Evaluation of drone imagery as a method for selection criteria in soybean breeding

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Abstract

Plant breeding is a process of manipulating plants, making them generally more useful. To incorporate beneficial attributes in cultivars, large segregating populations may be necessary. Evaluating large populations may create a bottleneck on plant selection. Sensor technologies have the potential to complement existing phenotyping criteria to improve the rate of genetic gain. This study compared the selection differential in seed yield, maturity, plant height, and lodging among F4-derived soybean lines selected in un-replicated progeny rows based on spectral imagery, visual observations, and random control selections. Spectral imagery was used to calculate a normalized difference red edge (NDRE), a red normalized difference vegetation index (NDVI), a thermal rating (TH), and canopy size (CC) indices for 5338 genotypes in 2017 and 6110 genotypes in 2018. The top 8% of the genotypes based on CC, NDRE, NDVI, TH, progeny row yield (PYLD) and visual (VIS) evaluations, along with random (RAND) selections were advanced to early and late maturing field trials (KPE and KPL) the following years. NDRE, NDVI, and TH selections were measured on mean values (X). Progeny row selections were evaluated in 2018 KPE, KPL, and 2019 KPE trials at three locations and in 2019 KPL trials were evaluated at two locations; all locations using a non-replicated, modified augmented design. Seed yield, maturity, lodging, and plant height were measured on all yield trial plots. Entry means were used to calculate the average seed yield, maturity, lodging, and height for each selection category. Selections based on XNDRE, XNDVI, PYLD, CC, and VIS showed significant yield improvement over RAND selections, however, these observations were not consistent across locations or years. XNDRE and XNDVI showed the greatest consistency across years. Height had shown to be significantly shorter for both XNDRE and XNDVI and lodging had shown to be significantly less severe amongst the XNDRE KPE selections when compared to the random

control selections (RAND). This association was supported by a significantly negative correlation between measurements of XNDRE (-.15) and XNDVI (-.07) in 2018 in addition to (-.31), and (-.21), respectively in 2019 to height means. XNDRE had shown a significant negative correlation (-.06) to lodging in 2018 and in 2019, both XNDRE (-.34) and XNDVI (-.21) were significantly correlated to lodging. These patterns were similar in the KPL trials. In 2018, XNDRE accounted for 44% more entries than RAND of the top 30% highest yielding lines, and XNDVI accounting for 47% more entries than RAND. In 2019, XNDVI accounted for 42% more of the top 30% highest yielding lines than RAND in the final population, and XNDRE accounted for 88% more entries than RAND. XNDRE and XNDVI both showed promising results as a selection method. In a program where visual selection is limited by trial size, spectral selection might prove beneficial; however, further research is needed to develop the selection criteria that will produce a consistent positive selection differential.

The second experiment consisted of four different locations and 5 different trials (two trials at the same location). Each trial ranged from 10 to 52 entries, set up in a randomized complete block design, planted in 4-row plots 3.7m long, spaced .76m apart. Seed yield and spectral measurements were measured from the center two rows of each plot. MicaSense, Sony, and FLIR cameras were used to make spectral measurements. MicaSense measurements evaluated were blue, green, red, red-edge, near-infrared (NIR), blue normalize difference vegetation index (BNDVI), green normalize difference vegetation index (GNDVI), red normalize difference vegetation index (NDVI), normalized difference red-edge (NDRE), and pigment index (PI). Sony measurements evaluated were blue, green, NIR, BNDVI, GNDVI, and PI. FLIR camera measurement analyzed was thermal (TH). MicaSense BNDVI, GNDVI, BNDVI, and NDVI showed a significant relationship to yield across multiple trials, however, these results showed to be variable, only showing a consistent measurement at two location. Sony cameras BNDVI, and GNDVI measurement had shown a significant relationship to yield across multiple sights as well but altered between positive and negative correlations. No physical plant characteristics were consistently associated with any significant yielding spectral measurements across all trials.

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Chapter 1 - Literature Review

Introduction

Plant breeding is primarily influenced by the ability to manipulate plant attributes, to make them generally more useful (Acquaah, 2007). It is from these plant manipulations that breeders often attempt to nudge nature into a specific direction as to enhance yielding attributes, to improve growth structures, harvest capabilities, and stress adaptations. The effects of these attributes are often the result of a genotype and its interaction with the environment (Acquaah, 2007). These expressions are generally referred to as phenotypes.

Phenotypic selection has largely been the basis of crop improvement long before modern technology. Even techniques that would be considered modern, such as marker assisted selection, utilize phenotypic characterizations to aid in selections made (Shakoor et al., 2017) The ability to find these beneficial attributes requires large segregating populations to find and select for desirable traits (Acquaah, 2007). In general, the larger the segregating population (more genotypes), the better the odds are to find a desirable and yield improving attribute. It is at this point of evaluating these large populations, that a general bottlenecking or restriction on plant selection develops (Araus and Cairns, 2014). These selections are labor-intensive, costly, and hold constraints in determining the genetics of stress associated variables. (Shakoor et al., 2017).

Genetic Gain

Breeding programs measure of success is highly influenced or determined by its genetic gain (Rife, 2016). This is highly dependent on a programs genetic diversity, selection accuracy, selection intensity, and selection cycle time. These programs allow for the development of new lines with valuable allele combinations superior to the parental genotypes. The accuracy of estimates throughout different variance components is important in determining the heritability and genetic gain (Baenziger et al., 2006). Thus, environmental variance, genotypic variance, error variance, and genotype by the environment are highly influential within a breeding program. In addition, progress from the selection can only be realized if superior genotypes are readily identified (Miller et al., 1958). The recognition of genetic variation among different genotypes is imperative for future improvement.

Plant breeding methods adhere to the creation, selection, and fixation of superior plant phenotypes so that improved cultivars complement the needs of farmers and consumers (Moose and Mumm, 2008). This can be found in the form of improved yields, nutritional qualities, or other traits of value. Examples of this are often recognized in the commercialization of hybrid maize, the green revolution with respect to wheat, and development of transgenic crops. Recent research had shown that cultivars from 1923 to 2008 had shown a 17.2 kg/ha annual increase in soybeans (Wilson et al., 2014).

Progeny Rows

In some crops, such as soybean, early testing is conducted with limited seed material, limiting the potential for replication of a given trial or entry (Moreira et al., 2019). The limited material often results in progeny-row trials being established as the first indicator of yield potential. These test plots are often un-replicated, one-row plots at one location. A small percentage of these lines are advanced for further evaluation and the remaining lines discarded. The legitimacy of early evaluations is highly dependent on the accuracy of the derived data and distinguishing the difference among the non-replicated genotypes and the selections stability, thereafter across multiple locations. The large effort invested in the evaluation of progeny rows can result in the elimination of potentially valuable lines.

Visual Selection

Visual ratings have been used as an important method for selection in progeny rows, but the ability of visual selection to predict yield has shown inconsistent, both positive and negative, results. Dahiya et al., 1984 compared selection based on both yield and visual ratings. They found that the low and high yield selection categories maintained relative performance in later yield trials, whereas visual selections for yield showed no improvement over random selections. Bowman et al., 2004 did note some merit for visual selection for yield in progeny rows, but did indicate that visual selection was unable to identify the highest yielding line in the trials. Kwon and Torrie, 1964 were able to visually discriminate between low and high yielding lines when yield differences were large, but not when yield differences were small. Ordas et al., 2012 found that visual selection was able to successfully select for better yielding and taller corn lines. Ud-Din et al., 1993 found that visual selection was not a successful method of selection for forage in wheat, and determined that yield was more beneficial as a selection method. It has been found that there is a potential to consciously or unconsciously select for characteristics that are uninformative of yield, such as height, and heading date. This making visual selection potentially inconsistent.

