for 12 GCPs (99 cm high) and a relative *RMSE* of 6.1% ($RMSE = 3.6$ cm) for soybean plots between the manually measured height and estimated height from a DEM generated by a set of RGB images

collected 15 m above ground in a soybean breeding field.

(2) Stresses

Plants may suffer various kinds of stress conditions during their growth, including abiotic stresses in adverse environments, such as drought, flooding, salt, heat and chill, etc., and biotic stresses, such as weed stress, pests, and disease stress (Abdel-Haleem et al., 2012). The stresses have significant adverse effects on plant growth and could reduce plant yield ultimately. Studying the response of plants to different stress conditions is essential to cultivating stress tolerant varieties in crop breeding (Valliyodan et al., 2016).

Plant canopy reflectance can be used as an indicator of plant health status under stress conditions because the reflected light directly depends on leaf pigment compositions (e.g., chlorophylls) (Jones & Vaughan, 2010). The reflectance in specific spectral bands could be captured by RGB, multispectral and hyperspectral cameras and then used to develop various VIs. One of the most widely used VIs is the Normalized Difference Vegetation Index (NDVI) that has been used to detect vegetations and evaluate plant ‘greenness’ (Gago et al., 2015):

$$NDVI = \frac{NIR - R}{NIR + R} \quad (1-2)$$

where *NIR* is the reflectance in the near-infrared band and *R* in the red band. Other image-derived VIs and geometric features were also investigated to detect plant physiological responses under stress. For example, drought is one of the most studied stress as its limiting factors in crop growth and production. It was reported that the yield of wheat, maize and soybean reduced 21%, 40% and 40%, respectively, due to drought on a global scale (Daryanto et al., 2016). Zhou et al. (2020) evaluated the differences in a group of VIs and

canopy temperatures between drought tolerant and sensitive soybean genotypes and the results show that drought tolerant genotypes had significantly ($p\text{-value} < 0.01$) higher NDVI, hue, saturation, canopy size, height, and lower temperature than drought sensitive genotypes. Those image traits were also shown to have higher broad-sense heritability (H^2) than yield.

(3) Maturity

Maturity time stands for the time length of soybean from planting to their physiological maturity. For some particular crop types, maturity time is a critical characteristic for breeders to select the crop genotypes with desired traits. Taking soybean as an example, soybeans with extended maturity time may take full advantage of the growing season to improve their yield (Staton, 2017). However, soybeans in delayed harvest days may suffer frost damages or high harvest losses. Therefore, a unique attribute-maturity group was given to crop whose productions related to their maturity time. For soybean, there are 10 maturity groups (MGs) from 0 to 9 according to their time length from planting to physiological maturity. MG 0 has the shortest maturity time and 9 has the longest maturity time. MG zones were developed accordingly to define where a soybean cultivar grows best (Mourtzinis & Conley, 2017).

Breeders need to figure out the maturity group of their new varieties and select those suitable for their targeted climate. To determine the MG of a new variety, breeders compare maturity dates of the new variety with checks that are in known MGs. Maturity date is the first day of the year when soybeans reach physiological maturity status. A soybean is considered mature when 95% of its pods achieve mature color. Conventionally, soybean breeders take notes of maturity date by visual observations. With tens of

thousands of breeding lines in a season, it is very tedious to take notes of every single lines.

Field HTP could be used to capture the difference between mature and immature plots and use the difference to predict the maturity date/MGs of breeding materials. Yu et al. (2016) developed a random forest model to classify mature and immature soybean lines using images in the blue and NIR band from a UAV-based HTP platform and achieved over 93% classification accuracy. Christenson et al. (2016) predicted the relative maturity of soybean lines using a PLSR model with three VIs derived from narrow spectral bands that were acquired by a UAV imaging system. The correlation between predicted relative maturity and observed maturity was 0.5 with an *RMSE* of 5.19 days.

(4) Yield estimation

Yield is the primary trait for selecting elite breeding genotypes, but a very complicated crop trait. Although the potential of estimating crop yield using image features has been reported in many studies, there are few studies conducted to predict the yield of soybean breeding lines in early breeding cycles. In breeding programs, tens of thousands of crosses from two parents are planted in progeny trials without replicates. Large variations in phenotypes (e.g. yield) can be caused by the conjunctions of their genetic and environmental variances (Fehr, 1991), consequently bringing large variances in image features and model predictions. Although a small percentage of selected genotypes are planted in following multi-location yield trials, the expansion of field networks across geographic locations increases spatial variances and consequently phenotypic variances in the same genotypes (Araus et al., 2018).

The most widely used method for yield estimation is linear regression that has high interpretability and shows good performance in some cases. Advanced data analytic

methods, such as ML and DL, show great potential in processing complicated data sets and extracting hidden information of data that are not easily acquired using restrictive models (Elavarasan et al., 2018). Maimaitijiang et al. (2020) predicted the yield of three soybean cultivars under two irrigation treatments with an R^2 of 0.72 and an $RMSE$ of $1054.9 \text{ kg} \cdot \text{ha}^{-1}$ using a deep neural networks (DNN) based on RGB, multispectral, and thermal images. Ashapure et al. (2019) reported a result of $R^2 = 0.7\text{-}0.89$ for predicting the yield of a set of tomato genotypes using a simple ANN model based on UAV-based RGB images.

1.2.3 Limitations and Challenges

Obtaining accurate and inexpensive estimates of the genetic value of breeding materials is central to improve breeding efficiency toward increasing genetic gain (Araus et al., 2018). While plant breeders have started using high-resolution genome information for germplasm characterization and genetic dissection of major agronomic traits, phenotypic traits such as maturity date and yield remain largely manual within breeding programs. Thus, the research bottleneck in crop breeding is shifting from genotyping to phenotyping (Mir et al., 2019). However, there are challenges limiting the adoption of the HTP advances for breeding purposes.

Data acquisition, processing, management, interpretation and modeling form the core of HTP to deliver accurate measurements of plant traits (D. Li et al., 2021). Each of the steps, more or less, currently requires manual interactions from those who have specific skills, such as remotely operating UAVs, programming, and statistical analyzing, etc. Although various software, tools, and online database have emerged to assist users with one or more steps in HTP pipelines, such as Coverage Tool, Plant 3D and Quantitative Plan, it is still being challenging for breeders to apply and customize these tools for their

specific research purposes. Thus, more user-friendly or ‘breeders preferred’ platforms are still needed to bridge the gap between breeders and the research community of ‘phenotypers’.

Besides, it is well noted that the current research in the field HTP largely focuses on developing HTP platforms, selecting input features and models, and validating the relationship between HTP data and ground data. When the platforms and models are under real breeding scenarios, they might not be as effective as they were tested due to large variations in field environmental conditions, breeding materials and their interactions. Therefore, there is an urgent need to integrate the current advances into a pipeline, gathering both image and ground data from yearly trials and training models to adapt to these variations, and eventually touching the goal of breeding programs to make selections of elite genotypes toward increased genetic gain (Araus et al., 2018).

1.3. Goals and objectives

Motivated by the need to bridge the gap between breeders and ‘phenotypers’, the goal of this research was to develop an integrated and automated HTP framework leveraging advanced technologies in remote sensing and artificial intelligence for estimating key traits and selecting elite genotypes towards improving the selection intensity and accuracy of conventional soybean breeding. To achieve this goal, this study features three objectives:

Objective 1: Develop an integrated and automated UAV HTP framework for measuring crop traits accurately and efficiently. The framework integrates a hardware module to acquire high-quality aerial images with accurate geo-references, as well as an applicational software module to automatically process the multi-dimensional images. The

framework can generate reliable measurements of plant geometric and morphological traits for further analysis.

Objective 2: Estimate soybean yield and maturity date of breeding materials using UAV image features and machine learning models. Yield is the primary trait for selecting elite soybean lines and the maturity date is decisive to select suitable lines for particular geographical regions. Accurate estimations of these two agronomic traits will increase the breeding population size that has been limited by the requirement of human input in field evaluations, and therefore increase the probability of selecting elite lines. Such estimations early in plant development allow selection decisions to be made before completing a growing season, eventually beneficial to saving breeding resources and reducing breeding circles.

Objective 3: Select elite soybean lines using UAV image features. The primary goal of soybean breeding is to select elite lines with high yield potential, meanwhile taking into consideration a few secondary traits, such as maturity date and lodging, etc. Additional to estimating the primary and secondary traits, the HTP framework can make selections on soybean lines by learning from the past breeder's selections and rank the candidate lines. With the initiative HTP suggestions on elite lines, breeders will be able to select a smaller group with higher yield potential to increase the selection intensity and accuracy towards improving genetic gain.

1.4 Dissertation outlines

The dissertation has five chapters:

Chapter One: Introduction. This chapter describes the background and justifications

to this research and a literature review of current HTP developments and their applications in crop breeding. It also listed the research goal and objectives, and an outline of the dissertation.

Chapter Two: Estimation of the maturity date of soybean breeding lines using UAV-based multispectral imagery. The physiological maturity date is a critical parameter for the selection of breeding lines in soybean breeding programs. This chapter was to investigate the potential of predicting maturity dates of soybean breeding lines using UAV-based multispectral imagery.

Chapter Three: Yield estimation of soybean breeding lines under drought stress using UAV-based imagery and CNN. This chapter was to evaluate the performance of a UAV imaging system and deep learning in estimating the yield of soybean breeding lines. A group of soybean breeding lines in three maturity groups was planted under rainfed conditions for testing their drought tolerance.

Chapter Four: Variety selection using UAV HTP towards improving selection accuracy and intensity for soybean breeding. This chapter was to model the breeder's selection using a group of agronomic traits and investigate the potential of selecting superior soybean varieties for soybean breeding programs using image-based secondary traits. The model selection was evaluated regarding selection and accuracy (future yield performance) and intensity (small group of selected genotypes with higher yield).

Chapter Five: Conclusion and future work. A summary of the results in this dissertation research and expectations for future work were presented in chapter five.

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CHAPTER TWO

ESTIMATION OF THE MATURITY DATE OF SOYBEAN BREEDING LINES USING UAV-BASED MULTISPECTRAL IMAGERY

2.1 Abstract

Physiological maturity date is a critical parameter for the selection of breeding lines in soybean breeding programs. The conventional method to estimate the maturity dates of breeding lines uses visual ratings of the pod senescence by experts, which is subjective by human bias, labor-intensive and time-consuming. Unmanned aerial vehicle (UAV)-based phenotyping systems provide a high-throughput and powerful tool to capture crop traits using remote sensing, image processing and machine learning technologies. The goal of this study was to investigate the potential of predicting maturity dates of soybean breeding lines using UAV-based multispectral imagery. Maturity dates of 326 soybean breeding lines were taken using visual ratings from the beginning maturity stage (R7) to full maturity stage (R8), and the aerial multispectral images were taken during this period on Aug. 27, Sept. 14, and 27, 2018. One hundred and thirty features were extracted from the five-band multispectral images. The maturity dates of the soybean lines were predicted and evaluated using partial least square regression (PLSR) models with 10-fold cross-validation. Twenty image features with importance to the estimation were selected and their changing rates between each two of the data collection days were calculated. The best prediction ($R^2 = 0.81$, $RMSE = 1.4$ days) was made by the PLSR model using image features taken on Sept. 14 and their changing rates between Sept. 14 and 27 with five components, leading to the conclusion that the UAV-based multispectral imagery is promising and practical in estimating maturity dates of soybean breeding lines.

2.2 Introduction

By 2050, the global population is expected to reach 9.8 billion (Hincks, 2018) and the current arable land is decreasing due to climate change, urbanization, soil degradation, water shortages and pollution (Breene, 2018). Food demand is expected to be 60% higher than it is today, resulting in limited global food supplies. Crop breeding is a promising solution for the food crisis by developing new crop varieties with improved traits, including high yield potential and resilience to biotic and abiotic stresses due to adverse environments (Sun, 2014).

The rationale of breeding programs is to select among a large number of genotypes crop varieties with high yield potential, tolerance to biotic and abiotic stresses and high efficiency in cultivation, harvest and processing (Breseghello & Coelho, 2013). Selection criteria usually include yield, lodging, flowering, maturity date, stress symptoms, severity, etc., with different emphasis depending on the purpose of breeding programs. Specifically, in soybean breeding programs, the physiological maturity date is a critical parameter for selecting soybean lines. Soybeans with extended maturity time may take full advantage of the growing season to improve their yield (Staton, 2017). However, soybeans in delayed harvest days may suffer frost damages or high harvest losses. Therefore, soybean cultivars are divided into maturity groups (MGs, e.g., MG III or MG IV) from 0 to 9 according to their time length from planting to the physiological maturity. MG 0 has the shortest maturity time and 9 has the longest maturity time. MG zones were developed to define where a soybean cultivar grows best (Mourtzinis & Conley, 2017).

Breeders determine the MG of a new variety by referring to the relative maturity (RM, by dividing an MG into 10 subgroups, e.g., RM3.0 to RM3.9 are 10 RMs in MG III)

of the new variety to commercially released soybean cultivars (referred to as checks) with a known MG. The RM is determined by the differences in the maturity dates (the first day of the year when soybeans reach maturity status) between the new variety and the checks. Conventionally, soybean breeders take measurements of the physiological maturity status of desired breeding lines using visual ratings by observing the senescence color of soybean pods. However, the visual rating is labor-intensive, time-consuming, and more importantly, it is subjective (Masuka et al., 2017). In conventional breeding programs, a breeder may need to scout tens of thousands of breeding lines in a progeny trial in a season (Yu et al., 2016), which makes it challenging to select the most desired lines. Therefore, there is a pressing need to develop an efficient and effective tool to take measurements of the maturity date of soybean progeny breeding lines in fields using novel approaches.

Maturity generally occurs when soybean pods have reached their physiological matured stages, showing gray, tan, or brown color in pods (Fehr et al., 1971). After entering the beginning maturity stage (R7), a soybean plant, including leaves and pods, loses its moisture quickly from around 60% to less than 30%, depending on the local air temperature, humidity, and soil conditions (Peske et al., 2004). In addition, with the plant approaching senescence, the chlorophyll content of leaves reduces and the color turns to yellow/gray (Fehr et al., 1971). This change might result in substantial changes in spectral reflectance. Therefore, it is possible to quantify the plant maturity level using the combinations of spectral reflectance in given wavebands that are very sensitive to crop canopy characteristics, such as chlorophyll content (Gitelson et al., 2003), nitrogen content or water status (Barnes et al., 2000).

Yu et al. (2016) developed a random forest model to classify mature and immature

soybean lines using images in the blue and near-infrared bands from an unmanned aerial vehicle (UAV)-based high-throughput phenotyping (HTP) platform, and achieved over 93% classification accuracy. Christenson et al. (2016) predicted the RM of soybean lines using a partial least square regression (PLSR) model with three vegetation indices (VIs) derived from narrow spectral bands that were acquired by a UAV imaging system. The correlation of determination (R^2) between the predicted RM and observed maturity was 0.5 with a root mean square error (*RMSE*) of 5.19 days. However, the difference between two relative maturity designations (e.g., RM 3.7 and RM 3.8) is usually only one to two days (personal communication with a soybean breeder-Dr. Andrew Scaboo). It is not sufficient for soybean breeding purposes by classifying breeding lines into only two classes of mature and immature lines or the prediction with 5 days error. To the best of our knowledge, there is no such study showing the ability to determine accurate maturity date for breeders.

Therefore, the goal of this study was to investigate a method to estimate the maturity dates of soybean breeding lines using a UAV-based imaging system. There were three supportive objectives to achieve this goal: (1) To investigate the best time (growth stage) to collect images, (2) to select image features important to estimate relative maturity date, and (3) to evaluate the accuracy of our estimates.

2.3 Materials and methods

2.3.1 Field experiment

The field experiment was conducted at an experimental field in the Greenley Research Center of the University of Missouri, Novelty, Missouri, United States (40°01'N, 92°11'W) in 2018. Eleven thousand, four hundred and seventy-three soybean lines were inbred from soybean varieties in MG III and IV, and planted on May 29, 2018, in a 3.64 ha

field. The soybean lines were planted in single-row plots, each being a progeny row derived from a single F4 plant, without replicates with 2.59 m in length and 0.76 m spacing in between rows. The breeding lines started maturing from Sept. 19 and were completed on Oct. 2, 2018.

Three hundred and twenty-six soybean lines were randomly selected before R8 stages as ground references for the estimation of maturity dates. The maturity dates of these soybean lines were determined by an experienced breeder using visual assessments. The measurements of maturity date were recorded every seven days starting from Aug. 31 to Oct. 5. Mature plots were not observed until Sept. 19. In each measuring day, a soybean line that has approximately 95% of its pods achieved mature pod color was determined to be mature and the date was recorded as its maturity date, and for those that have slightly less or more than 95% pods achieved mature color, the maturity dates were estimated as 1 to 3 days after or before the measuring day.