Early Generation Yield Testing

Often as a result of visual selection showing inconsistent results, these methods are used has process to screen out bad genotypes rather than used as a primary selection tool for progressing highly productive genotypes (Luedders et al., 1973). Luedders et al. found 27.5% of their highest yielding soybean lines were discovered in early yield testing, and that these lines were stable across years. They concluded that there is potential to remove up to 75% lines in early generations. DePauw and Shebeski, 1973 found similar results where they were able to select for heritable differences in yield in the F3 generation. Negative impacts from selection using yield data has been found as well. Briggs and Shebeski, 1971; and Knott and Kumar, 1975 reported that lines selected for yield in early generations were poor indicators of performance in different environments.

Early generation testing has the potential to be highly influential in a breeding program, allowing for the elimination of early undesirable lines. However, other publications (Ntare et al., 1984), have found that visual selection has proved to be a good selection method for yield improvement.

Leaf Pigment Properties

Crop energy production is largely influenced by the leaf biochemistry and how it relates to genotypic variation within different components such as pigment concentration and status (Reynolds et al., 2009). The concentrations of chlorophyll a and b and carotenoid have shown to be largely affected to both light and nutrient requirements (Moran et al., 2000). This suggests how photosynthetic pigments provide insight into the physiological status of the plant's vegetation. The remote sensing of these technologies allows for non-destructive capabilities for accessing these abiotic stress indicators (Hatfield and Prueger, 2010).

The understanding of how the plant and crop tissue respond to different wavelengths is directly related to remote sensing. Both plant tissue and soils absorb short wave energy (400-3000 nm) from the sun, and the remaining waves are emitted as longwave energy (Asner, et al., 1998). Apart from this, wavelengths can be identified on a visual scale with the naked eye. This is in relationship to chlorophyll a (red), and chlorophyll b (blue). As the chlorophyll a, and b absorb the red and blue wavelengths, leaving only green as it is reflected (Tucker and Sellers, 1986). The green wavelength reflected is what is visually observed with the naked eye. However, carotenoids (yellow pigments) contribute to the production of photosynthesis to plant as well, but when under stress carotenoids decline at a slower rate than chlorophyll, giving off chlorosis (Sims and Gamon, 2002). From a visual observation on plant color, we can make an interpretation of the plant health in relation to the "greenness" or chlorosis of the plant. Often this color change can be noticed as the plant matures through its flower stage. This is due to the natural destruction of the chlorophyll, and preexisting carotenoids become noticeable (Hendry et al., 1987).

Chlorophyll is the bases of converting sunlight into energy through the process of photosynthesis and thus is correlated to yield (Jin et al., 2012). From this, chlorophyll can be used as an indicator of a plant's photosynthetic capabilities and stress indications (Sun et al., 2018). Often chlorophyll b region (blue) is not used due to the close relationship with carotenoid absorbance, and so often the 550nm and 700nm regions (green and red) are used to eliminate issues with chlorophyll saturations (Sims and Gamon, 2002).

The measurement of spectral reflectance is the measurement of light reflectance observed from leaf tissues, cellular structures, and air-cell wall-protoplast-chloroplast interaction (Kumar and Silva, 1973). The visible portion of this falls between 400-730nm wavelength range, and have low reflectance, due to the plants absorbing the vast majority of energy in this range, using chlorophyll, carotenoids, carotenes, anthocyanins, etc. As an alternative wavelength near-infrared (NIR) has a high reflectance rate. The photosynthetic efficiency of a plant is a function of the plant's content of chlorophyll and other pigments. This can be used to estimate biomass production and photosynthetic capacity (Filella et al., 1995). Researchers have been able to develop predictions on plant health and yield using modeling from different vegetation indices, characterizing; photosynthetic capabilities and water status (Thomas and Gausman, 1977), chlorosis (Adams et al., 1999), green cover (Daughtry et al., 2009), and chlorophyll. This is in part due to chlorophyll being directly influenced by nitrogen (N), and chlorophyll can be used as an estimation of plant N status (Filella et al., 1995). From these measurements, researchers can make estimations on general plant health.

Anthocyanin's are considered an important leaf pigment for the plant's developmental process as it serves as an indicator of stress for many plant species (Hatfield et al., 2008). This pigment indicator along with carotenoid indicators have had a few variables causing issues for replicated results (Sims and Gamon, 2002). This is in relation to pigment adsorption and leaf cell structure problems. Models have been developed based on a red to a green ratio (Gamon and Surfus, 1999). However, these models were found to not be consistently reliable.

Photosynthesis has shown a strong relationship with yield (Dhanapal et al., 2016). Developing a method, that allows for manipulation of this relationship has been suggested as a way for yield advancement (Dhanapal et al., 2016). These results have been verified by using extraction-based methods, but these can be mildly destructive. As an alternative, spectral reflectance has developed for modeling plant phenotypes, specifically chlorophyll content in relation to light adsorption. Research has shown that spectral indices can be used to predict both disease pressure and yield potential within a trial, accounting for up to 11 to 77% variation in disease and 41 to 93% of the variation in yield (Menke, 2018). However, field-based research in relation to yield estimation using canopy reflectance and temperature has shown to be variable and inconsistent (Babar et al., 2006). Primarily this technology has been, useful in showing a relationship between yield and traits such as biomass and canopy reflectance. While many of these studies have had success in establishing a relationship between yield and reflectance relationship, there has been relatively no general understanding between their value and surrounding physiological and environmental factors that contribute to these interpretations(Christenson, 2016).

Selection to Reflectance Indices

The optimal spectral measurement for relative precession is best to utilize by ground cover methods (Ritchie et al., 2010). However, these readings take excessive time and high expertise for analyses. The biggest issue is in relation to spatial resolution and distance between cameras (Ritchie et al., 2010). So aerial imagery is being propagated to reduce manual labor and increase data measurements throughout replicated studies.

The absorbance of light energy is developed in both chlorophyll a and chlorophyll b. The highest absorbance is developed in the red (600-700nm) and blue (400-500) region (Sims and Gamon, 2002). Chlorophyll b generally absorbs light in the 460-650 regions, while chlorophyll a absorbs wavelengths in the 580-670 regions of the spectrum (Chappelle et al., 1992).The green and red regions at 550nm and 700nm are primarily used, because of high chlorophyll concentrations needed to saturate these wavelengths (Sims and Gamon, 2002). Shanahan et al. (2001) found that both the wave regions using green wavelength and NIR could account for 70 to 92% of yield variation in corn. NIR and visible wavelengths are used to create spectral ratios known as spectral reflectance indices (SRIs)(Jensen, 2007). These ratios are readily used to estimate biomass of different crops (Elliot and Regan, 1993; Babar et al., 2006).

One of the most common forms of SRIs used is the normalized difference vegetative index (NDVI) developed by Deering 1978 and Tucker 1986 and is formed from the ratio (R_{NIR}-R_{Red}/R_{NIR}+R_{Red}). This has been used to explain 44%-80% of the variation in soybeans, and to predict grain yield in soybeans, wheat, and corn (Ma et al., 2001; Shanahan et al., 2001; Aparicio et al., 2002). Similarly, blue normalized difference vegetation index (BNDVI) (R_{NIR}-R_{Blue}/R_{NIR}+R_{Blue}) and green normalize vegetation index (GNDVI) (R_{NIR}-R_{Green}/R_{NIR}+R_{Green}) show the reflective energy in the blue and green regions of reflectance (Hatton et al., 2019), and have been used in combination with one another creating a pigment index (PI) (BNDVI-GNDVI) to develop a close relationship with carotenoid concentration expressing plant health. Alternative indices had been developed to predict anthocyanin concentration using a red to green ratio (R_{600}-R_{700}/T_{500}-R_{600}) to determine yield estimations(Gamon and Surfus, 1999). Alternative forms of wavelengths have been used as stress indicators as normalized difference red edge index (NDRE) (Barnes et al., 2000). This has been used as alternative to using temperature as a measurement of stress.

Parameters have been established previously to account for biomass, leaf area index (LAI), fractional intercepted photosynthetic active radiation (fiPAR), and as canopy variable relate to crop yielding attributes (Serrano et al., 2000). This was found to be most useful in the form of the simple ratio (SR). This equation is derived from (NIR-Red/NIR+Red), and captures the ratio of NIR reflectance to reflectance in the red (Deering, 1978) and increased the difference between plants due to the increase adsorption in red energy and increase reflectance in the NIR energy for healthy plants.

Issues can arise in respect to how canopies affect the absorption and reflectance observed within different wavelengths. The leaf area index and leaf angle distribution can affect both the absorption and scattering of these properties. Shadowing within canopies can alter the reflectance properties as well. (Asner et al., 1998.) There is the ability to account for these different variables using different indexes such as NDVI. NDVI has the ability to capture plant health and estimate green biomass, that can be defined as (R_{NIR}-R_{RED}/R_{NIR}+R_{RED}) (Deering, 1978). NDVI allows for normalization and reduces the background reflectance, solar irradiance, and atmospheric effects (Ritchie et al., 2010). This index is specific to plants with relation to high adsorption of red and high reflectance of NIR. If the plant is starting to senesce or is under stress the adsorption of the light into the chlorophylls and other cellular components will start to decrease. Previous research has shown that NDVI does not show a linear relationship with chlorophyll content unless the chlorophyll content is at relatively low levels (Richardson et al., 2002). This has been used to explain 44%-80% of the variation in soybeans, and to predict grain yield in soybeans, wheat, and corn(Aparicio et al., 2002; Ma et al., 2001; Shanahan et al., 2001). In contradiction, it has been found that NDVI shows very little relationship to agronomic traits and that it shows the potential to account for genetic variation but doesn't attribute to drought tolerance (Clark, 2016).

Lower spectral reflectance values have been found for NIR and red wavebands in water-stressed canopies, and higher reluctance in no stressed canopies. However, the ability of the ratios developed from this study to detect water stress, was dependent on the growth stage, soil background, and atmospheric changes (Reynolds et al., 2009). In a study conducted by Andrew D. Richardson, they found that chlorophyll was highly correlated to the difference in wavelengths between 721 and 744 nm, and concluded that D730 was the single wavelength best to correlate to chlorophyll content (Richardson et al., 2002).

Selection from Thermal Indices

A crop's canopy temperature (CT) is related to the vascular system of the plant and its ability to extract water from the soil depending on demand, and photosynthetic potential through feedback on the stomatal opening, allowing for selection to be made upon the environment such as heat stress(Shakoor et al., 2017a). These studies have shown that thermal reading could be a response in the relationship to the crops efficacy to extract water from the soil, and photosynthetic efficiency on stomatal opening (Reynolds et al., 2009). This measurement has been used to determine parameters of plant physiological characteristics such as identifying drought tolerance (Aslan, 2015).

These traits have found to be applicable when phenotyping varieties for genetic gain. Under drought selection for cooler canopy temperatures. This has shown to select for genotypes with the ability to develop a rooting structure, to extract water from deeper soil profiles(Reynolds et al., 2009).

Thermal infrared is emitted and captured in the 3000-14000 nm electromagnetic spectrum (Jensen, 2007). This wavelength criterion has been used to create selection within wheat breeding programs (Reynolds et al., 1994), further showing a strong negative relationship between CT, as it relates to grain yield. Currently wheat-breeding programs use this technology to screen for dehydration resistance. This is due to the test being inexpensive, high association with performance, and there is little interaction with the crop growth stage or time of day (Shakoor et al., 2017).

Canopy Cover

The canopy development in soybean plays a large roll in crop development and grain yield (Hall, 2015). The rapid closure offers the ability for maximum light interception, and will contribute to improve total biomass and grain yield. Hall (2015) found that canopy closure characteristics showed high narrow senses heritability and high correlations to grain yield ranging from .61-.68. That study suggested that there is good potential for improvement of genetic gain in selection for this category. In a study conducted by Kantolic et al., (2013), they found that there was strong relationship between biomass accumulation, and crop growth rate in the reproductive stages (R3-R6) suggesting that there would be an improved yield upon selection of increased biomass growth in the later reproductive stages.

Throughput Phenotyping

Due to these constraints, there is a search to utilize modern technology to enhance the capabilities of a current-day breeding program. Modern remote sensing offers the potential to evaluate large populations in a snapshot style to identify beneficial characteristics through remote sensing (Shakoor et al., 2017; Jang et al., 2020; Zhang et al., 2019). Through manipulation and interpretation of these different wavelengths, there is potential to rapidly improve the attributes under selection in a breeding program.

High throughput phenotyping is highly sought to help improve upon the genetic exploration and to help gain access to genetic variation (Reynolds et al., 2009). These highly selective methods have shown to be an extremely important component for the improvement of a breeding program, and these selections can be related back to multiple traits such as: plant height, biomass, flowering time and seed yield (Ma et al., 2001; Furbank and Tester, 2011; Christenson et al., 2015, 2016; Keep et al., 2016; Bai et al., 2016; Xavier et al., 2017). Sensor technologies have the ability to expand and improve upon the existing phenotyping criteria to help produce a large set of data to select from and speed up the breeding process, along with improving the rate of genetic gain (Shakoor et al., 2017). There continue to be advancements in both research and general use of remote sensing methods continues due to the development and improvement of narrowband or hyperspectral sensors along with their ability to be mounted on various platforms, such as satellites, aircraft or small drones, often referred to as small unmanned aircraft (UAS) (Hatfield and Prueger, 2010, need a reference for UAS). This instrumentation can enable the screening of traits related to biomass, photosynthesis, transpiration, disease, and stress tolerance (Tattaris et al., 2016; Gehan and Kellogg, 2017; Shakoor et al., 2017).

The ability to collect phenotypic data with sufficient resolution and accuracy to plant characteristics has been a challenge in plant based science research (Bai et al., 2016). The typical collection of these plant attributes is labor intensive and expensive creating a bottleneck of linking data to selection variables. These visual observations are subject to potential bias interpretations (McKenzie and Lambert, 1961). With the advancements in technology, potential arises to connect genomic data with high throughput phenotypic data, to better advance genomic linkage (Furbank and Tester, 2011). This helps with reducing the cost and time constraints in collecting and characterizing plant phenotypes.

The adoption of using technologies in high throughput phenotyping provides an opportunity to aid in the selection of large populations of segregating progeny and could mitigate a phenotyping bottleneck associated with labor-intensive selection methods (Furbank et al. year). Research conducted by (Christenson et al., 2015) found that current breeding practices had indirectly selected for lines showing a relationship between spectral values and cultivar release date ranging from 1923 to 2010. This research found that newer cultivars had lower values in the visible to red-edge portion of the spectrum and higher values in NIR and concluded that there is potential to use spectral imagery as a method of selection based on spectral reflection criteria. They also noted that there was a high association with other physiological parameters, offering the possibility of the measurements being a result to different physiological plant parameters.

Similarly, in a study conducted by Xavier et al., 2017, they found that canopy growth derived from image collection had a high association with yield showing a (.87) correlation to yield and having a heritability (h^2) of .77. They suggested using canopy closure as a method of selection due to its ease of collection and association with to yield. In a study by Bai et al. 2017, they found strong correlations between both green NDVI, red edge NDVI and final grain yield in soybean and wheat, finding a similar conclusion that spectral reading would be a beneficial method of selection as its strong relationship to yield. Ma et al. 2001 evaluated the predictability of soybean yield collecting NDVI measurements on plots between maturities of R2 to R5 stages. They developed regression models that were able to account 44-80% of the variation in yield. In relation to canopy temperature, in a study conducted by Keep et al., 2016 they evaluated cultivars between two different maturity groups released between 1920 to 2010. They concluded that canopy temperature was highly associated with year of release, suggesting that these corresponding improvements could show potential as a method for selection as a means to improve selection towards seed yield. Further research has found a general positive relationship to spectral reading as it relates to yield (Aslan, 2015; Clark, 2016).