2.3.2 UAV data collection

Multispectral images were acquired using a multispectral camera RedEdge-M (MicaSense, Seattle, WA, USA) that has a resolution (number of total pixels) of 1260×960 pixels. The details of wavelength are shown in Table 2-1. An iPad mini 3 (Apple Inc., Cupertino, CA, USA) was used to configure the camera taking time-lapse images at 1 frame per second (fps) by connecting the camera using its Wi-Fi hotspot. A GNSS (Global Navigation Satellite System) unit was attached to the camera and pre-programmed to provide geo-referencing information for each frame of images. All images with the EXIF (Exchangeable Image File Format) metadata were saved to an SD card on the camera. Before each flight, a calibration reflectance panel (CRP) was imaged by holding the camera

at about 1 m above the CRP and looking vertically in an open area (to avoid shadow). The CRP has a factory calibrated reflectance in each of the five spectral bands and is used to convert the raw pixel values of multispectral images into reflectance during post-processing (MicaSense, 2017b). The reflectance in each band of the CRP used in this study is shown in Table 2-1.

Table 2-1. Wavelength information of the camera and the reflectance in each band of the calibration reflectance panel (CRP).

Band Name	Center Wavelength (nm)	Bandwidth* (nm)	Reflectance (%)
Blue (B)	475	20	49.2
Green (G)	560	20	49.3
Red (R)	668	10	49.1
Red edge (Re)	717	10	48.7
Near-infrared (NIR)	840	40	49.0

* Bandwidth is defined as the full width at half maximum (FWHM).

The multispectral camera was mounted on a UAV DJI Matrice 600 Pro (DJI, Shenzhen, China) with the camera facing the ground vertically (nadir view). The UAV platform was controlled using a flight control App Autopilot (Hangar Technology, Austin, TX, USA) that allows setting up flight path, flying speed, and elevation prior to the flights. Images were taken at 30 m above ground level (AGL) with a ground sample distance (GSD) of $20.8 \text{ mm} \cdot \text{pixel}^{-1}$. The desired flight speed and the number of paths were carefully determined to obtain sufficient overlaps (forward overlap of 87.5% and side overlap of 84.5% in this case) to cover the whole field. The images were acquired from 13:15 to 15:00 CDT on Aug. 27, Sept. 14 and Sept. 27, 2018.

2.3.3. Image processing

The geo-referenced multispectral images were downloaded from the SD card and uploaded to a UAV image processing software Pix4D Mapper (Pix4D, Lausanne, Switzerland) to generate orthomosaic images of the field. After all the images were

imported, the camera GNSS information was read automatically from the EXIF metadata. The images of the CRP were selected and the reflectance was inputted manually following an official instruction (MicaSense, 2018). The “Ag Multispectral processing” template was chosen for this processing. When the processing was completed, the orthomosaic image of each band was generated and exported as a *.tif* image, which was processed using the Image Processing Toolbox and Computer Vision System Toolbox of MATLAB (ver. 2016b, The MathWorks, Natick, MA, USA).

The background (soil and other non-crop) information was first removed from orthomosaic images using color thresholds. Figure 2-1 a and c are the colorful images with the red (R), green (G) and blue (B) channels of images acquired on Aug. 27 and Sept. 27 and they show different color contrasts between soil and soybeans at different growth stages. A blue normalized difference vegetation index (BNDVI) (Yang et al., 2004) that could signify the crop pixels and suppress non-crop pixels was used to remove the background of images on Aug. 27 and Sept. 14 as it had the highest contrast between soil and soybeans (Figure 2-1b). The blue-wide dynamic range vegetation index (BWDRVI) (Hancock & Dougherty, 2007) was used for images on Sept. 27 as it was found to have higher contrast than BNDVI when soybean leaves started turning yellow and similar to soil color (Figure 2-1d). From the histograms in Figure 2-1 e and f, pixels with BNDVI values higher than 0 or with BWDRVI values higher than 0.7 were considered as soybean lines.

$$BNDVI = \frac{nir - blue}{nir + blue} \quad (2-1)$$

$$BWDRVI = \frac{0.1 \times nir - red}{0.1 \times nir + red} \quad (2-2)$$

Figure 2-1. Orthomosaic images of part of the soybean field. (a) and (c) are the colorful images with the red (R), green (G) and blue (B) channels of images acquired on Aug. 27 and Sept. 27. (b) The grayscale image of blue normalized difference vegetation index (BNDVI) derived from images taken on Aug. 27 and (d) the grayscale image of blue-wide dynamic range vegetation index (BWDRVI) derived from images taken on Sept. 27. (e) and (f) are histograms of image pixel values in the two red rectangles.

After background removal, individual soybean lines were segmented from one of the five-band images (the blue band images were used in this case) by manually drawing vertical and horizontal lines in alleys between plots. The segmented individual plots were saved as binary masks and then applied to the rest bands to get images of individual plots in each band. Each segmented plot has a unique plot number associated according to its physical position in the field and the number was saved with images of the plots. The 326 soybean samples were recognized by matching plot numbers in ground reference data with

those in segmented images.

Fifty-four vegetation indices (VIs) were extracted from the five-band images using the formula summarized by Agapiou et al. (2012) and Henrich et al. (2009). The formula and a brief description of the selected VIs are included in Appendix Table A1. The mean and standard deviation (std) values of these VIs and the pixels of the five-band images of every single row were calculated as image features. In addition, we also calculated the mean and the std values of the hue, saturation (S) and value (V) in HSV color space and the L*, a* and b* in CIELAB color space, which was converted from R, G, and B channels using MATLAB function '*rgb2hsv*' and '*rgb2lab*'. In total, 130 image features were extracted for further processing.

2.3.4. Maturity date and adjusted maturity date

The status of maturity was determined when approximately 95% of the pods in a line had achieved mature pod color by visual assessment (Fehr et al., 1971), ignoring the delayed leaf drop and green stems that might introduce variances in canopy images of soybean lines with the same maturity date. Additionally, maturity dates of soybean lines are usually determined visually by breeders in an interval of seven days and the dates of the breeding lines matured within the intervals were estimated based on experiences, therefore, the maturity dates may allow a one-or two-day error (Yu et al., 2016). Figure 2-2 shows images of three soybean lines taken on Sept. 27. Although recorded with the maturity date of Sept. 25, they had different numbers of green leaves remaining on the canopy. Line 7344 (left) had rare leaves remaining so that it could be derived from the images that its pods have turned to mature color. However, a certain number of green leaves on the canopy of line 6913 (right) covered most of its stems. The real scenarios could be

that either its pods had reached the mature color but with delayed dropping leaves or its maturity date was estimated too early in the interval of seven days.

Figure 2-2. Illustration of variations in canopy images of the soybean lines matured on Sept. 25. Images were generated using R, G and B bands from the multispectral camera. Breeding line no. from left to right is 7344, 7335 and 6913.

In order to tolerate the variances in canopy leaves, we used a new parameter adjusted maturity date (adMD), calculated as Eq. 2-3. The adMD calculates the variances in canopy images of the soybeans matured on the same day and applied the variances to the manually measured maturity date. The original maturity dates would be extended to a more precise format (22.2, 23.7 and 25.6 for soybean lines in Figure 2-2 from left to right) considering human error and canopy variances.

$$adMD_i = \frac{1}{n} \left(\sum_{1}^n \frac{x_{in} - m_{in}}{std_{in}} \right) + MD_i \quad (2-3)$$

where $adMD_i$ is the adjusted maturity date of the i^{th} soybean line. i represents the i^{th} soybean line, $i = 1, 2, \dots, 326$ and n represents the n^{th} image feature used in an estimation model, $n = 1, 2, \dots, 130$. MD_i is the maturity date of the i^{th} soybean line that is determined by breeders. x_{in} is the value of the i^{th} soybean line in the n^{th} image feature. m_{in} is the mean of all soybean lines matured on the same day with the i^{th} soybean line in the n^{th} image features. std_{in} is the standard deviation of all soybean lines matured on the same day with the i^{th} soybean line in the n^{th} image features.

2.3.5. Data analysis

All statistical analyses were conducted using the statistical toolboxes in MATLAB. To evaluate the potential of UAV-based imagery in estimating maturity dates, PLSR

models were built using image features as predictors and maturity dates as responses for the data collected on three days. PLSR models create linear combinations (known as components) of the original predictor variables (image features) to explain the observed variability in the responses (measured maturity dates). Additionally, PLSR largely is able to reduce the variability and instability of estimated responses caused by multicollinearity among predictors (Ishtiaq & Abdul-Aziz, 2015). The PLSR was conducted using the '*plsregress*' function with 10-fold cross-validation. The estimation accuracy was evaluated using the *RMSE* of the estimated responses.

To develop a parsimonious and interpretable PLSR model for each data set, variable importance in projection (VIP) scores were used to select predictors by estimating the importance of each predictor in a PLSR model. A predictor with a VIP score close to or greater than 1 can be considered important in a given model (Eigenvector Research, 2018). The VIP scores of each predictor were calculated using MATLAB codes suggested by the MathWorks Support Team (2019).

The variance inflation factor (VIF) was also calculated to remove image features with high collinearity (James et al., 2013). The VIF is calculated as a dependent variable of the coefficient of determination (R^2) of an individual feature against all other features. R^2 was obtained by calling the parameter of '*lm.Rsquare.Ordinary*' in the '*fitlm*' function. This step was performed in a loop that the variable with the highest VIF was removed each time and the loop would not stop until the highest VIF is less or equal to 5 (Chen et al., 2014). The higher the VIF value an image features have, the higher the collinearity is between this feature with others.

To better interpret the PLSR models, the correlation between each selected

predictor and maturity dates was calculated using ‘*corr*’ function with the ‘*Pearson*’ option. As the multispectral images were collected on the three days at different growth stages of soybean lines, the changes in image features between every two days may indicate the transitions from the immature to mature status of soybean lines. The changing rate between each two data collection days was calculated using Eq. 2-4.

$$Changing\ rate(i) = \frac{P(i + 1) - P(i)}{P(i)} \quad (2-4)$$

where P is each selected predictor of single soybean lines on the $i = 1^{st}$, 2^{nd} and 3^{rd} time of data collection.

2.4 Results

2.4.1 Estimation of soybean maturity dates using PLSR

The maturity dates estimated using the PLSR models with 10-fold cross-validation on the three days are shown in Figure 2-3. Figure 2-3 a illustrates the percent variance explained in maturity dates with the number of PLSR components. Variance in maturity dates can be explained up to 59%, 77% and 83% using image features on Aug. 27, Sept. 14 and Sept. 27, respectively. Figure 2-3 b shows the changes of *RMSE* with the number of PLSR components and the optimal number of components for each day was determined when the *RMSE* reaches the minimum on that day. The estimation using image features on Sept. 27 had the lowest *RMSE* of 1.7 days with five components followed by the one on Sept. 14 (*RMSE* = 1.8 days) with 24 components and Aug. 27 (*RMSE* = 2.7 days) with seven components. Thus, the optimal numbers of components for the PLSR model on the three days are 7, 24 and 5, respectively.

Figure 2-3 c and e show the correlations between the manual maturity dates and

predicted maturity dates from PLSR models with the optimal numbers of components. When using image features of soybean lines in the middle of their maturity stage (R8, Sept. 27), there was the best agreement ($R^2 = 0.82$) of predicted maturity dates and manual measurements.

Figure 2-3. Estimation of maturity dates using the PLSR model. (a) Percent of variance explained in the maturity dates as a function of the number of components in the PLSR model. (b) Mean square error in the maturity dates as a function of the number of components. (c)-(e) The correlations between manual maturity dates and predicted maturity dates using images features taken on Aug. 27, Sept. 14 and Sept. 27, respectively. ncomp represents the optimal number of components.

2.4.2. Model parsimony

Figure 2-4 shows the VIP scores of 130 predictors of the PLSR models on the three days. There were 42, 56 and 73 predictors with VIP scores equal or greater than 1 in models on August 27, Sept. 14 and Sept. 27, respectively. VIFs of these predictors were then

calculated and those with VIFs less than 5 are shown in Table 2-2. Table 2-2 also shows the Pearson correlations between the maturity dates and the selected predictors (image features) from PLSR models on the three days. It can also be seen that few image features were overlapping in the PLSR models for all three days. The possible reason is that strong collinearities were existing among all the 130 image features so that those with high Pearson correlations were not selected due to their high VIFs (>5).

Figure 2-4. Variable importance in projection (VIP) scores of 130 predictors of the PLSR models on the three days.

Table 2-2. Pearson correlations between the predictors with VIP scores ≥ 1 and variance inflation factors (VIFs) < 5 and maturity dates on the three days.

Image features	Aug. 27	Sept. 14	Sept. 27
re_mean	-0.596*	—	—
S_mean	-0.501	-0.697**	—
CCCI_std	-0.487	-0.930***	—
CIrededge_mean	0.504	—	0.368
CVI_std	-0.389	—	—
CI_std	0.184	-0.662**	—
GDVI_std	0.177	0.861***	—
H_mean †	0.102	—	—
NormG_std	0.413	-0.831***	—
IF_std	-0.170	—	—
RRI2_std	-0.239	-0.227	—
CI_mean	—	-0.935***	—
H_std	—	-0.915***	—
GRVI_mean	—	0.922***	—
MTVI2_std	—	0.795***	—
hue_std ‡	—	—	0.959***

GEMI_mean	—	—	0.977***
GRNDVI_std	—	—	0.959***
BNDVI_mean	—	—	0.934***
IF_mean	—	—	-0.991***

*, ** and *** indicate the significance at $p = 0.05$, 0.01 and 0.001 levels, respectively. † and ‡ represent the image feature hue in HSV color space but was calculated using different methods. † uses the equation in Appendix Table A1. ‡ is converted from the RGB image using the ‘*rgb2hsv*’ function in MATLAB.

In order to understand the different growth trends of soybean lines matured sequentially, the Pearson correlations between maturity dates and the selected image features in Table 2-2 were calculated and shown in Table 2-3. It can be seen that only three image features re_mean, CI_mean and IF_mean had significant linear relationships with the maturity dates on Aug. 27, while the majority of image features on Sept. 14 and Sept. 27 are of significance.

Table 2-3. Pearson correlations between maturity dates and the means of image features at each maturity date on the three days.

Image features	Aug. 27	Sept. 14	Sept. 27
re_mean	-0.596*	-0.414	0.890***
S_mean	-0.501	-0.697**	-0.830***
CCCI_std	-0.487	-0.930***	0.605*
Cirededge_mean	0.504	0.928***	0.368
CVI_std	-0.389	-0.718**	-0.552*
CI_std	0.184	-0.662**	0.931***
GDVI_std	0.177	0.861***	0.845***
H_mean	0.102	-0.830***	-0.409
NormG_std	0.413	-0.831***	0.916***
IF_std	-0.170	-0.921***	-0.585*
RRI2_std	-0.239	-0.227	0.981***
CI_mean	-0.595*	-0.935***	-0.886***
H_std	-0.490	-0.915***	0.268
GRVI_mean	0.497	0.922***	0.499
MTVI2_std	-0.035	0.795***	0.989***
hue_std	-0.413	-0.931***	0.959***
GEMI_mean	-0.004	0.857***	0.977***
GRNDVI_std	0.242	-0.889***	0.959***
BNDVI_mean	0.356	0.879***	0.934***
IF_mean	-0.544*	-0.879***	-0.991***

*, ** and *** indicate the significance at $p = 0.05$, 0.01 and 0.001 levels, respectively.

Figure 2-5 shows the predicted maturity dates using three PLSR models with 20 selected image features and their changing rates. The estimations were performed using the

same method mentioned in section 2.5. The optimal numbers of components for the three PLSR models were 13, 5 and 4, respectively. Compared with Figure 2-3 d and e, the PLSR models with changing rates in the selected image features taken between Sept. 14 and Sept. 27 improved the estimation accuracy (Figure 2-5 b and c). The results indicate that there are certain patterns that image features of soybean lines with different maturity dates changed over time and these patterns could help to recognize the maturity dates. From Figure 2-5 a and b, the model with the changing rates between Sept. 14 and Sept. 27 had higher estimation accuracy than the one with the changing rates between Aug. 27 and Sept. 14, showing the challenges in estimating maturity dates at early stages.

Figure 2-5. Estimation of maturity dates using PLSR models with the selected image features and the changing rates. (a) The correlations between manual maturity dates and predicted maturity dates using the selected images features taken on Sept. 14 and their changing rates between Aug. 27 and Sept. 14. (b) The correlations using the selected images features taken on Sept. 14 and their changing rates between Sept. 14 and Sept. 27. (c) The correlations using the selected images features taken on Sept. 27 and their changing rates between Sept. 14 and Sept. 27.