Objectives and Hypothesis

Remote sensing is a resource to obtain phenotypic data on a field trial throughout the growing season and at a canopy level. These practices of acquiring numerical crop performance is often associated with the relationship to radiation, water, and nutritional efficacy. This can all be accomplished in a matter that is non-destructive and non-invasive to the natural crop production (Araus and Cairns, 2014).

Previous studies have characterized relationships between remote sensing data and phenotypic selection for soybean and wheat. Bai et al. 2016 reported correlations from .55 - .70 between grain yield remotely sensed data and concluded the measurements would be beneficial to utilize in a breeding program. Christenson, 2015 reported that spectral indices were related to cultivar year of release as seed yield improved indicated that breeding programs had been indirectly selecting for. This would indicate that newer versus older cultivars show a relationship to yield with respect to spectral reflectance (Christenson, 2015). Thus establishing that breeding programs have indirectly been selecting for increased spectral indices.

The objective of this study was to incorporate remote sensing phenotypic selection into the progeny row generation of a soybean breeding program, and compare phenotypic selection based on remotely sensed data to random and traditional selection practices. This study evaluated the relationship within a large population of non-replicated entries and the ability to evaluate spectral selection methods in comparison to conventional methods such as visual and yield selection and random control selection. This study determined the relationship between these practices and helped distinguish what selection techniques were the most optimal and have the largest improvement within a breeding program to progress entries to the later preliminary yield trials. In addition, we have reviewed spectral measurements in their relationship to phenotypic attributes as it relates to performance trials. This in an attempt to model predictor variables of trial performance. The hypothesis of the study was that spectral phenotypical selections would show a strong heritability, allowing the potential to account for variation using standardized checks within our early non-replicated progeny-row trials and determine yield ranking within performance trials. In addition, we have predicted that a variation of spectral values would show a linkage to improved yield among soybean genotypes.

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Chapter 2 - Soybean Progeny Row Phenotypic Selection Using Spectral Imagery

Abstract

Plant breeding is a process of manipulating plants, making them generally more useful. To incorporate beneficial attributes in cultivars, large segregating populations may be necessary. Evaluating large populations may create a bottleneck on plant selection. Sensor technologies have the potential to complement existing phenotyping criteria to improve the rate of genetic gain. This study compared the selection differential in seed yield, maturity, height, and lodging among F4-derived soybean lines selected in un-replicated progeny rows based on spectral imagery, visual observations, and random control selections. Spectral imagery was used to calculate a normalized difference red edge index (NDRE), a red normalized difference vegetation index (NDVI), a thermal rating (TH), and canopy size (CC) values for 5338 genotypes in 2017 and 6110 genotypes in 2018. The top 8% of the genotypes based on CC, NDRE, NDVI, TH, progeny row yield (PYLD) and visual (VIS) evaluations, along with random (RAND) selections were advanced to early and late maturing field trials (KPE and KPL) the following years. NDRE, NDVI, and TH selections were measured on mean values (X). Progeny row selections were evaluated in 2018 KPE, KPL, and 2019 KPE trials at three locations and in 2019 KPL trials were evaluated at two locations; all locations using a non-replicated, modified augmented design. Seed yield, maturity, lodging, and plant height were measured on all yield trial plots. Entry means were used to calculate the average seed yield, maturity, lodging, and height for each selection category. Selections based on XNDRE, XNDVI, PYLD, CC, and VIS showed significant yield improvement over RAND selections, however, these observations were not consistent across locations or years. XNDRE and XNDVI showed the greatest consistency across years. Height

had shown to be significantly shorter for both XNDRE and XNDVI and lodging had shown to be significantly less severe amongst the XNDRE KPE selections when compared to the random control selections (RAND). This association was supported by a significantly negative correlation between measurements of XNDRE (-.15) and XNDVI (-.07) in 2018 in addition to (-.31), and (-.21) respectively in 2019 to height means. XNDRE had shown a significant negative correlation (-.06) to lodging in 2018 and in 2019 both XNDRE (-.34) and XNDVI (-.21) were significantly correlated to lodging. These patterns were similar in the KPL trials. In 2018, XNDRE accounted for 44% more entries than RAND of the top 30% highest yielding lines, and XNDVI accounting for 47% more entries than RAND. In 2019 XNDVI accounted for 42% more of the top 30% highest yielding lines than RAND in the final population, and XNDRE accounted for 88% more entries than RAND. XNDRE and XNDVI both showed promising results as a selection method. In a program where visual selection is limited by trial size, spectral selection might prove beneficial, however, further research is needed to develop the selection criteria that will produce a consistent positive selection differential.

Introduction

An extensive effort is underway to increase seed yield to meet the growing demand for food throughout the world. Estimates predict that increases in seed yield are needed at a rate of 2.3% per year to meet demand, while current rates or improvement are only averaging 1.3% per year (Araus and Cairns, 2014). With predictions of increased atmospheric carbon dioxide causing altered weather patterns throughout the globe it is believed that the repercussions of climate change will exacerbate biotic stresses on agricultural plants (Walthall et al., 2012). As a result, current elite lines could be negatively affected, creating a need for a new highly adaptable combination of additive genetics.

To increase the rate of genetic gain large segregating populations are needed to identify progeny with desirable traits. Conventional methods of selection are often very labor-intensive and expensive, generally resulting in an independent measurement of yield replicated across different environments (Furbank and Tester, 2011). The accuracy of estimates of different variance components is important in determining the heritability and genetic gain (Baenziger et al., 2006). Environmental variance, genotypic variance, error variance, and genotype by environment are highly influential within a breeding program. Progress from the selection can only be realized if superior genotypes are readily identified.

In some crops such as soybean, early testing is conducted with limited seed amounts among large numbers of entries, thus limiting the potential for replication of a given trial or entry (Hegstad et al., 1999). The limited material often results in progeny-row trials being established as the indicator of agronomic suitability. The progeny rows are often non-replicated single-row plots at one location that limit the characterization of yield potential (Moreira et al., 2019). A small percentage of these lines are selected and advanced for further evaluations. Effective selection in progeny rows has the potential to be highly influential in a breeding program. The legitimacy of early evaluations is highly dependent on the accuracy of the derived data and distinguishing the difference among the non-replicated genotypes and the selections stability, thereafter across multiple locations.

Visual ratings have been used as a method for early generation testing but the predictability of these early generation methods can show confounding results (McKenzie and Lambert, 1961). It has been found that there is a potential to consciously or unconsciously select for characteristics that are uninformative of yield, such as height, and heading date, thus making visual selection potentially inconsistent. However, Ntare et al., 1984 found that visual selection proved to be a good selection method for yield improvement in cowpeas. In a study by Dahiya et al., 1984 they compared selection using both low and high yielding genotypes and compared them to visual selection categories. They found that the low and high yield selection categories continued in the same relationship to later yield trials, whereas visual selection showed no improvement over the random control variable suggesting limitations to visual selection methods (Dahiya et al., 1984).

Often as a result of showing inconsistent results, visual selection has been used to screen out bad genotypes rather than used as a primary selection tool for progressing highly productive genotypes (Luedders et al., 1973). Luedders et al. 1973, found 27.5% of their highest-yielding soybean lines were discovered in early yield testing, and that these lines were stable across years. They concluded that there is potential to remove up to 75% lines in early generations. DePauw and Shebeski, 1973 found similar results, when they selected for heritable differences in yield in the F3 generation. Negative impacts from the selection using yield data have been found as well, Briggs and Shebeski, 1971; Knott and Kumar, 1975, discovered that lines selected for yield in the early generations, were poor indicators of different environment influence on yield in later trials, but that yield selection within the same environment remained accurate.

As an alternative to yield and visual selection, imagery has been considered as a measurement to characterize plant attributes associated with yield. The adoption of using technologies in high throughput phenotyping provides an opportunity to aid in the selection of large populations of segregating progeny that could mitigate a phenotyping bottleneck associated with labor-intensive selection methods (Furbank and Tester, 2011). Research conducted by

(Christenson et al., 2015) found that current breeding practices had indirectly selected for lines showing a relationship between spectral values and cultivar release date. This research found that newer cultivars had lower reflectance values in the visible to the red-edge portion of the electromagnetic spectrum and higher values in near-infrared (NIR) and concluded that there is potential to use spectral imagery as a method of selection based on spectral reflection criteria. They also noted that there was a high association with other physiological parameters, offering the possibility of the measurements being a result of different physiological plant parameters. Similarly, in a study conducted by Xavier et al., 2017, they found that canopy growth derived from image collection had a high association with yield (r=0.87) and a heritability (h^2) of 0.77. They suggested using canopy closure as a method of selection due to its ease of collection and association with yield. In a study by Bai et al., 2016, strong correlations between both green NDVI, red-edge NDVI, and final grain yield in soybean and wheat were observed. Ma et al. 2001, evaluated the predictability of soybean yield using NDVI measurements on plots between maturities of R2 to R5 stages. They developed regression models that accounted for 44-80% of the variation in yield. Keep et al., 2016, noted that canopy temperature was associated with year of release among soybean cultivars in two maturity groups. They suggested that could be targeted as selection criteria to improve seed yield.

Due to noninvasive imagery collection having a positive relationship to beneficial plant attributes, studies are beginning to demonstrate that using spectral imagery as a selection method in a breeding program can prove beneficial, in that doing so would result in selection for beneficial characteristics. However, few studies have shown the difference between using spectral selection when compared to conventional methods of selection within a progeny row soybean trial. The objectives of this study were to: 1) determine the influence of using spectral imagery as a as a comparison to a control, 2) compare the selection differential between conventional selection methods such as visual and yield selection to spectral measurements of interest as a comparison of efficiency, 3) evaluate height, lodging and maturity association to selection variables, and 4) determine which spectral measurements are the most beneficial for each plant trait.

Materials and Methods

Experimental Field Design

This study evaluated 5,338 F_4-derived soybeans [*Glycine max.* (L) Merr.] lines in 2017 and 6110 lines in 2018 in progeny rows (Prows). The F_4-derived lines originated from crosses of elite by elite parents. The lines were developed from 51 different parental single-cross combinations in 2017 involving 55 different parental lines, and 78 different combination and in 2018 involving 22 different parental lines and 59 different combinations. Each cross contributed from 40 to 250 different lines to the progeny row trials. The average number of progenies per cross evaluated was 110 lines. Trial locations and soil characterization are show in Tables 2.1 and 2.2. Rainfall amounts in 2017 and 2018 were generally dryer than the 30-year average, and 2019 was wetter than the 30-year average (Table 2.3). Within a 30-year average, the hottest month of the growing season was July and the coldest month had been in May (Table 2.4). This had been consistent in the 2019 however in 2018 June was the hottest month and September was the coldest month in Manhattan and Onega.

Prows were planted in a modified augmented design with check plots every 10 plots in 2017 and every 15 plots in 2018. Plots consisted of a single row, 1.8 m in length spaced 76 cm apart. The plots were planted in fields that could be irrigated, but moisture stress was limited in the Prow trials. Irrigation of about 5 cm was applied once in 2017 and 2018 at the R1-R2 growth stages (Fehr and Caviness, 1977). Weeds were controlled by herbicides, cultivation, and hand-weeding.

Genotypic selections made from Prows were advanced to Kansas Preliminary Early (KPE) and Kansas Preliminary Late (KPL) yield trials following the year of selection. The KPE trials contained early maturity group (MG) III to mid IV lines. The KPL trials contained late MG IV to mid V lines. The KPE trials contained 1288 and 870 selected lines in 2018 and 2019 respectively. The KPL trial contained 243 and 465 entries in 2018 and 2019. Each KPE and KPL field trial was planted in a Modified Augmented Design (Type 2) at three locations, with one replication per location (Lin and Poushinsky, 1985). Incomplete blocks consisted of 9 to 15 entries, 2 or 3 columns and 17 to 32 rows depending upon the number of entries in the tests with one central control plot and 2 subplot control plots used in each experiment. In 2018, lines were planted in two-row plots, 3.7 m in length spaced 76 cm apart. In 2019, lines were planted in one-row plots, 3.7 m in length spaced 76 cm apart. The seeding rate of both years was approximately 26 live seed per meter of row.

Remote Sensing Data Collection

The spectral imagery was collected on Prows using a Matrice 100 DJI drone (DJI, Shenzhen, China). Gimbal attachments were added to permit mounting cameras of interest for data collection. The drone had a maximum load capacity of 1 kilogram allowing for a flight time of 25 minutes. The missions were created in Mission Planner and imported into “Litchi” for automated flight application. Thermal imagery was collected on a FLIR Vue pro R 13mm (FLIR Systems Inc, Wilsonville, OR, USA). For spectral imagery, a RedEdge camera by MicaSense (MicaSense Inc., Seattle, WA) was used. This camera collected spectral reflectance from five different wavelength bands including 475 nm (blue), 560 nm (green), 668 nm (red), 840 nm (near-infrared), and 717 nm (red edge). Both cameras collected imagery at 50 meters high with an 80% overlap between images among each flight pass. The flights were flown within a two-hour window of solar noon during cloud-free days to allow for limited interference. All flights were completed between R1 (beginning flowering) to R7 (beginning maturity). In 2017 five flights were collected on 6/20, 7/10, 7/20, 8/01, and 8/18. In 2018 four flights were collected on 6/27, 7/10, 7/24, and 8/09. The drone flight speed was at 3 m/s for all flights. The photos were stitched together using Agisoft PhotoScan Professional (Agisoft LLC 2018.). Then the resulting imagery was placed into ArcGIS Pro (Esri Inc, Redlands, CA) for raster calculations and extractions. Values were averaged across dates to give a single measurement of selection.

Phenotypic Traits Progeny Row Trials

The spectral and thermal phenotypes for the Prows were determined by calculating the mean pixel value in the plots after establishing plot polygons. Spectral imagery was used to calculate a normalized difference red edge index (NDRE) (Barnes at al., 2000), a red normalized difference vegetation index (NDVI) (Deering, 1978), a thermal rating (TH) (Jensen, 2007), and canopy size (CC) (Xavier et al., 2017) values. The NDRE was calculated as near-infrared (NIR) minus red-edge wavelengths, divided by NIR plus red-edge wavelengths. NDVI was calculated as near-infrared (NIR) minus red wavelengths, divided by NIR plus red wavelengths.

$$NDRE = \frac{NIR - RedEdge}{NIR + RedEdge}$$

$$NDVI = \frac{NIR - Red}{NIR + Red}$$

Daily observations were averaged across dates for each year and selections were made based on mean values of TH (XTH), NDRE (XNDRE), and NDVI (XNDVI). Within the thermal imagery FLIR camera measurements are associated with at-sensor radiometric temperatures showing low radiometric values, being associated with low temperatures and high radiometric values associated with higher temperatures (Sagan et al., 2019). Often to account absolute temperatures in degrees Celsius or Fahrenheit a conversion is necessary to account for atmospheric and emissivity corrections; however we were only interested in relative genotype thermal association and plot mean pixel ranking so radiometric value was sufficient. Canopy size (CC) was measured as the sum of all vegetative or green pixels within the plot area on one sampling date prior to canopy closure. Flights to determine CC were completed at or prior to the R1 growth stage (Fehr and Caviness, 1977) on 6/20 and 6/27 in 2017 and 2018, respectively. In 2017 visual ratings and seed yield (PYLD) were collected for Prows . Total plot seed weight was recorded for each single progeny row and visual interpretation by an experienced plant breeder was recorded for visual (VIS) selections. In 2018, due to disease and plant health issues relating to weather patterns, visual selection and yield were not collected.

Superior genotypes were considered those with high values for CC, XNDRE, XNDVI and seed yield, and low values for XTH. The top 8% of the genotypes based on CC, NDRE, NDVI, TH, PYLD and VIS evaluations, along with random (RAND) selections were advanced to early and late maturing field trials (KPE and KPL) the following years.