2.4.3. Adjusted maturity dates based on the variances in image features

Even though we can observe the monotonically increasing and decreasing trends in the selected image features against the maturity dates (Table 2-2), the variances in some days overlapped with other days (Figure 2-6 a and b), especially when those soybean lines were close to their maturity, for example, the large variances in NDVIrededge_mean taken on Sept. 14 were observed in those lines matured on Sept. 19 and 20, and the large variances

in CCCI_mean taken on Sept. 27 were observed from Sept. 21 to 27.

Figure 2-6 c shows canopy images in these two vegetation indices of soybean lines (as shown in Figure 2-1) that were determined by visual ratings maturing on the same day (Sept. 25). It can be seen that the dry stems had the highest CCCI values, while the green leaves had the lowest. When soybean lines had reached their R7 stages, the water content of the seeds is less than 50% and when they at the R8 stages, the water content is less than 30% (Fehr et al., 1971). As an indicator of water stress suggested by Barnes et al. (2000), CCCI could represent various water content in soybean lines.

The NDVIrededge was first proposed by Gitelson and Merzlyak (1994) and found that it was highly proportional to crop leaf chlorophyll content. It was calculated using the R (668 nm) band with the Re (717 nm) band and has shown a strong linear proxy ($R^2 = 0.94$) of the green portion of the fraction of absorbed photosynthetically active radiation (fAPAR) that is sensitive to chlorophyll content in maize and soybean canopy (Viña & Gitelson, 2005). It is consistent with our observation that the soybean line with more remaining leaves (No. 6913) had higher NDVIrededge values while the dry lines (e.g., No. 7344) had very low values.

Figure 2-6. Vegetation indices CCCI and NDVIrededge. (a)-(b) The relationships between maturity dates and NDVIrededge_mean and CCCI_mean collected on Sept. 14 and 27. (c) Canopy images of the soybean lines matured on Sept. 25 in RGB, CCCI and NDVIrededge.

The maturity dates of soybean lines were adjusted based on the variances of NDVIrededge_mean and CCCI_mean taken on Sept. 14 and 27. The relationships between maturity dates and the adjusted maturity dates were shown in Figure 2-7. The PLSR models were used to predict the maturity dates using the same method mentioned in section 2.5 and the estimations are shown in Figure 2-7 b and c.

Figure 2-7. Adjusted maturity dates. (a) The relationship between maturity dates and the adjusted maturity dates based on the variances of NDVIrededge_mean and CCCI_mean taken on Sept. 14 and 27. (b) The correlations between adjusted maturity dates and predicted maturity dates using the selected image features taken on Sept. 14 and their changing rates between Aug. 27 and Sept. 14 (c) The correlations using the selected image features taken on Sept. 14 and their changing rates between Sept. 14 and 27. (d) The correlations using the selected image features taken on Sept. 27 and their changing rates between Sept. 14 and 27.

2.5 Discussion and future work

2.5.1. Estimation of soybean maturity dates at different growth stages

The estimation of soybean maturity dates using the PLSR model with image features collected on different days in Figure 2-2 shows that the best agreement of predicted maturity dates and manual measurements was made by image features in the middle of

their maturity stage, while the worst is by those collected when none of the soybean lines started maturing.

From Table 2-2, only one image feature (re_mean) had a significant linear relationship with the maturity dates on Aug. 27, while there were nine and five image features highly correlated (p -value < 0.05) with the maturity dates for Sept. 14 and 27, respectively, indicating the challenge in estimating maturity date at the early stages. We can also see that there were six common image features selected for PLSR models on Aug. 27 and Sept. 14, and five of them had significant linear relationships on Sept. 14. These image features had the potential to be used as predictors, but they have not shown the trends at the early stage when mostly soybean lines remained green (Figure 2-1a).

Again, it can be seen from Table 2-3 that only three image features re_mean, CI_mean and IF_mean had significant linear relationships with the maturity dates on Aug. 27, while the majority of image features on Sept. 14 and 27 were of significance. It may indicate a higher accuracy in estimation of maturity date using data from later stages than early stages, as implied by the estimations from the PLSR models (Figure 2-3).

2.5.2. Selected features for the parsimonious models

For all three days, five image features (CIrededge_mean, GDVI_std, GRVI_mean and BNDVI_mean) had positive linear relationships with the maturity dates, and four image features (S_mean, CVI_std, IF_std, CI_mean and IF_mean) were negatively related to the maturity dates, suggesting that these image features of all soybean lines kept increasing or decreasing from the beginning to the full maturity stage so that they can maintain consistent trends no matter which stages the image features were collected at. As shown in Figure 2-8a, for all the soybean lines, the changing rates in CI_mean were above

0 due to the increased CI_mean in all soybean lines from Sept. 14 to 27. It was also observed that the changing rates in soybean lines that matured later were greater than those in lines that matured early, leading to a positive linear relationship between the changing rates in CI_mean and the maturity dates.

It should be noticed that the Pearson correlation coefficients of the other 11 image features in Table 2-3 had opposite signs for the three days, especially for the latter two days, such as re_mean, CCCI_std, CI_std, NormG_std, hue_std and GRNDVI_std. The reason may be that for the soybean lines matured on different days, there were different changing rates in such features. From Figure 2-8a, the changing rates in hue_std were significantly less than 0 for lines matured before Sept. 24, indicating the hue_std of these lines decreased from Sept. 14 to 27. For lines matured during Sept. 24 to 27, the changing rates in hue_std were very close to 0, suggesting that the hue_std in these lines barely changed between two data collection days. For lines matured after Sept. 27, the changing rates were significantly greater than 0, showing that there were increased hue_std in these lines. It might be due to the gradual occurrence of yellow leaves when the soybean lines were closed to their maturity (Figure 2-8b), leading to high variances in hue, while those lines that matured late still had more green leaves (Figure 2-8c). When hue was calculated on Sept. 27, matured soybean lines had more dry leaves than fresh leaves (Figure 2-8d), leading to low variances, while the late-matured lines were stepping into their R8 stages (Figure 2-8e), resulting in high variances. Similar situations could happen in other image features with opposite linear relationships for the latter two days.

Figure 2-8. The changing rates in CI_mean and hue_std from Sept. 14 and 27. (a) The changing rates in CI_mean and hue_std. (b) and (d) are RGB images of a line matured on Sept. 19, and they were taken on Sept. 14 and 27, respectively. (c) and (e) are RGB images of a line matured on Oct. 1, and they were taken on Sept. 14 and 27, respectively.

2.5.3. Adjusted maturity dates

In Figure 2-7a, the adjusted maturity dates for both image collection days were extended around their observed maturity dates, which might help to tolerate the variances of canopy green leaves as well as errors from subjective judgments and estimations of breeders. By using the adjusted maturity dates as a ground reference, the *RMSE* dropped from 1.7 to 1.4 and the R^2 increased to 0.81 using the selected images features taken on Sept. 14 and their changing rates between Sept. 14 and 27, while nearly no changes occurred on the other two models. It might be because these two image features captured more variances on Sept. 14 than those on Sept. 27.

By personal communication with breeders, *RMSE* within 1.5 days can be considered as a tolerable error in soybean breeding programs. Compared to previous

studies, our study shows improvements in predicting the exact maturity dates instead of the classification between mature and immature lines (Yu et al., 2016), as well as making a more practical prediction (with an acceptable accuracy) for breeding programs (Christenson et al., 2016). Therefore, it can be concluded that it is a promising method to predict soybean maturity dates using UAV-based multispectral image features by screening the soybean lines once at the beginning of maturity stage to quantify the variances in canopies and another one at the middle of the full maturity stage to track the canopy changes.

As the adjusted maturity dates are first proposed in this study to tolerate the variances of canopy green leaves for the purpose of predicting soybean maturity dates using canopy image features, the maturity dates were adjusted based on the variances in two image features with known matured dates in a single environment. In soybean breeding programs, RMs of a variety from around 40-50 environments are required to assign an MG to the variety. Therefore, the variances need to be quantified in future studies by repeating the experiments in multiple environments and documenting the image features.

Errors could be introduced by many factors regarding image features. Due to the limited field of view of the multispectral camera, the 3.64 ha field was fully screened by three flight missions during the time period of more than 2 h, including taking off and landing the UAV, changing batteries and calibration. Light changes during image collection were conjunctions of many factors, such as the changing relative position between sun and drone, various light diffusions caused by the thin cloud in the sky and the random exiting thick clouds, which resulted in cloud shadows in the images (not observed in this study) (MicaSense, 2017a). Another inevitable source of variation is some blurred

portions of orthomosaic caused by image stitching. At the later stage of soybean growth, soybean lines with big canopies can touch and overlap with their neighbors and leaves on immature lines are yellow or in the transition to yellow, which were hard to be distinguished with soil. Under this scenario, blurs and even undetectable misalignments of breeding lines might occur in the orthomosaic.

2.5.4. Future work

In future studies, the variances in image features to adjust the maturity dates need to be quantified and derived by repeating the experiments in multiple environments and documenting image features. There were also two major issues expected to be addressed to reduce errors introduced during the image collection and processing pipeline. First, as an image pixel value is the reflectance of incoming solar irradiance, the variation in light conditions could introduce variations to image-derived features. The relationship between light changes during UAV flight missions and camera responses (reflectance) should be further explored and flight dynamics (drone pose, sun's position, light diffusion, clouds, etc.) need to be better integrated to describe the light changes. Second, variations are inevitably introduced when images are stitched based on feature recognition. Instead of localizing individual breeding lines from an orthomosaic, efforts have to be made to investigate the potential of direct georeferencing that the geometric position of a breeding line is the conjunction of the relative position of the line in an image and the UAV position where the image was taken. With direct georeferencing, flight attitude and speed can be liberated from high image overlaps ($\geq 70\%$ for creating orthomosaic images) so that the time issue and light variation can be moderated consequently.

2.6. Conclusions

The potential of predicting maturity dates of soybean breeding lines using UAV-based multispectral imagery was investigated in this paper. Maturity dates of 326 soybean breeding lines were taken using visual ratings from the beginning maturity stage (R7) to full maturity stage (R8), and the aerial multispectral images were also taken during this period on Aug. 27, Sept. 14 and Sept. 27. One hundred and thirty features were extracted from the five-band multispectral images. The maturity dates of soybean lines were predicted and evaluated using PLSR models with 10-fold cross-validation. The results showed that the estimations at later stages had better accuracy than those at earlier stages. Twenty image features with importance were then selected to simplify the PLSR models. The changing rates in image features between each of the two collection days were calculated. The estimation accuracy was improved by the simplified PLSR models with the selected image features and their changing rates. The adjusted maturity dates were proposed to tolerate the variances of canopy green leaves and calculated based on the variances in $NDVI_{rededge_mean}$ and $CCCI_mean$. The best prediction ($R^2 = 0.81$, $RMSE = 1.4$ days) was made by the PLSR model using selected image features taken on Sept. 14 and their changing rates between Sept. 14 and 27 as predictors and the adjusted maturity dates as responses with five components. This study outperformed other similar studies in terms of prediction accuracy and practical usefulness. Another contribution of this paper is that based on our observations from canopy images, the adjusted maturity dates were proposed to tolerate the variances of canopy green leaves as well as errors from subjective judgments and estimations of breeders.

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CHAPTER THREE

YIELD ESTIMATION OF SOYBEAN BREEDING LINES UNDER DROUGHT STRESS USING UNMANNED AERIAL VEHICLE-BASED IMAGERY AND CONVOLUTIONAL NEURAL NETWORK

3.1 Abstract

Developing new crop varieties with improved traits through breeding programs is one of the key approaches to secure food supply for the growing global population of 10 billion in 2050. In breeding programs, yield is always used as a primary trait to select superior genotypes and evaluate breeding efficiency. However, yield trials have to be conducted at multiple locations for several years to evaluate the performance of numerous breeding lines and their adaptability to environments. The efficiency of conventional breeding programs is limited by the labor-intensive and time-consuming field phenotyping procedures, which can be improved by developing high-throughput field phenotyping systems using emerging technologies, including Unmanned Aerial Vehicle (UAV) imagery and deep learning technologies. The goal of this study was to evaluate the performance of a UAV imaging system and convolutional neural network (CNN) in estimating yield of soybean breeding lines. In this study, 972 soybean breeding plots in three maturity groups were planted under rainfed conditions for testing their drought tolerance. Aerial images were taken at the late vegetation (V6), early (R1), and late reproductive (R6-R8) growth stages. Seven image features associated with plant height, canopy color, and canopy texture were selected to estimate the yield of each breeding plot. A mixed CNN model was built to estimate soybean yield by taking the seven image features and two categorical factors, i.e. maturity group and drought tolerance, as predictors. Results show that image features collected at the early and late reproductive growth stages are comparably promising in

estimating soybean yield. The prediction model could explain 78% of measured yield with a root mean square error of 391.0 kg·ha⁻¹ (33.8 % to average yield), indicating that the UAV imagery and deep learning models are promising in estimating yield for soybean breeding purposes.

Keywords: Soybean breeding; drought stress; yield estimation; UAV multispectral imagery; convolutional neural network

3.2 Introduction

Development of new crop varieties with improved traits through crop breeding programs is one of the promising solutions for the estimated food crisis in 2050 when agricultural production needs to double its current growth rate to feed 10 billion people in the world (Hincks, 2018). Soybean (*Glycine max*) seed has high protein and edible oil content and is an important and widely used crop for human food, animal feed, biofuel production, and many other products (Valliyodan et al., 2016). Global soybean production reached 336.11 million metric tons in 2019 (USDA-FAS, 2020) and world trade in soybean is projected to increase by 22% in 2025 (Westcott & Hansen, 2014). Desired crop traits for soybean include improved yield potential and other important agronomic traits such as disease resistance and stress tolerance, which can be obtained through breeding programs. Conventionally, a large number of progenies are made from a given population derived from a designed cross and grown in single-row plots. A certain percent of progenies (typically 10%) are then selected based on visual evaluations of desired traits and grown at multiple locations over several years to further select a smaller group of genotypes that perform well under different environmental conditions (BATS Center, 1995; Sleper & Poehlman, 2006). The conventional breeding procedure is labor-intensive and low efficient

and may take seven to eight years to develop a small number of new soybean varieties from tens of thousands of progeny plots (Krueger, 2019).

Genetic gain is used to evaluate the efficiency of a breeding process (Fehr, 1991) and can be increased by increasing breeding population size, improving selection accuracy, and shortening the breeding cycle (Moreira et al., 2019). A larger population, including more progeny lines, more seeds, and larger fields, increases the chance of identifying the best combination of favorable alleles with high yield potential. However, the maximum population for a breeding program is limited by available labor, materials, resources, facilities, and time to handle all breeding lines. With the aid of accurate and automated high-throughput field phenotyping systems, breeders would be able to efficiently collect crop traits over a large population, evaluate soybean performance, increase selection intensity, and improve breeding efficiency (Araus et al., 2018). Leveraging emerging technology to predict yield in progenies or early breeding cycles will revolutionize the breeding program and improve the breeding efficiency significantly.

Recent studies in UAV-based plant phenotyping showed that phenotypic variances in crops can be quantified by one or combinations of image features, such as plant height (Feng et al., 2019), canopy cover (Chu et al., 2016), chlorophyll content (Kanning et al., 2018), stress symptoms (Zhou et al., 2020) and maturity dates (Zhou et al., 2019). Those image features representing important crop traits were found significantly correlating with crop yield. However, yield is a very complicated crop trait, and there are few studies conducted to predict the yield of soybean breeding lines in early breeding cycles according to a literature survey on Mar 3, 2020. As shown in Figure 3-1, there were 102 articles written in English related to the keywords of ‘UAV’ and ‘yield prediction’ or ‘yield

estimation' in the Scopus database (www.scopus.com), excluding reviews and erratum. Among them, there were 59 articles published during 2014-2020 that are related to crop yield, with 45 research papers were published in peer-reviewed journals and 14 were presented in conferences and symposiums. Most of the 59 articles attempted to prove the concept that UAV-based image features related to crop yield with R^2 (correlation of determination) from 0.4 to 0.92. Some other studies were testing the potential use of image features in qualifying yield variations in single varieties of certain crops due to controlled experimental variables, such as irrigation, fertilization, or other management practices in field conditions. There is even less number of studies involving two or more crop varieties is even less (orange bars in Figure 3-1) among which only two studies directly estimated the yield of hundreds and more breeding materials using UAV imaging systems (Moreira et al., 2019; Yu et al., 2016).

Figure 3-1. Publications about crop yield using UAV imagery.

The most widely used method for yield estimation is linear regression that has high interpretability and shows good performance in some cases. For example, Geipel et al. (2014) used a linear model to predict corn yield of one hybrid at four treatments of nitrogen fertilization using plant height and canopy coverage from UAV red-green-blue (RGB) images. The results showed that the prediction accuracy of yield (R^2) reached up to 0.74.

However, prediction performance varied when linear models were applied to different scenarios even with similar predictors. For example, the yield prediction accuracy reported by Chu et al. (2016) was only up to 0.53 for predicting the yield of 35 cotton varieties using plant height and canopy coverage as predictors in their linear model.

Advanced data analytic methods, such as machine learning and deep learning, show great potential in processing complicated data sets and extracting hidden information of data that are not easily acquired using restrictive models (Elavarasan, et al., 2018). One of the examples is Partial Least Square Regression (PLSR) that was successfully used to estimate the yield of 20 commercial maize hybrids ($R^2 = 0.73$) (Herrmann et al., 2019) and 1103 soybean breeding lines ($R^2 = 0.59$) (Zhang et al., 2019). Recently, deep learning methods have gained more and more attention thanks to high-performance computation systems. With the availability of convenient application programming interfaces (APIs), e.g. TensorFlow and Keras, more and more popular deep learning models, such as deep neural networks (DNNs), convolutional neural networks (CNNs), and recurrent neural networks (RNNs), have been used to address agricultural problems in four categories, i.e. identification of plant stresses, classification based on stress symptoms and signatures, quantification, and prediction of plant traits (Singh et al., 2018).

In breeding programs, tens of thousands of crosses from two parents are planted in progeny trials without replicates. Large variations in phenotypes (e.g. yield) can be caused by the conjunctions of their genetic and environmental variances (Fehr, 1991), consequently bringing large variances in image features and model predictions. Although a small percentage of selected genotypes are planted in following multi-location yield trials, the expansion of field networks across geographic locations increases spatial

variances and consequently phenotypic variances in the same genotypes (Araus et al., 2018).

Yield estimation for breeding purposes is challenged by the complexity in genotypes and environments, and only a few studies have been reported. Maimaitijiang et al. (2020) predicted the yield of three soybean cultivars under two irrigation treatments with an R^2 of 0.72 and root mean square error (*RMSE*) of 1054.9 kg·ha⁻¹ using a DNN based on RGB, multispectral, and thermal images. Ashapure et al. (2019) reported a result of $R^2 = 0.7-0.89$ for predicting the yield of a set of tomato genotypes using a simple Artificial Neural Network (ANN) model based on UAV-based RGB images. However, yield estimations for trials containing a large number of breeding varieties with unknown traits have not been reported.

This study was motivated by the need for accurate yield estimation of a large population of genotypes in soybean breeding programs for efficient selection of breeding lines with high yield potential. The goal of this study was to investigate the potential in estimating the yield of a large number of soybean breeding lines under drought stress using UAV multispectral imagery and a CNN model. The specific objectives were to (1) quantify the variance in yield due to environmental and genotypic variations in a typical breeding field trial setup, (2) evaluate the dynamics between soybean yield and image features collected at different growth stages, and (3) develop and evaluate a mixed CNN model for yield estimation.

3.3 Methods and materials

3.3.1 Field experiment and plant materials

A field experiment was conducted in a rainfed experimental field at Clarkton,

Dunklin County, Missouri, United States in 2018. The experimental field locates in a humid subtropical climate region (Rubel et al., 2017) with sandy soil that facilitates moisture stress development in a few days after any rainfall event. The field has been used as an ideal location for drought research by the University of Missouri scientists for the last 10+ years. Figure 3-2 b shows the weather conditions during the growing season, including daily average temperature and accumulative precipitation. The data were retrieved from a nearby weather station that is part of Missouri Mesonet - Weather Station Network (<http://agebb.missouri.edu/weather>).

Figure 3-2. The field experiment. (a) The test site location. (b) Daily average temperature and cumulative precipitation during the growing season (c) Illustration of the experimental blocks in the field

The breeding field in this study was designed to develop drought tolerant soybean

cultivars that are suitable for the mid-west and mid-south states of the USA. A group of 116 soybean advanced breeding genotypes and checks (commercial varieties) in maturity group (MG) 3, 4 and 5 were planted in three breeding blocks (Block I, II, III) of the field as shown in Figure 3-2 c. Block I included two breeding groups, i.e., I-MG4 and I-MG5. The I-MG4 group consisted of 15 advanced breeding lines, three commercial checks, one tolerant and one moderately susceptible check in MG 4. The I-MG5 group consisted of 11 advanced breeding lines, three commercial checks, one tolerant check and one susceptible check in MG 5. The Block II (II-MG3) contained 28 recombinant inbred lines (RILs) selected from two mappings derived from crosses involving one elite cultivar (drought susceptible) and one drought tolerant exotic soybean line. Four parental lines (drought susceptible and tolerant) involved in the crosses and four Near Isogenic Lines (NILs) bearing drought tolerance quantitative trait locus (QTLs) in MG 3. In addition, Block III also had two groups, with III-MG4 consisting of 29 varieties/lines in MG 4 and III-MG5 consisting of 12 varieties in MG 5 (proprietary cultivars of seed companies). Each group had one drought susceptible, one drought tolerant and one commercial check (P44A49X).

The soybean test entries in the Block I were planted in four-row plots, while those in the Block II and III were in two-row plots leading to 972 single-row soybean plots in total. All rows were 3.65 m in length and 0.75 m between rows. All breeding plots were planted on May 8, 2018, with three replications following a randomized complete block design. The test field was irrigated once at the vegetative stage (V6) to prevent a foreseeable death of soybean plots due to the severe drought. Plots were harvested using a plot combine during mid-October 2018. The seed yield of each plot was calculated based on the weight of two center rows of the four-row plots or all two rows of the two-row plots

in kilograms per hectare ($\text{kg}\cdot\text{ha}^{-1}$). Under the drought environment, the average yield of all the experimental materials was $1156.8 \text{ kg}\cdot\text{ha}^{-1}$.

3.3.2 Visual wilting scores of soybean plots

Canopy leaf wilting is one of the most important visual symptoms that are used by breeders to determine the drought-tolerant soybean varieties (Carter Jr, 1999; Pathan et al., 2014). The drought-sensitive plants wilt quickly (referred as fast-wilting) while the tolerant plants show delayed (referred as slow-wilting) canopy wilting under drought stress. Genotypes with the slow-wilting trait were found yielding more than those with the fast-wilting trait under drought stress environments (Valliyodan et al., 2016).

The canopy wilting was rated visually during 1-3 pm in the first week of July 2018 when the field was experiencing drought stress and plants showed noticeable canopy wilting symptoms. Canopy wilting was rated on a scale of 1 to 5 using the method described in Zhou et al. (2020). The slow and fast-wilting genotypes were classified by taking 2.5 as a midpoint, i.e., genotypes with an average wilting score (mean of three replicates) lower than 2.5 were categorized in the slow-wilting group and those with scores higher than or equal to 2.5 were in the fast-wilting group (Ye et al., 2019). Slow- and fast-wilting genotypes of three MGs were scored resulting in six groups with two factors ($\text{MG} \times \text{wilting traits}$). The number of soybean breeding plots in each group is shown in Table 3-1.

Table 3-1. The number of soybean breeding plots in the six groups ($3 \text{ MGs} \times 2 \text{ wilting traits}$).

Wilting traits	MG			Total
	3	4	5	
Slow	110	176	152	438
Fast	112	256	166	534
Total	222	432	318	972

3.3.3 UAV imagery data collection

Imagery data were acquired using a UAV imaging system consisting of a UAV platform (model: DJI Matrice 600 Pro, DJI, Shenzhen, Guangdong, China) and two types of cameras, i.e., a multispectral and a visible RGB camera. Data were collected on June 11, July 7 and Aug. 22, 2018 when soybeans were at the late vegetation stage (V6), the early flowering stage (R1) and late reproductive stages (R6-R8). The RGB images were taken using a digital camera (GoPro Hero 5 Black, GoPro. Inc., San Mateo, CA, USA). It has a resolution of 4000×3000 pixels and was set to take time-lapse photos at 2 fps (frame per second). The multispectral images on June 11 and July 7 were acquired using a customized camera that was modified by replacing the spectral filter in the blue channel of a digital camera (PowerShot ELPH 300, Canon U.S.A., Inc., Huntington, NY, USA) with a near-infrared (NIR) filter (800-900 nm) (MaxMax.com - Llewellyn Data Processing, Carlstadt, NJ, USA). The multispectral camera has a resolution of 4608×3456 pixels and was controlled with a third-party firmware Canon Hack Development Kit (CHDK, <http://chdk.wikia.com>) to take images at 0.5 fps. The multispectral images on Aug. 22 were acquired using a five-channel (Blue-Green-Red-RedEdge-NIR) camera (model: RedEdge-M, MicaSense, Seattle, WA, USA) that has a resolution of 1260×960 pixels. The five-channel camera was configured to take time-lapse images at 1 fps and calibrated using a spectral standard board before flights following the procedure described in Zhou et al. (2019).

The UAV imaging system flew at 15 m above ground level at a nadir view for all data collections, resulting in ground sampling distances (GSDs) of 7.7, 4.7 and 10.4 mm·pixel⁻¹ for the RGB, three-channel multispectral and five-channel multispectral

images, respectively. Prior to the flights, the flight speed was set to $7 \text{ km}\cdot\text{h}^{-1}$ and the flight paths were designed to ensure a forward overlap of $\geq 70\%$ and side overlap of $\geq 65\%$ for all images using a flight control App Autopilot (Hangar Technology, Austin, TX, USA).

Seven ground control points (GCPs) were placed in the field in each data collection. The GCP was made of a 30×30 cm wood square covered with a cross-patterned vinyl sheet that was mounted on the top of a 1.1 m plastic fence post. The GCPs were manually stepped into the soil before each data collection and each had a height of 99 cm above the ground. In addition, the plant height of nine randomly selected soybean plots was manually measured from the ground to the base of the highest fully expanded leaf in every data collection. The measured plots were identified in images using the location information on their labels.

3.3.4 Image processing

Orthomosaic images and digital elevation models (DEMs) of the field were generated using the software Agisoft PhotoScan Pro (Agisoft LLC, St. Petersburg, Russia), following the procedures described in Zhou et al. (2019). The DEM was built based on the principle of structure from motion (SfM) and included elevation information (relative to mean sea level) of soybean canopy, bare soil surface and GCPs (Feng et al., 2019). The geo-reference information of the five-channel multispectral images was obtained using the GPS information from their metadata. The geo-reference information of the RGB and three-channel multispectral images was manually assigned using the telemetry data of UAV flight logs. The orthomosaic images and DEMs were then processed using the Image Processing Toolbox and Computer Vision System Toolbox of MATLAB (ver. 2019a, The MathWorks, Natick, MA, USA).

The pixel values of orthomosaic images from RGB and multispectral images are linearly correlated to the light intensities that are closely related to imaging time (Yu et al., 2016). To make the data collected at different dates comparable, all images were calibrated using the calibration color boards (Figure 3-2 c) included in each data collection. Using images collected on June 11 as a baseline, linear equations for each image channel were obtained by regressing digital numbers of the black and blue color calibration boards. The linear equations were applied to the images collected on other dates.

Individual soybean plots were separated from the orthomosaic and DEMs by manually drawing vertical and horizontal lines in alleys between plots and then resized to 20×100 (the input size of a mixed CNN model). Soybean plants were segmented from the background by detecting projected canopy contours using the ‘*activecontour*’ function (Whitaker, 1998) with the ‘*Chan-Vese*’ method (Chan & Vese, 2001) and 400 maximum iterations. Pixels within a full contour were considered as foreground (soybean plants) while those outside contours were background (soil and residues). Contours with extremely small regions were identified as noises using the ‘*regionprops*’ function and then removed from the foreground.

Six image features, including Hue (H) and Saturation (S), plant height and three VIs, were extracted to evaluate the best growth stages in estimating yield. The image features H and S were extracted from the HSV color space converted from the RGB orthomosaic images using the function ‘*rgb2hsv*’ of MATLAB. The HSV color space has attributes corresponding directly to the basic color concepts, which makes it conceptually simple in image processing (Shipman, 2012). The height of soybean plots at three dates was derived from the DEMs built from RGB images. Outliers in each DEM of soybean

plots and the GCPs were detected and removed using the '*rmoutliers*' function with the default options. Height of the GCPs and plant was then calculated as the difference between the 95% percentile of the DEM (considered as the elevation of plants or GCPs) and the 5% percentile of the DEM (considered as the elevation of ground/soil) of each plot. Three VIs, including Normalised Difference Vegetation Index (NDVI), green NDVI (gNDVI) and Excess Green (ExG), were calculated from the processed three-channel and five-channel multispectral images using Eq. 3-1 to 3-3.

$$NDVI = \frac{NIR - R}{NIR + R} \quad (3-1)$$

$$gNDVI = \frac{NIR - G}{NIR + G} \quad (3-2)$$

$$ExG = 2 \times G - R - B \quad (3-3)$$

where, NIR, R and G are the digit number of each image pixel acquired by the multispectral cameras. The VIs were designed to combine two or more spectral bands to signify vegetation and suppress non-vegetation objects in the images.

3.3.5 Data analysis

All data analyses were performed in RStudio (Ver. 1.1.414, RStudio, Boston, MA, USA). A two-way analysis of variance (ANOVA) with an honest significant difference (HSD) Tukey test was conducted to evaluate the effects of MG, wilting trait, and their interactions on yield. A one-way ANOVA was conducted to compare the changes in image features over the three growth stages. ANOVA tests were conducted at a significance level of 5% using the '*aov*' function and the Tukey tests were performed using the '*TukeyHSD*' function. The correlation between each image feature and yield was calculated using the '*corr*' function in RStudio.

3.3.6 Yield estimation using a mixed CNN model

A mixed CNN model was designed to estimate the yield of each soybean breeding plot. Predictors of the model included seven input images extracted from the processed images for each soybean plot as well as two vectors containing the categorical attributes of MG and wilting traits associated with the soybean plots. Manually measured yield data were used as training outputs to the model.

(1) Architecture of the mixed CNN model

The architecture of the mixed CNN model is illustrated in Figure 3-3, which consists of two branches with one (Branch 1) taking the seven input images and another (Branch 2) taking the categorical vectors as inputs. Each of the branches generated a vector of outputs. The two vectors were concatenated into one single vector that was directly connected to the output layer. Branch 1 (Figure 3-3) consisted of 20 layers to process the seven input images for each soybean plot. The input images were processed using six convolutional layers with a number of randomly initialized filters each with a size of [3, 3] in each layer. The numbers of filters were incremented exponentially to generate more sophisticated filters as the network goes deeper. A Rectified Linear Unit (ReLU) activation function that defines the output of neurons given a set of inputs from previous layers was selected for each convolutional layer. All the convolutional outputs less than 0 will be transformed to 0, while those greater than 0 will be outputted themselves (LeCun et al., 2015). A batch normalization layer was then introduced to prevent arbitrary parameters (weights and biases) that were added in the convolutional layers from being imbalanced with extremely high or low values. It was proved that batch normalization can greatly increase the speed and training accuracy and reduce the influence of the outliers with large

weight (Ioffe & Szegedy, 2015). An average pooling layer was added after every two batch normalization layers to reduce the dimensionality of the output from previous layers.

Figure 3-3. The architecture of the mixed CNN model.

A fully connected layer was then added to the end of all the convolutional layers to transform 2D image features of each soybean plot into a 1D vector that can be connected to their yield. A dense layer with the ReLU activation was used to further create trainable parameters related to the vector. To prevent overfitting, a dropout layer was added to randomly ignore the neuron units that could be possibly co-dependent on each other. Finally, another dense layer was added to match the number of nodes from both branches.

In Branch 2 (Figure 3-3), two categorical vectors each with the size of $1 \times n$ (n is the number of soybean plots) contained codes for MG and wilting traits of each soybean sample. A dense layer with 16 kernels (weight matrices) and the ReLU function were used to activate the categorical vectors with a set of trainable parameters, followed by a dense layer with four kernels. After trained by two dense layers, the categorical outputs were concatenated with the image outputs and connected to the regression layer.

The 972 soybean plots were randomly split into training (70%) and testing (30%)

datasets to train and validate the model during the training process. The parameters associated with all layers were trained and optimized iteratively according to the setup of the model's hyperparameters (the top right corner of Figure 3-3). In flexible modelling methods such as CNN models, overfitting commonly happens during model training when model parameters are tuned to closely fit the training set and the training loss is much lower than the testing loss (James et al., 2013). Overfitting could be prevented by carefully monitoring the training epochs (Goodfellow et al., 2016). The optimal number of epochs was determined when the testing loss stops decreasing while the training loss keeps increasing. The mixed CNN model was developed in Keras (<https://keras.io/>), an open-source deep learning API written in Python.

(2) Data set preparation

Images features extracted from the 5-band multispectral images on Aug 22 (R6-8) were used as predictors to the mixed CNN model. The input for each soybean plot has seven images, including the red edge (705-729 nm) band of the multispectral images, three VIs (Table 3-2), a DEM and two texture features that were used to quantify the roughness or smoothness of soybean canopy, including contrast and entropy. The image texture features were calculated based on CVI (Table 3-2) using the '*graycomatrix*' function in MATLAB.