Yield Trials

Data collected for the KPE and KPL trials each year included seed yield, maturity, plant height, and lodging. Maturity was recorded as the number of days after August 31st when 95% of the pods reach a fully matured color. Plant height was measure as the average length from the ground to the tip of the main stem. Lodging was scored at harvest on a scale of 1-5, with 1 = all plant upright, 2 = 20º lean, 3 = 45º lean, 4 = 60 º lean, and 5 was assigned to plots when plants were prostrate. Modified Augmented Design (MAD) analysis was used to calculate the individual location means, and PROC MIXED was used to calculate the grand means within maturity group by location within a given trial year.

Evaluation of Preliminary Yield Trials Differential Across Selection Methods

In early preliminary yield trials, VIS, CC, PYLD, XTH, XNDRE, and XNDVI were compared to a control (RAND) in 2018. In 2019 CC, XTH, XNDRE, and XNDVI were compared to RAND. Analysis of variance was use to compare differences between the selection methods. PROC GLM (SAS Institute, 2018) was used as a method to fit general linear models. PROC CORR was used to calculate the Pearson's correlation matrix to evaluate relationships between physical attributes. The same selections were used as treatments and compared in the PROC GLM function of the grand mean of the 30% top performing entries for both KPE and KPL trials in 2018 and 2019. The review of the top 30% was a method to retroactively review selections within this trial as a means for future line progression. Variables being reviewed were the same as the primary evaluation; yield, height, lodging, and maturity.

Results and Discussion

Kansas Preliminary Early Trials

Preliminary Early 2018

Yield entries differed when averaged across locations with a low of 1681 to a high of 3665 kg/ ha (Data not shown). Average maturities ranged from 15 to 56 days after August 30. Lodging scores ranged from a low of 1 to a high of 3.7. Average plant heights ranged from 63 to 128 cm. Entry means across three locations for XNDRE, CC, XNDVI, PYLD, and VIS selections of 2834, 2802, 2825, 2827, and 2845 kg/ha, respectively, were significantly higher than RAND selections at 2727 kg/ha (Table 2.5). It is shown that the standard deviation for

Location 1 presents a high standard deviation. This reflects the higher yield for that location across all selection methods. PYLD, CC, XNDRE, and XNDVI were all significantly ($P <.05$) correlated with each other (Table 2.6). Correlations tended to be the largest between XNDRE, XNDVI and CC. On average, selections for PYLD were significantly later in maturity than RAND, but similar in height and lodging (Table 2.5). PYLD showed a positive correlation to maturity (.18*) (Table 2.6). XNDRE and VIS selections tended to be shorter and have lower lodging scores than RAND (Table 2.5). Small, negative correlations between XNDRE and both lodging (-0.06*) and height (-0.15*) showed that as XNDRE increased, height and lodging decreased (Table 2.6).

At Location 1, XNDRE and VIS selections were greater in seed yield, shorter in plant height, and more lodging resistant than RAND selections (Table 2.4). XNDRE values were positively correlated to yield (0.14*) and negatively correlated to maturity (-0.07*), lodging (-0.06*) and height (-0.12) (Table 2.6). At location 2, PYLD and VIS selections were higher yielding than RAND at 2275 and 2287kg ha, respectively. PYLD selections tended to be 2 days later in maturity, and VIS average 3 cm shorter in plant height than RAND selections. PYLD was significantly correlated to yield (0.15*) and maturity (0.18*) at location 2 (Table 2.6). At location 3, CC, XNDVI, PYLD, and VIS selected averaged significantly higher yields at 2550, 2560, 2568, AND 2555 kg/ha, respectively, when compared to RAND at 2441 kg/ha (Table 2.5). VIS selections averaged 4 cm shorter than RAND entries. Entry values for CC, and PYLD showed significant correlations up to (0.18*) to yield at Location 3 (Table 2.6).

Preliminary Early 2019

Yield among entries differed across locations with average yields ranging from 1966 to 4565 kg/ha. Average maturities ranged from 12 to 50 days after August 30, lodging scores ranged from 1 to 3, and plant height ranged from 58 to 105 cm (Data not shown). Base on the three location means, XNDRE and XNDVI showed significant (0.05 or 0.1) increases over RAND selections at two location and in the 2019 grand mean (Table 2.7). Average yields of both these criteria were significantly higher than XTH, but were not significantly different from CC selections. At locations 4 and 6, XNDRE and XNDVI showed a significant yield improvement over RAND selections. No differences in seed yield were observed among the selection criteria at Location 5. On average and across locations, XNDRE and XNDVI selections tended to be shorter in plant height and more lodging resistant than RAND selections. Both XNDRE and XNDVI were positively correlated to yield (r values ranged from 0.11* to 0.31*), and negatively correlated to lodging and plant height (r values ranged from -0.15 to -0.34), on average and across locations (Table 2.8).

KPE Discussion

When comparing the performance of the top 30% highest yielding lines of the grand mean, the average phenotypic values among the selection categories tended to be similar and show no significant difference, except for plant height, where the highest yielding lines tended to be shorter in plant height in the VIS selections compared with RAND. However, XNDRE, and XNDVI in 2018 and 2019 and PYLD in 2018 accounted for over 25 more observations than RAND in the top 30% of the grand mean (Table 2.9, 2.10). NDVI and NDRE measurements have shown to have potential in a soybean breeding program by Bai et al., 2017 finding a significant correlation between plot yield and NDRE and NDVI measurements. Similarly, Christenson et al., 2015 found that individual wave bands associated with NDVI and NDRE measurements would have the same effect. In this study XNDRE variable showed a significant improvement in yield at only location 1, 4, and 6, which all were located near the origin of p-row selection. This result is supported in research by Briggs and Shebeski, 1971; Knott and Kumar, 1975, suggesting that selections made within early generations of lines are primarily representative of the location they were selected at. This could show constraints of selection as being restricted by line genetic stability (Fehr, 1939). Some cultivars are more adapted to a broad range of environments, while others are more limited by their location. This stability is influenced by the cultivars ability to withstand stress conditions. Cultivar stability is less prominent as homogeneity increases within the cultivar being that F1 hybrids are more stable than their homozygous parents. Of the 30% best performing entries, XNDRE accounted for 101 of the entries in 2018 and in 2019 accounted for the most entries of 113. Improvement outside of the origin of selection amongst XNDVI, CC, PYLD, and VIS. CC selection had shown similar results to Xavier et al., 2017; where a positive response to selection was shown to yield. Of the significant spectral measurements, CC had and the least significance with an alpha level of 0.10 of the 2018 grand mean (Table 2.4). XNDVI significant improvement relationship to yield is similar to research founded by (Ma et al., 2001) showing that this measurement is indicative of yield, however, this significant relationship showed up at only one location and the grand mean in 2018 but had a greater response in 2019 showing a significant improvement over the control at two locations and the grand mean. This variable did account for 103 of the entries in 2018 and 85 in 2019 of the top 30% yielding lines (Table 2.9, 2.10). PYLD and VIS had shown a significantly positive response in yield to selection and this response had shown at multiple locations. VIS had shown the most consistent response showing a significant improvement over RAND at all three locations in 2018. Of the 286 highest yielding lines in 2018 (top 30% of the grand mean) PYLD accounted for the greatest amount at 111 lines (Table 2.9). Of the spectral measurements, XNDVI accounted for the most in 2018 at 103 lines and the second most in 2019 (Table 2.10) with 85 lines. In comparison, the RAND control had accounted for 70 lines in 2018 and 60 lines in 2019 suggesting that these selection methods had made an impact over the top 30% of the population.