Table 3-2. Descriptions of three vegetation indices and texture features used in the mixed CNN model. The variables in the formulas of VIs are the pixel values of different channels of the 5-band multispectral. p_{ij} is the pixel value of the i^{th} row and the j^{th} column in an image. N is the dimension of the moving window (kernel) when calculating the grey level co-occurrence matrix of each image for texture features

Index Name	Full name	Formula
CCCI (Barnes et al., 2000)	Canopy Chlorophyll Content Index	$\frac{\text{NIR} - \text{Re}}{\text{NIR} + \text{Re}}$ NDVI

CVI (Hunt et al., 2011)	Chlorophyll VI	$\text{NIR} \times \frac{R}{G^2}$
MTVI2 (Haboudane et al., 2004)	Modified Triangular VI	$\frac{1.5 \times (1.2 \times (\text{NIR} - G) - 2.5 \times (R - G))}{\sqrt{(3 - \text{NIR})^2 - 6 \times \text{NIR} + 5 \times \sqrt{R} - 0.5}}$
Contrast (Hall-Beyer, 2017)	-	$\sum_{i,j=1}^N p_{i,j} (i - j)^2$
Entropy (Hall-Beyer, 2017)	-	$\sum_{i,j=1}^N p_{i,j} (-\ln p_{i,j})^2$

The model datasets, i.e. images as input and yield as output, were normalized to balance the training parameters and improve converge efficiency (Moghimi et al., 2020). To avoid removing relative differences between images of different soybean plots, the image normalization was conducted for each channel separately using the mean and standard deviation of image pixel values of all soybean plots in that channel, as shown in Eq. 3-4 (Freedman et al., 2007).

$$p'_{ijnk} = \frac{p_{ijnk} - \mu_k}{\sigma_k} \quad (3-4)$$

where p_{ijnk} is the pixel value of the i^{th} row and the j^{th} column in an image of the n^{th} soybean plot in the k^{th} channel, p'_{ijnk} is the normalized value of p_{ijnk} , μ_k is the mean and σ_k is the standard deviation of the image pixel values of all soybean plots in the k^{th} channel. Yield data were normalized using Eq. 3-5 to mitigate the slight right skewness in its distribution (Kutner et al., 2005).

$$y'_n = \frac{\sqrt{y_n} - \mu}{\sigma} \quad (3-5)$$

where y_n is the original yield for the n^{th} soybean plot, y'_n is the normalized yield for the n^{th} soybean plot, μ is the mean and σ is the standard deviation of the square root yield of all soybean plots. $\mu = 32.46$ and $\sigma = 10.82$ in this case.

3.4 Results and discussion

3.4.1 Accuracy of plant height from DEM

The measurement accuracy of DEMs was evaluated by comparing the DEM-based height measurement with the rated height of the GCPs and manually measured height of soybean plants. As shown in Figure 3-4a, the overall measurement *RMSE* of the height for the GCPs was 2.9 cm in all three data collections. The largest error was 8 cm for the sixth GCP collected on June 11, which might because the fence post was not fully stepped into the dry soil so that its actual height was larger than 99 cm. In addition, uneven ground surface or tilt GCPs may have caused errors in the height from DEMs.

Figure 3-4. Comparison of the height from DEMs with manual measurements. (a) The deviations of the GCPs height from DEM. (b) Agreement of the height from DEMs and manual measurement of nine soybean plots collected on July 7 and Aug. 22. Black solid line: a reference line ($y = x$). Orange dash line: a least square linear fit of the orange dots ($y = 1.8x - 28.5$). Blue dot line: a least square linear fit of the blue dots ($y = 1.1x - 9.1$).

Figure 3-4 b shows the agreement between the DEM-derived and manually measured plant height of nine soybean plots on July 7 and Aug. 22. In general, both data had high agreements with $R^2 = 0.88$ and 0.90 for the data on July 7 and Aug. 22 with the relative *RMSE* to the average of 10.8% ($RMSE=4.0$ cm) and 6.1% ($RMSE=3.6$ cm), respectively. It was noticed that the measurement errors of soybean plots were larger than

those of the GCPs, which might be due to the greater height variations in individual soybean plots than these of the GCPs with flat surfaces, and it might also be caused by personal biases during manual operations. Additionally, plant heights from the DEM on July 7 were overestimated in the lower range, but underestimated for the higher range, compared to the manual measurements of these nine soybean plots. The errors could result from the difficulties in both generating DEMs and keeping human sights as horizontal as soybean canopies on short objects (28-48 cm) in field conditions. The adequacy of using plant height from the DEM at the early stages will be further evaluated using lower standard objects (GCPs) during data collection.

3.4.2 The effects of the maturity and wilting trait on yield

The results of the two-way ANOVA test (Figure 3-5) show significant effects of maturity, wilting trait, and their interactions on soybean yield ($p < 0.001$). Significant differences were also observed in yield between two wilting groups across the three MGs, with the slow-wilting group producing on average $1395.0 \text{ kg} \cdot \text{ha}^{-1}$ and the fast-wilting group only $986.5 \text{ kg} \cdot \text{ha}^{-1}$ (Figure 3-5a). Such yield benefit from the slow wilting trait has also been noted in other studies (Pathan et al., 2014; Ye et al., 2019) and is likely associated with other underlining physiological traits. Specifically, it has been observed that higher production resulted from the complex drought tolerance mechanism behind the symptom of slow canopy wilting, including the uptake of water from deep soil horizon, improving water use efficiency, and limiting transpiration rate (Passioura, 2007; Sadok & Sinclair, 2010).

Figure 3-5. The bar plots of the effects of maturity group, wilting trait, and their interactions on yield. (a) is the yield means of the fast- and slow-wilting groups. (b) is the yield means of three MGs. (c) is the yield means of three MGs with different wilting traits. The lower-case letters above bars indicate the significant difference among these means at a 0.001 significance level.

No significant difference was found in yield between MG 3 ($1018.0 \text{ kg} \cdot \text{ha}^{-1}$) and MG 4 ($976.8 \text{ kg} \cdot \text{ha}^{-1}$) across wilting traits, while the yield of MG 5 ($1540.0 \text{ kg} \cdot \text{ha}^{-1}$) was significantly higher than that of MG 3 and MG 4 (Figure 3-5 b). Generally, soybean genotypes in later MGs, thus with extended maturity time may take full advantage of the growing season to improve their yield (Zhou et al., 2019). The longer growing period of MG 5 plots may offer opportunities and time for plants to recover from stress conditions such as drought, thereby resulting in higher yields. The interactive effects of MGs and wilting traits were also significant on yield, implying that reductions in yield loss due to soybean drought tolerance depend on its maturity stages, i.e., the stage that soybeans are under water stress. The finding is similar to the conclusion drawn by Kron et al. (2008) that responses of soybean differed to water stress induced at different stages. In addition, Desclaux et al. (2000), and Rosolem et al. (2005) also observed that the growth period of pod initiation (R3) to 50% yellow leaves (R7) is the most critical stage for productivity and thus soybean showed greater susceptibility to water stress during these stages. In our case, the absence of yield improvement of the MG 4 genotypes (Figure 3-5 b) might be due to the average yield loss caused by the severe drought during their reproductive stages.

3.4.3 Image features collected at three growth stages

ANOVA tests were conducted to evaluate their variations on different growth stages based on the six image features extracted from two types of images. Image features included three RGB derived features (hue, Saturation, and ExG), two VIs related to the NIR band (NDVI and gNDVI), and a dimensional parameter (height). The results of ANOVA as shown in Figure 3-6 show a significant difference among each of these image features collected at three growth stages. It can be seen from Figure 3-6a that the mean H of all soybean plots collected on July 7 was significantly higher than both earlier and later dates. Hue resembles pure colors and the range from 0.15 to 0.40 in Figure 3-6a covers the colors from yellow-green to dark-green (Smith, 1978). Soybean plants were in light green color at vegetative stages (extreme low values could be due to remaining soil background around small plants) and reached the highest green (hue) during reproductive stages (full canopy coverage). The decrease in hue at later stages might be because the more seed pods achieved mature color, i.e. grey, tan or brown color as soybean entered maturity status (Zhou et al., 2019).

Figure 3-6. Image features collected at three growth stages. (a) - (f) are boxplots of six image features, i.e. hue, saturation, ExG, height, NDVI, and gNDVI collected at three growth stages.

The lower-case letters indicate the significant difference among these means at a 0.05 significance level. (g) Pearson correlations between yield and image features collected at three dates. NS indicates No Significance. **, *** indicate the significance levels of $p = 0.01$ and 0.001, respectively.

In addition, Figure 3-6 b and f show that the mean values of the other five image features gradually increase as soybean maturing. It is straightforward to understand that soybeans were averagely taller at later stages (no lodging was observed in this experiment). It should be noted that soybean height at the latest stage varied more than earlier stages Figure 3-6 d, which might be due to the genotype variations as well as water stress induced in the late vegetation stage (V4) (Desclaux et al., 2000). As NDVI and gNDVI are widely used to quantify chlorophyll content in plants (Gago et al., 2015; Gitelson & Merzlyak, 1996), Figs. 3-6 e and f imply that soybeans at later stages had higher chlorophyll generally. It was observed by Zhou et al. (2019) that mature soybean plots had significantly lower NDVI values than immature plots, leading to high variations in NDVI and gNDVI values.

Pearson correlations between yield and each of the image features collected were

calculated to evaluate the potential growth stages in estimating yield (Figure 3-6 g). Except for plant height on June 11, all other image features at three dates had significant positive relationships with yield. Image features on Aug. 22 had the best overall performance with r ranging from 0.41-0.65, followed by the features on July 7 with r from 0.35-0.62, and those collected on the earliest stage presented the poorest performance with r from 0-0.37. On June 11, soybean plants were at the V6 stage and started experiencing drought after 18 days without any precipitation and irrigation. As yield could be severely reduced by drought conditions during the flowering stage by limiting the number of pods produced per plant and seed filling stages by reducing seed weight (Corn Agronomy, 2015; Desclaux et al., 2000), image features at the early stages might not be reliable to predict yield.

It should be noticed that S and NDVI on July 7 had higher r values compared to those on Aug. 22, and two r values for gNDVI on both dates were surprisingly close to each other. Additionally, right skewness that might underestimate r in linear regressions (Kutner et al., 2005) can be observed from the box plots of the three image features on Aug. 22. It is possibly because the genotypes in early MGs had entered the maturity status (95% of pods achieved mature pod color) and halted parts of their physiological changes (Yu et al., 2016), leading to the aggregation at the top of boxes. Therefore, the comparison suggests that image features collected at the early and late reproductive stages are comparably promising in estimating soybean yield.

Although strong linear correlations between image features and yield were observed in similar studies for breeding purposes (Maja et al., 2016; Yu et al., 2016), the performance of yield estimation was limited using specific image features with single data points representative for soybean plots. Underlying features beyond the commonly used

features were expected to be explored using more complicated models, e.g. CNN models.

3.4.4 Yield estimation using the mixed CNN model

The mixed CNN model was trained for 100 epochs and the results are shown in Figure 3-7. The model loss for the training and testing set (Figure 3-7 a) generally decreased over epochs. It was noticed that the testing loss exhibited a slightly increasing trend at around the 60th epoch, while training loss kept decreasing, which may be caused by overfitting (Ashapure et al., 2019). Therefore, the 60th epoch was considered as an optimal point for training the model. The performance for yield estimation with the optimal mixed CNN model achieved an R^2 of 0.78 with the RMSE of 391.0 kg·ha⁻¹ (33.8 % to average yield).

The CNN branch of the mixed model (Branch 1) was trained separately for 50 epochs to compare its performance with the mixed model, i.e. comparing the performance between the estimations with and without the categorical factors. As shown in Figure 3-7 c and d, the estimation performance of the CNN branch yielded a slightly lower R^2 (0.76) and higher RMSE (439 kg·ha⁻¹) than the mixed model, implying the necessity of including MGs and wilting traits as categorical factors in the model.

Figure 3-7. The performance of yield estimated by the CNN branch and the mixed CNN model with seven image features as predictors. (a) and (c) are the scatter plots of losses against epochs. (b) and (d) are the scatter plots of the predicted vs. the measured yield. Blue dot lines: least square linear fits of the dots ($y = 1.4x - 393$ and $y = 1.5x - 399$ for the lines in (b) and (d), respectively). (a) and (b) are for the mixed CNN model. (c) and (d) are for the CNN model.

The proposed mixed CNN model is competitive among similar studies that focused on yield estimation for soybean breeding purposes. For example, the estimation accuracy with $R^2=0.72$ and $RMSE=1054.9 \text{ kg} \cdot \text{ha}^{-1}$ was reported by Maimaitijiang et al. (2020) for predicting the yield of three soybean cultivars under two irrigation treatments. Zhang et al. (2019) reported an estimation accuracy of $R^2=0.59$ for 1103 soybean breeding lines using a PLSR model and image features from hyperspectral imagery. Based on personal communication with soybean breeders, the estimation accuracy is an important factor to improve the selection intensity of superior soybean lines. The evidence in this study that image features at early reproductive stages have great potential in estimating yield will help breeders to adjust experimental cycles in future trials and thus accumulatively improve the

efficiency of soybean breeding programs. The framework and proof of concept developed in this study may provide evidence of yield estimation using key image features and a mixed CNN model, which might be potentially useful in differentiating yield components in crops for other studies.

Figure 3-8 lists images of five soybean plots that had the yield of 235.4, 853.4, 1537.4, 2346.9 and 2836.3 kg·ha⁻¹ from the top to bottom. They were divided into five levels from L0 to L4 corresponding to their yield levels. From the RGB image in Figure 3-8 a, it is observed that soybean plots with higher yield had a darker green color canopy. As chlorophyll content has shown a positive relationship with yield in rice (Liu et al., 2017) and wheat (Kanning et al., 2018), the darker green might imply higher chlorophyll content in a soybean plot. In addition, MTVI2, CCCI and CVI are VIs related to chlorophyll content (Barnes et al., 2000; Vincini et al., 2008), the darker green canopy had higher values in these channels too.

Figure 3-8. Images of soybean plots with yield in five levels. (a) - (e) are images in RGB, MTVI2, DEM, CVI_contrast, and CVI_entropy, respectively.

It is also obvious that the high-yield soybean plots, such as L4, had a full and wide canopy shape, while those with low yield, e.g. L0, were slim and discontinuous. In the DEM images in Figure 3-8c, the center part (green color) of L4 showed a complete runway shape and covered the majority of the image, while as yield decreasing, the center part starts shrinking and clustered. Compared to the images in VIs, a DEM image contains only depth information excluding color details and other noises, leading to a relatively clear contour of the soybean canopy.

Based on our observations, canopies of soybean plots with lower yield were less uniform than plots with a higher yield. For example, the soybean plot L0 shown in Figure 3-8 had an inconsistent canopy width which may be due to poor germination or missing plants. There were also two clusters of weeds near two ends of the plot, taking away nutrition and water from crop plants. In the L3 plot, some dots with a light green color

might because that drought sensitive varieties showed canopy wilting symptoms causing the lower side to show up (Zhou et al., 2020).

Texture contrast can signify the amount of contrast in local regions of an image by exponentially increasing the difference between neighbor pixels (Hall-Beyer, 2017). The texture contrast images were able to highlight the non-uniform canopies, such as weed clusters in L0, light green dots in L1 to L3, which showed as bright dots as shown in Figure 3-8 d. The texture feature calculated using entropy is used to quantify the level of disorder in an image and larger entropy indicates greater disorder (Hall-Beyer, 2017). As shown in Figure 3-8 e, the L4 with uniform canopy had a low and dense fog texture image, while the texture values in L0 were much higher due to the variations in its canopy.

One outlier was identified in Figure 3-7 b and d. From the illustration in Figure 3-8, soybean plots with high yield are bulky, dark green and have a smooth canopy texture, while those with low yield are slim, light green and varied. Comparing the outlier with these soybean plots that have similar yield but were predicted with small residuals, the large residuals came from their canopy appearances that were different from the normal features in their peers. The outlier rarely had productions but had a wider canopy and darker color than L1. It is possibly because the soybean plot failed to gain seed weight due to severe drought stress during its seed filling stages (Zhou et al., 2020), even though with equivalent flowers, nodes and vigor to other fairly yielded plots.

3.5 Conclusions

The potential of estimating the yield of soybean breeding plots under drought conditions using UAV imagery and convolutional neural network was investigated in this study. A population of 972 soybean breeding plots in three maturity groups was planted

under rainfed conditions to select soybean genotypes with improved traits of drought tolerance and yield potential. The visual wilting score was given to each plot by breeders to evaluate their drought tolerance and classify the soybean wilting traits (slow- and fast-wilting). RGB and multispectral images were acquired at the late vegetation (V6), early reproductive (R1) and late reproductive growth stages (R6-R8). The correlations between the yield and the six image features extracted from the images collected at three stages were evaluated, and statistical significance was observed in the correlations. Seven image features representing plant height, canopy color and canopy texture were used to develop a mixed CNN model for yield estimation. The predictors included the seven image features and two categorical factors, i.e. maturity groups and drought tolerance. Results show that image features collected at the early and late reproductive growth stages were comparably promising in estimating soybean yield. The agreement of predicted yield and the measured yield was high with the R^2 up to 0.78 and a root mean square error of 391.0 kg·ha⁻¹ (33.8 % to average yield), leading to the conclusion that the UAV imagery and deep learning methods are promising in estimating yield for soybean breeding purposes.

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CHAPTER FOUR

VARIETY SELECTION USING UAV HIGH-THROUGHPUT PHENOTYPING TOWARDS IMPROVE SELECTION ACCURACY AND INTENSITY FOR SOYBEAN BREEDING

4.1 Abstract

The efficiency of a crop breeding strategy is evaluated by genetic gain in the primary trait, i.e. yield, achieved in one year through artificial selection of advanced breeding materials. Selection for yield by measuring yield itself is a labor-intensive procedure, especially for progeny rows, and sometimes inaccurate due to its complex genetic behavior and high genotype-environment interaction. Indirect selection by considering crop secondary traits may improve genetic gain when the traits are less expensive but more accurate to measure than yield, or by accelerating decision-making steps and consequently decreasing the breeding cycle if they can be selected in earlier developmental stages or generations. There has been a number of applications of a secondary trait that can be precisely measured with a High-throughput phenotyping platform. However, the selection criteria have not been well established for crop breeding due to the lack of knowledge about the phenotypes responsible for the growth and development of superior genotypes. The goal of this study is to model the breeder's selection using a group of agronomic traits and investigate the potential of selecting superior soybean varieties for soybean breeding programs using image-based secondary traits. A total of 11473 progeny rows were planted in 2018 (PT) and 1773 among them were selected for the preliminary yield trial (PYT) in 2019 and 238 were then selected for the advance yield trial (AYT) in 2020. Seven agronomic traits, including yield, plant height, maturity data, flower and pubescence color, moisture and lodging were manually

measured for soybeans in the two yield trials. Unmanned Aerial Vehicle-based images were collected every two weeks over the growing seasons, and a group of image features was extracted from five-band multispectral images for each trial. Results show that yield is the primary trait for selecting superior soybean lines as there were significant differences in yield between the selected and non-selected group for both yield trials. Profile analysis on the image features showed that progeny rows had the most variation among three trials and the images collected at earlier stages (before R5) explained more variation than those at later stages. The lasso model for selecting soybean lines with image features correctly identified 71% and 76% of the breeder's selection for the PT and PYT and the model's selections were shown to have a significantly higher yield than the breeder's selection regarding yield in the following year's trial. The model selections in PT and PYT had respectively 4% and 5% higher yield, comparing the breeder's selection. It could be concluded that the proposed model is promising in making selections on soybean breeding trials.

Keywords: high-throughput phenotyping; soybean breeding; machine learning; profile analysis; variety selection

4.2 Introduction

Crop breeding is the art and science of changing the traits of crops in order to produce desired characteristics (Sleper & Poehlman, 2006). From 10,000 years ago, plants with preferable phenotypes have been selected and bred by human for higher productions and environmental adaptivity (Ahmar et al., 2020). Since the early 1900s, crop development has been systemized with the introduction of Mendelian laws, followed by cross-breeding and hybrid breeding that are referred to as conventional breeding strategies.

It usually takes one or two decades to create a new cultivar by conventional breeding, given the many phases of crossing, selection, and testing involved in the production of new plant varieties, (Ahmar et al., 2020). With the availability of high-throughput genome information, molecular breeding strategies were developed to shorten conventional breeding cycles with the aid of genetic markers (Xu et al., 2017). For example, marker-assisted selection (MAS) and genomic selection (GS) rely on marker-trait associations and are both routinely applied in modern plant breeding with the objective of selecting individuals with superior performance (Arruda et al., 2016).

Despite genomics and technical advances, identifying plants with desirable traits is the initial and most important step in breeding pipelines (Ahmar et al., 2020). It is well acknowledged in the breeding community that phenotyping efficiency still represents a bottleneck in crop breeding (Araus & Cairns, 2014; Araus et al., 2018; Furbank & Tester, 2011), as phenotypes are the bases to make selections in conventional breeding and a lack of suitable phenotyping data has led to poor results in gene/quantitative trait locus (QTL) discovery, limiting progress in genomics-assisted crop improvement programs (Mammadov et al., 2012).

The goal of crop breeding is to improve genetic gain that is the amount of increase in performance of a population achieved in one year through artificial selection (Fehr, 1991). Genetic gain can be increased by increasing population size (or selecting fewer numbers of varieties) to increase selection intensity, shortening the breeding cycle, ensuring suitable genetic variation in the population, and obtaining accurate estimates of the genetic values (Araus et al., 2018; Moreira et al., 2019; Xu et al., 2017). Among them, the selection intensity (or population size) is limited by the phenotyping capacity on

measuring key agronomic traits of breeding materials and selection accuracy is limited by the lack of objective and efficient phenotyping tools.

In conventional breeding, there are several procedures following the timeline (as shown in Figure 1-1) to combine desired crop traits into individual crops and eventually select superior varieties. After identifying a key problem in a specific program, breeders select from parent lines from given populations that have desired traits to potentially address the problem. Then the selected parent lines are combined to obtain crosses that probably would exhibit simultaneously desired traits from both parents. The cross lines are advanced for a couple of years to obtain enough seeds for field evaluations. Normally 10,000 to 30,000 cross lines are made at the beginning of soybean breeding programs, depending on the scale of the programs.

In the first year of the field evaluations, the cross lines are planted in progeny trials (PTs). With the large number of lines and limited numbers of seeds for each entry, progeny lines are planted in one-row plots without replicates (Orf et al., 2004). Manual phenotyping of all such one-row plots in PTs requires a large investment of resources but is inaccurate due to the boundary effects of single rows (Moreira et al., 2019). As an alternative, the plots in PTs are visually examined by experienced breeders and a certain percentage of them (normally 10-15%) will be selected for a combination of the desired traits eliminating those inheriting undesired traits from parents.

The selected progeny lines are kept planting in multi-location yield trials (YT_s). In YT_s, soybean lines are planted in four-row plots with two center rows as effective rows while two side rows as protection rows. Key traits, such as yield, plant height and maturity group, etc., are manually measured for each plot in YT_s. This procedure will be repeated

in many locations for 2-3 years. Breeders could keep selecting a certain percentage of lines (normally 10% each year) that are adapted either widely across the regions or specifically for targeted regions, depending on the purpose of the breeding program.

High-throughput phenotyping (HTP) has been demonstrated the ability to estimate various agronomic traits for different crops for breeding purposes, such as yield (J. Zhou, J. Zhou, et al., 2021), height (Watanabe et al., 2017), the maturity date (Zhou et al., 2019), detecting crop components (Makanza et al., 2018; Zhang et al., 2018) and stress symptoms (J. Zhou, H. Mou, et al., 2021; Zhou et al., 2020). With higher accuracy and efficiency, the estimates by HTP could be used as secondary traits additionally to yield to select a small group of superior lines, thus increasing the population size and selection intensity (Richards, 2000). Those that could be estimated at earlier growth stages or generations would accelerate the selection procedure and eventually shorten the breeding circles (Moreira et al., 2019).

Motivated by the need to improve the selection accuracy and intensity for soybean breeding, the goal of this study was to investigate the potential of selecting superior soybean progeny using the unmanned aerial vehicle (UAV)-based image features. There were three objectives to achieve this goal: (1) modeling breeder's selection (selected/non-selected) using manually measured crop traits (flower color, pubescence color, height, lodging, maturity date, moisture and yield) to quantify weights of the traits on the final selections, (2) evaluate the difference in image features between selected and non-selected genotypes, (3) identify the growth stages when the image features are effective in making selections by profiling the time-series image features, and (4) evaluate the performance of model selections by comparing their yield performances with breeder's selection in the

following YTs.

4.3 Material and Method

4.3.1 Plant material and field experiment

A three-year breeding trial was conducted at three locations in Missouri, United States from 2018-2020, including a progeny trial (PT), a preliminary yield trial (PYT) in 2019 and an advanced yield trial (AYT) in 2020. The PT with 11473 soybean progeny rows derived from F4 plants was conducted in a 3.64 ha field at the Greenley Research Center of the University of Missouri (MU), Novelty, Knox County, Missouri. The progeny rows were planted without replicates in one-row plots of size 2.59 m with 0.76 m row spacing and planted on May 29, 2018.

A group of 1860 lines was selected from the PT among which 1773 were advanced as a PYT in a 4.09 ha field at the Bradford Research Center of MU, Columbia, Boone County, Missouri. The PYT lines were planted without replicates in four-row plots on June 3, 2019. A group of 238 lines selected from the PYT was evaluated in an AYT in a 1.76 ha field at Missouri Soybean Association, Columbia, Boone County, Missouri. The lines were planted with two replicates in four-row plots on June 2, 2020. Soybean plots in the PYT and AYT were in the same size and row spacing as those in the PT. In each of the three trials, replicated checks in MG III and IV were planted over the field as maturity references.

Figure 4-1. Illustration of the experimental fields. (a) The progeny trial in 2018. (b) The preliminary yield trial in 2019. (c) The advanced yield trial in 2020. (d) Locations of the fields.

4.3.2 Manually measured agronomic traits

Seven agronomic traits were manually measured for the four-row soybean plots in the PYT and AYT, including flower color, pubescence color, plant height, lodging, maturity date, seed moisture and grain yield. There were three flower colors noticed, including purple, white, and segregating (mixed colors) and four pubescence colors, including tawny, gray and segregating in the PYT and tawny, light tawny and gray in the AYT. Plant height was measured from the ground level to the leaf base of the highest fully expanded leaf by holding a measuring tape close to the stem of soybean lines vertically. Lodging is visually rated on a scale from 1-5 with 1 = plants standing upright while 5 =

plants prostrating on the ground. Maturity dates were visually determined and recorded as the number of days after September 1st (day 1) in each of 2019 and 2020. A soybean plot was not considered mature until approximately 95% of its pods achieved mature pod color (Zhou et al., 2019). Grain yield and seed moisture were measured during harvest in real-time with a combine.

4.3.3 UAV data collection

Imagery data were acquired using a UAV imaging system consisting of a UAV platform (model: DJI Matrice 600 Pro, DJI, Shenzhen, Guangdong, China) and a five-channel multispectral (Blue-Green-Red-RedEdge-NIR) camera (RedEdge-M, MicaSense, Seattle, WA, USA) camera. The multispectral camera has a resolution of 1260×960 pixels and was configured to take time-lapse images at 1 fps (frame per second) and calibrated using a spectral standard board before flights following the procedure described in (Zhou et al., 2019). The multispectral camera has a Global Navigation Satellite System (GNSS) receiver unit embedded and pre-programmed to provide geo-referencing information. The information was stored in EXIF (Exchangeable Image File Format) metadata associated with each frame of the images. Before each flight, a calibration reflectance panel (CRP) was imaged by holding the camera at about 1 m above the CRP and looking vertically in an open area (to avoid shadow).

Data were collected on four times (75, 90, 108 and 121 day after planting (DAP)) in the 2018 PT, six times (45, 57, 74, 87, 109 and 120 DAP) in the 2019 PYT and six times (51, 64, 78, 94, 107 and 120 DAP) in the 2020 AYT. The UAV imaging system flew at 30 m above ground level at a nadir view for all data collections, resulting in ground sampling distances (GSDs) of 20.8 mm·pixel⁻¹ for the multispectral images. Before each flight, the

flight speed was set to $7 \text{ km}\cdot\text{h}^{-1}$ and the flight paths were designed to ensure a forward overlap of $\geq 70\%$ and side overlap of $\geq 65\%$ for all images using a flight control App Autopilot (Hangar Technology, Austin, TX, USA).

Twelve, 15 and 18 ground control points (GCPs) were placed evenly for each data collection in the PT, PYT and AYT field, respectively. The GCP was made of a $30\times 30 \text{ cm}$ wood square covered with a cross-patterned vinyl sheet that was mounted on the top of a 1.1 m plastic fence post. The GCPs were manually stepped into soil before each data collection, and each had a height of 99 cm above the ground. The GNSS coordinates of the GCPs were obtained using a Reach Real-Time Kinematic (RTK) GNSS receiver system (Reach RS+, Emlid, St. Petersburg, Russia). The system consists of a base station and a rover receiver. The base station was mounted on a tribrach that was fixed in an open area in the fields to ensure sufficient satellite reception for each data collection. As accurate absolute GNSS accuracy is not required for this study, but precise relative accuracy of the rover to the base station, the base position with an averaged single accuracy ($\sim 2.5 \text{ m}$) was obtained for each field by accumulating its GNSS coordinates for 5 min (Emlid, 2021). The base station was maintained stationary and horizontally during every flight and its position was fixed over a growing season. The rover receiver was mounted on a monopod that was placed vertically in the holes after the GCPs were pulled out. The GNSS coordinate of each GCP was recorded after accumulating for 10 sec using ReachView that is a manufacturer-developed App for users to take full control of the Reach modules.

4.3.4 Image processing

The images were processed using a semi-automatic pipeline to generate numeric inputs for data analysis and modeling. The pipeline mainly includes (1) Generating

Orthomosaic images; (2) Plot separation and (3) Feature calculation.

Figure 4-2. The image processing pipeline

The multispectral images were processed using Pix4D Mapper (Pix4D, Lausanne, Switzerland) to generate orthomosaic images. After all the images were imported, the geo-referencing information was read automatically from the EXIF metadata. The images of the CRP were manually cropped for reflectance calibration (Zhou et al., 2019). The generated orthomosaic images and DEMs were then exported as the tag image file format (tiff) that allows GNSS position tagged with each pixel being exported together. The exported images were then processed using the Mapping Toolbox and Image Processing Toolbox of MATLAB (Version 2019a, The MathWorks, Natick, MA, USA).

GNSS positions (i.e., Latitude and Longitude) are stored in the exported

orthomosaic images as two matrices that have the same dimensions as the images. One matrix has only latitude values and the other has only longitude. The coordinates were read using the '*pixcenters*' function with a '*makegrid*' option in the Mapping toolbox in MATLAB. The projected geolocation coordinate system of the multispectral images was converted into the World Geodetic System 1984 (WGS84) using the function '*projinv*' with required information read from the orthomosaic images.

Individual four-row plots were manually separated from the orthomosaic image when soybean plants had the bulkiest canopy. A rectangle mask was given to each plot and then visually examined to ensure the plot was separated correctly regarding its positions and boundaries. The mask was applied to the coordinate matrices to obtain latitude and longitude values of the plot. New rectangle masks were generated by looking for pixel positions of the previous latitude and longitude values of the plot from the coordinate matrices of other orthomosaic images and then applied to separate the plot from those images. Directional and heading shifts might occur when matching coordinates for different images due to GPS and stitching errors. The shifts were identified by visual examination and measured by calculating the difference between the matched coordinates and the manual adjustments of the masks at four corners. The saved coordinate values were applied to separate plots from the images on other days with the same procedure for adjusting shifts.

The separated images containing a single-row soybean were used to calculate image features. The center part of each row was cropped by removing the image edge (a quarter of image width on each width side and a quarter of image length on each length side). Thirty-eight image features were calculated from the five-band images, including the band

of green and red edge, as well as 36 vegetation indices listed in Appendix A.

4.3.5 Model training and validation

Soybean plots in the PT and PYT were randomly split into training (80%, i.e., 9178 and 1418 plots for the PT and PYT, respectively) and testing (20%, 2295 and 790 plots for the PT and PYT, respectively) datasets to train and validate the variety selection model. Two Lasso (Least absolute shrinkage and selection operator) models were trained separately for the PT and PYT. The model was built using the '*cv.glmnet*' function in the '*glmnet*' R package by specifying the model type argument $\alpha = 1$. The shrinkage parameter lambda was tuned in the model regarding test errors by 5-fold cross-validation. The trained models were applied on the test sets to predict if the plots were selected (1) or non-selected (0). The model output for each plot was a probability (p) of it being selected or non-selected. The plot was classified to the selected group if $p > 0.5$, or the non-selected group, otherwise.