<u>Kansas Preliminary Late Trials</u>

Preliminary Late

Yield among entries differed across locations with a range in yield of 2376 to 4330 kg/ha in 2018, and from 2161 to 4571 kg /a in 2019. Maturities ranged from 37 to 66 in 2018 and 51 to 65 days after August 30 in 2019. Lodging scores ranged from 1 to 3.3 in 2018, and 1 to 4.5 in 2019. Plant height ranged from 64 to 140 cm in 2018, and 71 to 135 cm in 2019 (Data not shown). In 2018 and 2019, the average values of selections for PYLD, VIS, CC, XNDVI, and XNDRE were not significantly different from RAND (Table 2.11, 2.12). Average of the selection criteria were not significantly different from each other. This could be a result of the location of original selection not being indicative of the environment the lines were tested in (Briggs and Shebeski, 1971; Knott and Kumar, 1975), showing similar constrains to KPE trials of genetic stability implications (Fehr, 1939). Within the KPL trials 4 of the 5 trials had a relatively lower latitude than the origin of spectral measurements collected within the p-rows. This change of environment could influence growth and senescence as a response to photoperiod sensitivity being non-indicative the location in comparison to the section criteria (Cober and Morrison, 2010). Of the top 30% of the entries, all of the selection criteria accounted for more entries than RAND (Table 2.13). In 2018 VIS accounted for the most entries showing 39 entries in the top 30% and of the spectral measurements XNDRE showed the most accounting for 21 entries. In 2019 the response was stronger where RAND accounted for 40 entries when compared to XNDRE accounting for the most entries at 122 (Table 2.14).

These selection responses found that within the early maturity trials XNDRE and XNDVI showed a consistent relationship to yield in both years. These results differed in their significance level in 2019, suggesting that XNDVI could potentially be a more reliable measurement for yield improvement. CC showed a significant relationship to yield in 2018 but was relatively inconsistent across years and locations. These results are constant with the study conducted by (Christenson et al., 2015) suggesting that measurements in the visible portion of the spectrum could make for good selection criteria within a breeding program showing consistent responses in yield in early maturity trials. Similarly, less response was found in later maturity entries suggesting a lesser response that could be due to physiological growth differences between maturity groups. Similarly to a study conducted by (Benjamin, 2015) selection by CC showed a positive response to yield but the results were inconstant across environments.

KPE and KPL Trial Review

Where XNDRE had shown a significant correlation to yield in the KPE trials, and the 2019 KPL trial, this variable, also showed a significant correlation to height and lodging (Table 2.2.6, 2.8, 2.15). It is possible that these measurements are selecting the beneficial plant characteristics, rather than being tightly linked to yield and that separation from yield and growth characteristics could develop. XNDVI had shown less association with these physical attributes. Interestingly even where the spectral measurements had not shown a significant mean value increase, they accounted for 40% more of the observations than the control in the top 30% of the performing entries in 2018, and 41 – 175% (XNDVI) more in 2019. It is possible that even though the population sizes are adequate that larger population sizes can show an influence on selection differential based on degrees of freedom. This is noticed in that significant improvements are recognized in the KPE trials where entries ranged from 870-1288, and no significance is found in the KPL trials with lesser lines of 243-465 entries. When considering the average annual genetic gain of soybean of cultivars from 1923 to 2008 is 1.25% increase (Wilson et al., 2014), it puts into perspective potentially why large populations could influence the evaluation of the selection differential. Interestingly in both trials, the same variables showed an increase in observations compared to the control (RAND) when evaluating the top 30% of the grand mean (Table 2.9, 2.10, 2.13, 2.14).

Tandem Selections

To examine the interaction and potential synergism of selecting superior genotypes based on combinations of selection criteria, the impact of selection was examined by evaluating the performance of entries based on multiple selection criteria. In 2018 and 2019, selection combinations of XNDRE plus XNDVI, XNDRE, plus XNDVI, plus XTH, and XNDRE, plus XNDVI, plus CC were evaluated within each maturity group. The mean seed yield in all of the tandem selection combinations was not significantly greater than the selection categories based on the best single criterion (Data not shown). .

Conclusions

Spectral measurements were used to make genotype selections within the progeny row trials of a soybean-breeding program. The yield of these selections was evaluated to determine the measurable influence based on those selections. Significant improvements in yield were found in using both XNDVI and XNDRE measurements. These recognized yield increase from the control RAND had developed primarily in the KPE trials. CC had shown to have significant improvement over RAND in 2018 KPE trial, however this was at an alpha set to 0.10 and was non repeatable across years. Within these trials XNDVI had shown to develop an improved yield at a higher alpha level than XNDRE, suggesting that this variable is more reliable as a measurement for selection. The conventional selection methods of PYLD and VIS showed significant improvement over the control. VIS had shown to have the highest average yield over all selection methods. In the KPL trials, the average yield for the spectral selections in regards to NDVI and NDRE did result in having a higher overall yield, but it did not develop at a level of significance. TH had shown to have no influence on yield or any other plant characteristic from the selection. Both PYLD and VIS were not significantly different from RAND, however VIS had shown a similar pattern as within the KPE trials, in that the average yield was higher than all other variables. Within tandem selections, many of the combinations did influence the baseline yield; however, the influence did not change with any significant level.

Selections based on spectral measurements had also shown an association with plant height and lodging. It was found that XNDRE and XNDVI had a significantly shorter height than the control in addition to having a lower lodging score in the KPE trials. Similarly, VIS selections were significantly shorter and had significantly better lodging scores. PYLD had shown a significant association with maturity, in that this variable would select for later maturing lines. In the KPL trials XNDRE and XNDVI had shown significantly lower lodging in 2019. Both PYLD and VIS had shown to be significantly shorter than RAND and VIS had not only a significantly lower lodging score than RAND but over all other variables. It is possible that these selection criteria could be influenced by this criterion and in the case of VIS could be open to potential biases.

Using spectral measurements within a soybean-breeding program has shown to be effective. Both XNDRE and XNDVI had shown to have strong associations with yield, making them good candidates for spectral selection variables. XNDVI was less associated with physical parameters suggesting that this variable would have a stronger relationship to yield without having secondary influences. VIS had shown to have the greatest opportunity amongst all the selection variables; however, this variable has the greatest opportunity for error given that this selection is completely open to interpretation of “good lines”. It is worth noting a well-experienced breeder with many years of experience made VIS selections improving the likelihood of selection for good candidates. It is possible that in a situation of lesser experienced breeder with less familiarity of the given crop, that this criteria could be more open to apparent biases within the given data. Observationally, tandem selections did not appear to influence selection, but it is possible that where XNDVI and XNDRE had shown to have a significant improvement at alternative environments, that tandem selection could allow for normalization between measurements and less environmental influence. This would need to adhere to practice to observe the actual selection response as opposed to the observational response.

Table 2.1. Trial location is specified by reference name and latitude-longitude location. Trials soil type and taxonomic classification are given for each location.

Trial	Year	Location	reference	plant date	Latitude	Longitude
P-Rows	2017	Manhattan	na	25-May	39° 7'56.86"N	96°37'4.92"W
P-Rows	2018	Manhattan	na	17-May	39° 8'25.06"N	96°37'46.76"W
KPE	2018	Manhattan	Location 1	23-May	39° 8'35.19"N	96°37'46.76"W
KPE	2018	Onega	Location 2	10-May	39°24'55.46"N	96° 9'16.94"W
KPE	2018	Ottawa	Location 3	15-May	38°32'25.20"N	95°14'51.91"W
KPE	2019	Manhattan	Location 4	31-May	39° 8'29.77"N	96°37'46.38"W
KPE	2019	Riley	Location 5	4-Jun	39°21'26.92"N	96°48'26.28"W
KPE	2019	Manhattan	Location 6	5-Jun	39° 8'29.77"N	96°37'46.38"W
KPL	2018	Manhattan	Location 7	22-May	39° 8'35.19"N	95° 3'8.32"W
KPL	2018	McCune	Location 8	6-Jun	37°23'40.41"N	95° 3'8.32"W
KPL	2018	Pittsburg	Location 9	5-Jun	37°20'27.08"N	94°35'43.62"W
KPL	2019	Pittsburg	Location 10	1-Jul	37°15'46.98"N	94°37'59.55"W
KPL	2019	Ottawa	Location 11	3-Jun	38°32'31.55"N	95°14'52.00"W

Table 2.2. Trial soil type and taxonomic classification are given for each location.