The model performance was evaluated by the classification recall (Eq. 4-1) of the testing sets. As shown in Figure 4-3, the true positive (TP) class represents the overlapping between the model selections and breeder's selection, while the false negative (FN) class contains those who were not selected by the model but selected by the breeder. In this study, the FN class needs to be avoided, i.e., the minimum type 2 error. Recall measures the proportion of the TP class out of all the true classes. In breeding programs, it is always preferred that the selected genotypes have the increased yield from one generation to the next (Moreira et al., 2019). To evaluate the model performance, yield means in the following yield trials of the TP class (the model selections) were compared to the yield means of the FN class (the model non-selected group but selected by breeders).

$$Recall = \frac{TP}{TP + FN} \quad (4-1)$$

Figure 4-3. Illustration of two metrics for evaluating model performance for selecting superior soybean plots

4.3.5 Data analysis

Data analyses were conducted using the R language in RStudio (Ver. 1.3.1093, RStudio, Boston, MA, USA). A one-way analysis of variance (ANOVA) with an honest significant difference (HSD) Tukey test was conducted to evaluate the differences in manually measured agronomic traits between the selected and non-selected soybean varieties. Principal component analysis (PCA) was performed to estimate the relationship among the manually measured agronomic traits and model the breeder's selection criterion.

A multivariate ANOVA (MANOVA) was conducted to test the null hypothesis that no difference in image features averagely for multiple data collections between the two groups. Profile analysis was conducted to evaluate the time behavior of image features for the selected and non-selected groups. The null hypothesis of the profile tests is defined as:

$$H_0: \mathbf{LBM} = \mathbf{0} \quad (4-2)$$

where $\mathbf{L} = [1, -1]$, $\mathbf{B} = \begin{bmatrix} \mu_{11} & \mu_{12} & \dots & \mu_{1p} \\ \mu_{21} & \mu_{22} & \dots & \mu_{2p} \end{bmatrix}$, and $\mathbf{M} = \begin{bmatrix} 1 & 0 & \dots & 0 \\ -1 & 1 & \dots & 0 \\ \vdots & \vdots & \ddots & \vdots \\ 0 & 0 & \dots & -1 \end{bmatrix}$. $\mathbf{L}$ is the

indictor matrix of comparisons between the selected and non-selected groups. $\mathbf{B}$ is the matrix of the mean responses in an image feature between the two groups over time, and p

represents the times of data collection in each trial. In this study, $p = 4, 7$ and 7 for the PT, PYT and AYT, respectively. M is the matrix of comparisons across time. The profile analysis was performed using the '*linearHypothesis*' function in the '*car*' package (S. Zhou et al., 2021).

4.4 Results

4.4.1 Differences in the manually measured agronomic traits between the selected and unselected groups

Figure 4-4 shows the result of one-way ANOVA tests on the manually measured agronomic traits that were collected in the yield trials. In the PYT, significant differences were observed in yield between the two groups, with the selected group averagely producing 2804.2 g seed weight and the non-selected group 2427.1 g (Figure 4-4 a). The yield difference was observed consistently in the AYT, with the selected group averagely producing 2605.1 g seed weight and the non-selected group 2330.7g (Figure 4-4 a). In the PYT, plant height had a dynamic range from 45 to 125 cm and the selected group (89.6 cm) was significantly taller than the non-selected one (87.9 cm), while in the AYT there was no difference in plant height (55-105 cm) between the two groups. No significant differences were observed between the two groups in lodging and moisture for the PYT. Similarly, there is no significant difference in moisture for the AYT. It is noted that the selected group had higher lodging readings than the non-selected group, which were possibly caused by the fewer variations in lodging, as most of the plots in the AYT had the lodging readings within the range of 1-2, except for a few plots over 3. For the maturity date, the selected groups were mostly located between 25-37 and 30-37 days after August 1st. From Figure 4-4 f, g and 4-5f, g, flower pubescence colors were not the decisive factors

because certain percentages of selection were made from each of the colors. However, it is obvious that color segregating is an indicator of the non-selection groups.

Figure 4-4. Comparisons of manually measured agronomic traits between the selected and non-selected groups in the PYT. (a) – (d) Boxplots of the comparisons in yield, height, lodging and moisture, respectively. The lower-case letters above bars indicate the significant difference among these means at the above significance levels (p -values). (e) Histogram of the maturity dates. (f) and (g) Bar plots of the flower and pubescence colors. For (e)-(g), the blue bars represent all plots, while the orange bars represent the selected plots.

Figure 4-5. Comparisons of manually measured agronomic traits between the selected and non-selected groups in the AYT. (a) – (d) Boxplots of the comparisons in yield, height, lodging and moisture, respectively. The lower-case letters above bars indicate the significant difference among these means at the above significance levels (p -values). (e) Histogram of the maturity dates. (f) and (g) Bar plots of the flower and pubescence colors. For (e)-(g), the blue bars represent all plots, while the orange bars represent the selected plots.

4.4.2 Modelling breeder's selection criterion

The PCA analytical results on the manually measured agronomic traits for the PYT and AYT are shown in Figure 4-6a and 4-7a. The PCA revealed three principal components (PCs) with eigenvalues >1 capturing 65.3% of the total variation for the PYT and three PCs capturing 62.4% for the AYT. In both yield trials, PC1 was primarily influenced by yield, height and maturity date. While PC2 was greatly influenced by moisture and lodging in PYT, and by maturity date and flower color in AYT. Yield and height had the highest contributions with consistent directions to PC1 for both trials. Moreover, it was noted from Figure 4-6c and 4-7c that height was significantly correlated with yield with positive linear coefficients. Lodging, maturity date and moisture contributed secondly. No consistent

linear relationship was found between lodging and yield, as well as between the maturity date and yield over two years. Flower and pubescence color had very little contribution to the selection and no particular color was observed to have a higher yield than others. In PYT, soybean plots with the white flower color had a higher yield than those with purple and segregating, while those with the pure pubescence colors had a higher yield than segregating lines. In AYT, however, no significant difference was found among different colors. The relationship between yield and other agronomic traits shown in Figure 4-6 and 4-7 generally confirms the variable contributions found in the first two PCs in the PYT and AYT.

From the scatterplots of sample distribution based on PCA regression scores, it is obvious that the selected groups were mainly in the second and third quadrant, negatively directing to the axis of PC1. However, the selected group highly overlapped with the non-selected group in the first two PCs in both trials, and in each trial, the two PCs collectively accounted for less than 50% of the variations.

Figure 4-6. Manually measured agronomic traits in PYT. (a) are the visualization plots of the variables' contributions and directions in the plane of the first two PCs. (b) is the projection of sample variables on the first two PCs. (c)-(d) are scatterplots of yield versus height, lodging and maturity date, respectively. (h) is a scatterplot of the relationship between moisture and maturity date. Eight outliers with moisture values higher than 20% were removed to ensure the adequacy of linear regression. (g)-(h) are boxplots of the yield of three groups of flower color and three groups of pubescence color. The p-value above (c)-(h) indicates the significance level of estimation.

Figure 4-7. Manually measured agronomic traits in PYT. (a) are the visualization plots of the variables' contributions and directions in the plane of the first two PCs. (b) is the projection of sample variables on the first two PCs. (c)-(e) are scatterplots of yield versus height, lodging and maturity date, respectively. (f) is a scatterplot of the relationship between moisture and maturity date. (g)-(h) are boxplots of the yield of three groups of flower color and three groups of pubescence color. The *p*-value above (c)-(h) indicates the significance level of estimation.

4.4.3 Differences in image features between selected and non-selected varieties

Figure 4-8 shows the differences in 38 image features collected multiple times in the PT, PYT and AYT. Each square indicates the existence of a difference in the means of

an image feature (all listed above) between the selected and non-selected groups. The DAPs for collecting images in each trial was on the left side. There were image features from PT, PYT and AYT from top to bottom. In the PT, there were 37, 35, 31 and 31 image features significantly different between the two groups, collected on DAP 75, 90, 108 and 120, respectively. There were 31, 32, 24, 4, 28 and 7 image features in the PYT and 34, 33, 28, 26, 18, 3 image features in the AYT that showed significant differences from the early to late stages. Among the 38 image features, 17 had consistently significant patterns (i.e., the selected group consistently higher or lower than the non-selected group) for the three trials.

Figure 4-8. Illustration of the differences in image features between the selected and non-selected groups. Each square represents if there is a significant difference in an image feature collected at single times (p -value < 0.05) between the two groups and which had higher values.

4.4.4. Profile analysis results in image features

Results of the profile analysis on the 38 image features show that all the image

features in the PT displayed different patterns ($p\text{-value} < 0.05$) over time between the selected and non-selected groups, while 37 image features (except for IO) in the PYT and 31 features (except for red-edge, GLI, IF, RGR, and TGI) in the AYT had different patterns ($p\text{-value} < 0.05$). Figure 4-9 shows the plots of GNDVI as an example of the profile analysis. The profile plots look parallel between the two groups due to the extended dynamic ranges over three years. A group of profile plots in Appendix Figure A1 was plotted with different GNDVI scales. It can be seen that the non-selected group usually had higher variations than the selected group. The variations of GNDVI collected at single times in the PT were higher than those in the PYT and AYT at similar times. In both PT and AYT, the non-selected group had a sharper increasing slope before the 90 DAP (around the R5 stage). The profiles showed that the 90 DAP was a changing point that the GNDVI values slowly increased before the stage and then started decreasing sharply.

It is noted that in the PYT, the GNDVI values had extremely low values among all data points. Appendix Figure A2 shows an RGB image and a GNDVI map collected at the 120 DAP for each of the three trials. It can be seen that the PT had a very large variation in the canopy greenness, i.e., maturity status. There were a number of lines have matured for some time (yellow and dry canopy), while most of the plots still had many green leaves. The PYT and AYT obviously had less vegetation than the PT and higher uniformity in maturity dates (mostly in 20-40 days after Sept. 1st). The GNDVI values for soil background in the three maps were consistently within a range of 0.3-0.45 and those for the light green leaves were around 0.6. However, the mature plots in the PYT contributed a lot to the low GNDVI values with some had similar values to the soil background.

Figure 4-9. Profile plots of GNDVI for the selected and non-selected groups over the growing seasons. (a) – (c) are plots for the PT, PYT and AYT, respectively. The lower-case letters above bars indicate the significant difference among these means at a 0.05 significance level. The p-value above each plot indicates the significance level of the null hypothesis that the two groups had a parallel pattern over time in GNDVI. Appendix Figure A1 shows the profile plots with different GNDVI scales.

4.4.5 Performance of the model selections

The performance of the model selections for the PT and PYT is shown in Figure 4-10. In all the breeder's selections of progeny rows, the part selected by the lasso model had a significantly higher yield in PYT than the non-selected part, when the model used image features collected at 75, 90 and 108 DAPs. No significance was observed when using the image features on the 120 DAP. However, the significance was not observed in yield from the AYT between the two groups. In the breeder's selection of the PYT lines, significant differences were observed in the PYT yield when using image features before the R5 stage (the 45, 57 and 74 DAP) and in AYT yield only at the 57 DAP. The numbers in blue show the classification recalls of model selections, representing the proportions of the breeder's selection that were also selected by the model. For both PT and PYT, the model was able to identify over 60% of the breeder's selections from the testing sets. The highest recalls were 0.71 (90 DAP) and 0.76 (102 DAP) for the PY and PYT, respectively.

Comparing the model selected part with all the lines of breeder's selections of progeny rows, yield in the PYT of the model selection (90 DAP) were up to 4% (106 g/plot)

higher than the breeder's selection and 1% (133/plot) higher in the AYT. While comparing for the selections of PYT lines, yield of the model selection (57 DAP) was up to 2% (59 g/plot) higher than the breeder's selection in the PYT and 5% (107/plot) higher in the AYT. It is noted that the highest recall (0.76) in the PYT selections was obtained using image features on the 102 DAP, but the PYT yield of the model selection was not significantly higher than the remaining lines in the breeder's selection. On contrary, the AYT of the model selection was clearly lower than the remaining lines. A similar situation was observed for PT selections on the 120 DAP. Therefore, the high classification recall did not necessarily lead to higher yield performance in the following trials.

The significantly higher PYT yield of the model selections was achieved by the image features before the maturity stage (R8). Although there was no significant difference in the AYT yield in the corresponding DAP due to higher variations, the yield in model selections on the 75 (R4: the full pod stage) and 90 DAP (R5: the beginning of seed fill stage) was clearly higher. Similarly, the significance was observed in the PYT model selections before the 87 DAP (R5: the beginning of seed fill). Thus, image-based crop traits collected before maturity tended to perform better in selecting higher yielding progeny lines and, the appropriate time point for making selections for yield trials should be even earlier due to the less variation in these trials.

Figure 4-10. Model performance on selecting superior soybean lines from the PT and PYT. (a) and (c) Comparison of the PYT yield means. (b) and (d) Comparison of the ATY yield means. (a)-(b) are for the PT selections, while (c) and (d) are for the PYT selections. Orange and blue bars represent the model selections (the TP class) and model non-selections but selected by breeders (the FN class), respectively. Error bars show the standard deviations. *, **, *** indicate the significance levels of $p = 0.05$, 0.01 , and 0.001 for testing the hypothesis that there is no difference in yield means between model selections and non-selections. NS represents no significance. The numbers in blue are the classification recall.

4.5. Discussion

Grain yield is the only trait that had significant differences (Figure 4-4a and 4-5a) in both yield trials, and it consistently and highly contributed (Figure 4-6a and 4-7a) to explain the variations in collected agronomic traits. As the most important trait for soybean breeding, yield has demonstrated its primary position among all the agronomic traits in this study. Yield estimation using remote sensing technologies has been a hot topic in the area of high-throughput phenotyping and yield has shown high correlations with image features

(Herrmann et al., 2019; Maimaitijiang et al., 2020; Moreira et al., 2019). In one of our previous study (J. Zhou, J. Zhou, et al., 2021), the yield was highly correlated with VIs, such as GNDVI, and CCCI that were also shown to be significantly distinguishable for selecting superior soybean lines in all the three trials (Figure 4-8), implying the reliability of using these features make selections in scenarios without actually knowing yield, i.e., the PT.

Similar to yield, plant height is another agronomic trait that highly contributed to explaining the variations in both yield trials, which might be due to the significantly positive correlation between yield and height (Figure 4-6c and 4-7c). The selected group was taller than the non-selected group in the AYT, while with less variation and fewer outliers, there was no significant difference in plant height in the AYT. Compared to grain yield, plant height is easier to be measured using photographic methods with high accuracy and thus often used as an alternative to yield in agriculture studies. For example, Hassan et al. (2019) reported a significantly high correlation of $R^2 = 0.96$ with a 5.75 cm root mean square error between UAV-based plant height estimates and ground measurements in wheat, and Feng et al. (2019) reported a Pearson correlation coefficient of 0.96 between and cotton yield and UAV-based plant height.

It should be noted that the soybean materials in this study were indeterminate (IND) varieties that continue their vegetative growth and producing nodes on the main stem until the beginning of seed fill (R5) (Purcell et al., 2014). Compared to those with a determinate (DET) growth habit, i.e., stop vegetative growth and producing nodes before the flowering stages, IND soybeans usually have tall plants and averagely high yield, however, they are more susceptible to lodging. Height VS yield between indeterminate (IND) and determined

soybean. It was reported by Wilcox and Sedyama (1981) that seed yield increased by 350 kg/ha and lodging score increased by 0.008 for every 10 cm increase in plant height among the determinate lines, while seed yield increased by 112 kg/ha and lodging score by 0.3 for every 10 cm increase in plant height among indeterminate lines. Therefore, plant lodging is nonnegligible in soybean breeding and those with severe lodging have to be avoided, especially among the IND varieties because of its tall plant height due to elongation of the main stem after flowering (Kato et al., 2015).

Severe lodging was not observed in this study. There were only six and seven plots having the lodging scores above 3 in the PYT and AYT, respectively. And there was not a consistently incremental or decremental relationship shown between lodging and yield (Figure 4-6d and 4-7d). It has been shown that lodging from R3 (beginning pod) to R5 (beginning seed) have the greatest impact on yield, while lodging before R2 (full flowering) and after R6 (full seed) does not significantly impact yield but lodging at the maturity stage can have negative impacts on harvestability (Koester et al., 2014). As the lodging scores were taken when soybeans reached their R8 stages, they did not necessarily reflect the negative effects on the yield of potential lodging in the middle of the growing seasons.

Figure. 4-8 gives a general idea of how differentiable the image features were between two groups over the growing seasons and how consistently the differences in multiple years and locations. Significant differences were observed in many image features between the selected and non-selected groups in the three trials. The PT had consistent differences from the reproductive to maturity stages. On the contrary, the PYT and AYT plots had less distinguishable features after the R5 stage. In soybean breeding, the selection

on progeny row is usually based on the breeder's visual examinations. The plots with a similar appearance to the surrounding checks would be preferable and have desired performance regarding yield potential and maturity date. Therefore, the selected progeny rows are expected to have low variations, compared to all the materials planted in the PT. Yield of soybean plants is mainly formed at before their R5 stages, as the results of conjunctive effects of genotype and environments. The bloom (R1-R2) and pod stages (R3-R4) are critical for gaining seed yield by determining the numbers of flowers, pods and beans, seed size and pod dry weight. Any stress at these stages could cause more yield reduction than at any other time. The model performance was benefited by the most significance in canopy coverage and colors displayed at these stages, rather than the appearances of yellow and dry stems at the maturity stages.

GNDVI was proved to be more sensitive to the variation of chlorophyll content in crops and highly correlated with nitrogen content in leaves (Gitelson & Merzlyak, 1996). The profile analysis on GNDVI clearly exhibited how soybean physiology changes over time. Despite the extremely lower value collected at 120 DAP in the PYT, the profile patterns were very consistent for all three years. Moreover, it is straightforward to see image features collected at early stages were more powerful in splitting the selected and non-selected groups.

The selection confirms with the profile analysis that image features at early stages had better performance in selecting lines because the early model selections tended to have significantly higher productions in the next year, compared with the breeder's selections. Due to higher variations in the PT materials, superior progeny rows were easier to be identified with image features collected through reproductive stages (75-108 DAP). With

the model selection, nearly 30% of soybean lines could be cut off in the following yield trials, consequently saving 30% of land usage, human labor and other resources. With the aid of efficient data collection and selection, human interactions could be eliminated a lot from the traditional breeding pipeline and the limitation on the number of progeny rows could be boosted. Hence the genetic gain could be increased by a higher selection intensity benefited from higher population size.

4.6 Conclusion

This study evaluated the breeder's criterion on selecting superior soybean for soybean breeding programs varieties using a group of agronomic traits and investigated the potential of making the selections using image-based secondary traits. A total of 11473 progeny rows were planted in 2018 (PT) and 1773 among them were selected for the preliminary yield trial (PYT) in 2019 and 238 were then selected for the advance yield trial (AYT) in 2020. Seven agronomic traits, including yield, plant height, maturity data, flower and pubescence color, moisture and lodging were manually measured for soybeans in the two yield trials. Unmanned Aerial Vehicle-based images were collected every two weeks over the growing seasons, and a group of image features was extracted from five-band multispectral images for each trial. Results show that yield is the primary trait for selecting superior soybean lines as there were significant differences in yield between the selected and non-selected group for both yield trials. Profile analysis on the image features showed that progeny rows had the most variation among three trials and the images collected before the beginning of seed fill stage (R5) explained more variation than those at later stages, consistently for the PT, PYT and AYT. The lasso model for selecting soybean lines with image features correctly identified 71% and 76% of the breeder's selection for the PT and

PYT. The PYT yield in PT model selections before 120 DAP (the maturity stage R8) and the PYT model selection before 87 DAP (the beginning of seed fill stage, R5) was significantly higher ($p\text{-value} < 0.05$) than the model non-selections but selected by breeders. The model selections in PT and PYT had respectively 4% and 5% higher yield in the following year's trial, comparing the breeder's selection. It could be concluded that the proposed model is promising in making selections on soybean breeding trials.

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CHAPTER FIVE

CONCLUSIONS AND FUTURE WORK

In this research, an integrated and automated UAV HTP platform was developed to deliver image-based crop traits for soybean breeding programs. It was equipped with multispectral and digital cameras to take images with high spatial and temporal resolutions, as well as GNSS units to provide precise geo-referencing for image stitching and processing. Images collected at different days were calibrated to eliminate the effects of light variations on image pixel numbers and thus ensure consistent spectral reflectance. An automated pipeline was developed to process the time-series images and extract a number of image features for each soybean plot. To evaluate the potential of improving breeding efficiency using the UAV-based HTP platform and pipeline, a typical soybean breeding program was traced for three years from the progeny trial to the preliminary yield trials and then to the advanced yield trials. Two key agronomic traits, i.e., maturity date and yield were estimated using image features and machine learning models. A group of elite soybean genotypes was selected by learning from the breeder's selections. The performance of the proposed framework was evaluated by comparing the image estimates with the manually measured traits. Detailed conclusions are as follows.

5.1 Conclusions

The analysis on image features and estimation of soybean maturity dates showed that the best agreement of predicted maturity dates and manual measurements was made by image features collected roughly when soybean was at the beginning maturity status, i.e., starting to have fallen leaves and yellow pods. It was found that four image features (CIrededge_mean, GDVI_std, GRVI_mean and BNDVI_mean) had positive

linear relationships with the maturity dates, and five image features (S_mean, CVI_std, IF_std, CI_mean and IF_mean) were negatively related to the maturity dates. The adjusted maturity dates proposed in this study were able to tolerate the variances caused by the conjunction of genetic and environmental variances. The maturity dates were predicted using a partial least square regression (PLSR) model and the results showed that the image predicted maturity date highly agreed ($R^2 = 0.81$) with the visually rated value and the root mean square error (*RMSE*) of 1.4 days. By personal communication with breeders, *RMSE* within 1.5 days can be considered as a tolerable error in soybean breeding programs. Compared to previous studies, the proposed methods showed improvements in predicting the exact maturity dates instead of the classification between mature and immature lines, as well as making a more practical prediction for breeding programs.

A CNN model was developed to estimate soybean yield taken seven image features representing plant height, canopy color, and canopy texture as the predictors. The analysis on the RGB and multispectral image features acquired at three growth stages showed that the image features collected at the early and late reproductive growth stages were comparably promising in estimating soybean yield. It was also observed that high-yield soybean lines had a darker green color with a full and wide canopy shape, a higher plant height, and a more uniform canopy texture. As the breeding materials were in three maturity group and under drought stress, two categorical traits, i.e. maturity groups (III, IV and V) and drought tolerance (fast- and slow-wilting) were also included in a mixed version of the CNN model. The improved prediction model could explain 78% of the measured yield with a root mean square error of 391.0 kg·ha⁻¹ (33.8 % to average yield).

To model the breeder's selection criteria, seven agronomic traits, including yield,

plant height, maturity data, flower and pubescence color, moisture and lodging were manually measured for soybeans in two yield trials. The principal component analysis (PCA) showed that yield is the primary trait for selecting superior soybean lines as there were significant differences in yield between the selected and non-selected groups for both yield trials. In addition, plant height is another agronomic trait that highly contributed to explaining the variations in both yield trials due to the significantly positive correlation between yield and height. It was noted that for indeterminate (IND) materials (the kind that used in this soybean in this study, plant lodging is a nonnegligible factor for selecting elite genotypes and those with severe lodging have to be avoided. The profile analysis showed that image features at early stages had better performance in selecting lines as soybean plants at early stages exhibited larger variances in their appearances. Due to higher variations in the PT materials, superior progeny rows were easier to be identified with image features collected through early reproductive stages (75-108 DAP). compared the yield trials. A Lasso model for selecting soybean lines with image features correctly identified 71% and 76% of the breeder's selection for the PT and PYT. The model selections in PT and PYT had respectively 4% and 5% higher yield, comparing the breeder's selection.

The developed UAV HTP platform is able to efficiently collect multi-dimensional images on soybean breeding materials, for example, it can cover a 9-acres breeding field within 2 hours. The automated pipeline can generate tabulated image features for each soybean plot within 24-48 hours after data collection. For images collected multiple times in a field, the pipeline can segment soybean plots and output their image features from orthomosaic images efficiently within 10 min for over 10,000 plots, leveraging the precise

georeferencing information. By comparing with manual/ground data, the accuracy of estimating the maturity date and yield using image features and machine learning models is sufficient for breeding purposes. The proposed framework largely increases the efficiency of scouting the fields and measuring plant performances for breeders, thus breaking the constraints of the numbers of breeding lines in a trial due to limited human labors. Breeding efficiency would be improved by the higher probability to identify elite lines as more materials could be tested in a trial. The model selections of elite lines confirmed near 70% of the breeder's selections and their intersections had better yield performance than the remaining. The HTP framework can successfully assist breeders to identify lines with better yield performance and select a smaller group than the regular percentages. Therefore it helps to increase the selection intensity and eventually genetic gain. Moreover, with a smaller group of candidates, land usage, human labor and other resources will be saved correspondingly, allowing breeding resources to be relocated for more efficient strategies. With all respects, it is concluded that the proposed framework is promising in improving the soybean breeding efficiency by increasing the selection intensity and accuracy, and saving resources.

5.2 Future work

Based on the observations and results from this research, future work should focus on: (1) There were two major issues expected to be addressed to reduce errors introduced during the image collection and processing pipeline. First, as an image pixel value is the reflectance of incoming solar irradiance, the variation in light conditions could introduce variations to image-derived features. The relationship between light changes during UAV flight missions and camera responses (reflectance) should be further explored and flight

dynamics (drone pose, sun's position, light diffusion, clouds, etc.) need to be better integrated to describe the light changes. Second, variations are inevitably introduced when images are stitched based on feature recognition. Instead of localizing individual breeding lines from an orthomosaic, efforts have to be made to investigate the potential of direct georeferencing that the geometric position of a breeding line is the conjunction of the relative position of the line in an image and the UAV position where the image was taken. With direct georeferencing, flight attitude and speed can be liberated from high image overlaps ($\geq 70\%$ for creating orthomosaic images) so that the time issue and light variation can be moderated consequently.

(2) The proposed models for yield estimation and maturity date should be further validated by repeating the experiments in multiple environments and documenting image features. To make the model parameters robust and universal for other soybean breeding programs, soybean materials with more genetic variations should be added to the training dataset by collecting both UAV-based images and ground data. Moreover, the models could be further improved by adding variables related to environmental factors, such as soil conditions, temperature, and precipitation, etc.

(3) The image-based model selections of soybean elite progeny rows should be further evaluated by side-by-side comparisons of the yield-potential in following yield trials between all the model selections and the breeder's selections. Other than solely trained by the breeder selections, the model would learn to process other information from the provided image features, i.e., estimating each of the factors that are critical to a specific breeding program, such as yield, lodging and maturity dates, etc., and selectively integrating these factors to make selections.

APPENDIX

Table A1. Vegetation indices.

No.	Index Name	Descriptions	Formula
1	NDVI	Normalized difference VI*	$\frac{nir - red}{nir + red}$
2	ATSAVI	Adjusted transformed soil-adjusted VI	$1.22 \times \frac{nir - 1.22 \times red - 0.03}{1.22 * nir + red - 0.0366 + 0.08 \times (1 + a^2)}$
3	ARVI2	Atmospherically Resistant VI 2	$-0.18 + 0.17 \times \frac{nir - red}{nir + red}$
4	BWDRVI	Blue-wide dynamic range VI	$\frac{0.1 \times nir - red}{0.1 \times nir + red}$
5	CCCI	Canopy Chlorophyll Content Index	$\frac{nir - re}{nir + re}$
6	CIgreen	Chlorophyll Index Green	$\frac{NDVI}{nir}$
7	CIrededge	Chlorophyll Index RedEdge	$\frac{green - 1}{nir}$
8	CVI	Chlorophyll VI	$\frac{red}{re - 1}$
9	CI	Coloration Index	$nir \times \frac{red}{green^2}$
10	CTVI	Corrected Transformed VI	$\frac{red - blue}{red}$
11	GDVI	Green Difference VI	$\frac{NDVI + 0.5}{ NDVI + 0.5 } \times \sqrt{ NDVI + 0.5 }$
12	EVI	Enhanced VI	$\frac{nir - green}{nir - red}$
13	EVI2	Enhanced VI 2	$2.5 \times \frac{nir - red}{nir + 6 \times red - 7.5 \times blue + 1}$
14	EVI22	Enhanced VI 2-2	$2.4 \times \frac{nir - red}{nir + red + 1}$
15	GEMI	Global Environment Monitoring Index	$2.5 \times \frac{nir - red}{nir + 2.4 \times red + 1}$
16	GARI	Green atmospherically resistant VI	$a = \frac{2 \times (nir^2 - red^2) + 1.5 \times nir + 0.5 \times red}{nir + red + 0.5}$
17	GLI	Green leaf index	$GEMI = a \times (1 - 0.25 \times a) - \frac{red - 0.125}{1 - red}$
18	GBNDVI	Green-Blue NDVI	$\frac{nir - (green - (blue - red))}{nir + (green + (blue - red))}$
19	GRNDVI	Green-Red NDVI	$\frac{2 \times green - red - blue}{2 \times green + red + blue}$
20	H	Hue	$\frac{nir - (green + blue)}{nir + (green + blue)}$
21	IPVI	Infrared percentage VI	$\frac{nir - (green + red)}{nir + (green + red)}$
			$\tan^{-1} \left(\frac{2 \times red - green - blue}{30.5} \times (green - blue) \right)$
			$\frac{nir}{2 \times (nir + red)} \times NDVI$

22	I	Intensity	$\frac{red + green + blue}{30.5}$
23	LogR	Log Ratio	$\log\left(\frac{nir}{red}\right)$
24	MSAVI	Modified Soil Adjusted VI	$\frac{1}{2} \times \left(2 \times nir + 1 - \sqrt{(2 \times nir + 1)^2 - 8 \times (nir - red)}\right)$
25	NormG	Norm Green	$\frac{green}{nir + red + green}$
26	NormNIR	Norm NIR	$\frac{nir}{nir + red + green}$
27	NormR	Norm Red	$\frac{red}{nir + red + green}$
28	NGRDI	Normalized green red difference index	$\frac{green - red}{green + red}$
29	BNDVI	Blue-normalized difference VI	$\frac{nir - blue}{nir + blue}$
30	GNDVI	Green NDVI	$\frac{nir - green}{nir + green}$
31	NDRE	Normalized Difference Red-Edge	$\frac{nir - re}{nir + re}$
32	RI	Redness Index	$\frac{red - green}{red + green}$
33	NDVIrededge	Normalized Difference Rededge/Red	$\frac{re - red}{re + red}$
34	PNDVI	Pan NDVI	$\frac{nir - (green + red + blue)}{nir + (green + red + blue)}$
35	RBNDVI	Red-Blue NDVI	$\frac{nir - (red + blue)}{nir + (red + blue)}$
36	IF	Shape Index	$\frac{2 \times red - green - blue}{green - blue}$
37	GRVI	Green Ratio VI	$\frac{nir}{green}$
38	DVI	Difference VI	$\frac{nir}{red}$
39	RRI1	RedEdge Ratio Index 1	$\frac{nir}{re}$
40	IO	Iron Oxide	$\frac{red}{blue}$
41	RGR	Red-Green Ratio	$\frac{green}{red}$
42	SRRedNIR	Red/NIR Ratio VI	$\frac{red}{nir}$
43	RRI2	Rededge/Red RedEdge Ratio Index 2	$\frac{re}{red}$
44	SQRTIRR	SQRT(IR/R)	$\sqrt{\frac{nir}{red}}$
45	TNDVI	Transformed NDVI	$\sqrt{\frac{nir - red}{nir + red}} + 0.5$

46	TGI	Triangular greenness index	$-0.5 \times (0.19 \times (red - green) - 0.12 \times (red - blue))$
47	WDRVI	Wide Dynamic Range VI	$\frac{0.1 \times nir - red}{0.1 \times nir + red}$
48	MSR	Modified Simple Ratio	$\frac{\sqrt{\frac{nir}{red}}}{\sqrt{\frac{nir}{red}} + 1}$
49	MTVI2	Modified Triangular VI	$\frac{1.5 \times (1.2 \times (nir - green) - 2.5 \times (red - green))}{\sqrt{(3 - nir)^2 - 6 \times nir + 5 \times \sqrt{red}} - 0.5}$
50	RDVI	Renormalized Difference VI	$\frac{nir - red}{\sqrt{nir + red}}$
51	IRG	Red Green Ratio Index	$red - green$
52	OSAVI	Optimized Soil Adjusted VI	$\frac{nir - red}{nir + red + 0.16}$
53	SRNDVI	Normalized Difference Vegetation Index	$\frac{nir^2 - red}{nir + red^2}$
54	SARVI2	Soil and Atmospherically Resistant Vegetation Index 2	$2.5 \times \frac{nir - red}{1 + nir + 6 \times red - 7.5 \times blue}$

*VI: Vegetation index.

Figure A1. Profile plots of GNDVI for the selected and non-selected groups over the growing seasons. (a) – (c) are plots for the PT, PYT and AYT, respectively. They were plotted with different GNDVI scales to show the differences in profile patterns. The error bars represent the standard deviation. The p-value above each plot indicates the significance level of the null hypothesis that the two groups had a parallel pattern over time in GNDVI.

Figure A2. RGB images of the partial experimental fields in the three trials. The blue rectangles mark the selected plots. (a) and (b) were from the PT. (c) and (d) were from the PYT. (e) and (f) were from the AYT. (a), (c) and (e) are RGB bands of the multispectral images. (b), (d) and (f) were in the GNDVI. Images were collected on the 120 DAP for all three trials.

VITA

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