Trial	Year	Location	reference	%	Soil Type	Taxonomic Class
P-Rows	2017	Manhattan	na	100	Eudora silt loam	*mesic Fluventic Hapludolls*
P-Rows	2018	Manhattan	na	100	Bismarck-Kimo Complex	*shallow Typic Dystrudepts*
KPE	2018	Manhattan	Location 1	94.1	Belvue silt loam	*mesic Fluventic Hapludolls*
				5.9	Eudora silt loam	*mesic Fluventic Hapludolls*
KPE	2018	Onega	Location 2	64.3	Chase silty clay loam	*mesic Aquertic Argiudolls*
				35.2	Wabash silty clay	*mesic Cumulic Vertic Endoaquolls*
KPE	2018	Ottawa	Location 3	100	Woodson silt loam	*thermic Abruptic Argiaquolls*
KPE	2019	Manhattan	Location 4	13	Belvue silt loam	*mesic Fluventic Hapludolls*
				87	Eudora silt loam	*mesic Fluventic Hapludolls*
KPE	2019	Riley	Location 5	100	Whmore silty clay loam	*mesic Aquertic Argiudolls*
KPE	2019	Manhattan	Location 6	13	Belvue silt loam	*mesic Fluventic Hapludolls*
				87	Eudora silt loam	*mesic Fluventic Hapludolls*
KPL	2018	Manhattan	Location 7	94.1	Belvue silt loam	*mesic Fluventic Hapludolls*
				5.9	Eudora silt loam	*mesic Fluventic Hapludolls*
KPL	2018	McCune	Location 8	100	Parsons Silt Loam	*thermic Mollic Albaqualfs*
KPL	2018	Pittsburg	Location 9	8.8	Medoc Silt Loam	*thermic Aeric Albaqualf*
				91.2	Cherokee silt loam	*thermic Typic Albaqualfs*
KPL	2019	Pittsburg	Location 10	1.7	Helper Silt Loam	*frigid Typic Haplustepts*
				98.3	Parsons Silt Loam	*thermic Mollic Albaqualfs*
KPL	2019	Ottawa	Location 11	100	Woodson silt loam	*thermic Abruptic Argiaquolls*

Table 2.3. Rainfall accumulation by trial location from May 1st to September 30th in comparison to the 30-year average.

Trial	Year	Location	reference	5/1 -9/30 rainfall	30 YR average	Rainfall Difference
P-Rows	2017	Manhattan	na	377.44 mm	533.15 mm	-155.71 mm
P-Rows	2018	Manhattan	na	506.98 mm	533.15 mm	-26.17 mm
KPE	2018	Manhattan	Location 1	506.98 mm	533.15 mm	-26.17 mm
KPE	2018	Onega	Location 2	489.71 mm	594.61 mm	-104.9 mm
KPE	2018	Ottawa	Location 3	368.05 mm	591.57 mm	-223.52 mm
KPE	2019	Manhattan	Location 4	788.92 mm	533.15 mm	255.77 mm
KPE	2019	Riley	Location 5	887.22 mm	578.1 mm	309.12 mm
KPE	2019	Manhattan	Location 6	788.92 mm	533.15 mm	255.77 mm
KPL	2018	Manhattan	Location 7	506.98 mm	533.15 mm	-26.17 mm
KPL	2018	McCune	Location 8	496.32 mm	605.54 mm	-109.22 mm
KPL	2018	Pittsburg	Location 9	496.32 mm	605.54 mm	-109.22 mm
KPL	2019	Pittsburg	Location 10	998.98 mm	605.54 mm	393.44 mm
KPL	2019	Ottawa	Location 11	1216.15 mm	591.57 mm	624.58 mm

Table 2.4. Temperature averages by month through growing season for soybean of May through September in comparison to the 30 year average.

Trial	Year	Location	reference	comparison	May	Jun.	Jul.	Aug.	Sep.
P-Rows	2017	Manhattan	na	Average	17.60	23.20	26.1	25	19.9
				Actual	17.90	24.10	26.9	22.3	22.1
P-Rows	2018	Manhattan	na	Average	17.60	23.20	26.1	25	19.9
				Actual	22.30	26.50	25.9	24.8	20.9
KPE	2018	Manhattan	Location 1	Average	17.60	23.20	26.1	25	19.9
				Actual	22.30	26.50	25.9	24.8	20.9
KPE	2018	Onega	Location 2	Average	17.10	22.20	25	24.2	19.3
				Actual	23.20	25.30	25.2	24.9	20.9
KPE	2018	Ottawa	Location 3	Average	18.10	23.10	25.9	25.1	20.2
				Actual	23.00	25.80	25.9	25.2	21
KPE	2019	Manhattan	Location 4	Average	17.60	23.20	26.1	25	19.9
				Actual	16.80	23.00	25.7	24.3	24.1
KPE	2019	Riley	Location 5	Average	18.40	23.70	26.6	25.6	20.4
				Actual	17.10	23.40	26.3	24.7	24.4
KPE	2019	Manhattan	Location 6	Average	17.60	23.20	26.1	25	19.9
				Actual	16.80	23.00	25.7	24.3	24.1
KPL	2018	Manhattan	Location 7	Average	17.60	23.20	26.1	25	19.9
				Actual	22.30	26.50	25.9	24.8	20.9
KPL	2018	McCune	Location 8	Average	18.80	23.70	26.4	26.3	21.3
				Actual	22.10	25.90	26.6	25.2	22.4
KPL	2018	Pittsburg	Location 9	Average	18.80	23.70	26.4	26.3	21.3
				Actual	22.10	25.90	26.6	25.2	22.4
KPL	2019	Pittsburg	Location 10	Average	18.80	23.70	26.4	26.3	21.3
				Actual	18.90	23.30	25.6	25.2	25.3
KPL	2019	Ottawa	Location 11	Average	18.10	23.10	25.9	25.1	20.2
				Actual	17.5	23.3	25.5	24.2	24.1

Table 2.5. Comparison of mean and standard deviation (SD) for seed yield, maturity, plant height, and lodging among selection criteria for soybean entries in Kansas Preliminary Early trials in 2018.

Selection criterion[a]	Mean across three locations		Location 1		Location 2		Location 3		No. of entries W/I criterion
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	n
					Seed yield (kg/ha)				
RAND	2727c	351	3596b	753	2192b	354	2441b	411	323
XTH	2759bc	336	3629b	647	2218ab	363	2495ab	422	349
XNDRE	2834ab	304	3810a	576	2214ab	360	2518ab	421	348
XNDVI	2825ab	339	3665ab	680	2268ab	373	2560a	410	348
CC	2802abc*	343	3641b	676	2236ab	359	2550a	420	348
PYLD	2827ab	365	3692ab	724	2275ab*	370	2568a	420	348
VIS	2845a	375	3746ab*	668	2287a	359	2555a	457	297
					Maturity (days after August 31)				
RAND	29b	9.31	40ab	12.59	31bc	9.79	23ab	7.68	323
XTH	30ab	9.49	34b	12.48	32ab	10.12	24ab	8	349
XNDRE	29b	8.25	33b	11.49	30c	8.77	22b	6.78	348
XNDVI	30ab	9.36	34ab	12.55	32ab	9.95	24ab	7.92	348
CC	29b	8.94	33b	11.98	31bc	9.5	23ab	7.58	348
PYLD	32a	9.46	37a*	12.9	33a	10.04	25a	7.98	348
VIS	31ab	9.72	35ab	12.76	33ab	10.62	24ab	8.08	297
					Plant height (cm)				
RAND	90a	10.77	108a	13.28	80a	11.61	82a	11.63	323
XTH	88ab*	10.01	105ab	13.86	78ab	11.18	79ab*	10.98	349
XNDRE	87b	9.17	104b	12.88	77b	10.05	78b	9.79	348
XNDVI	88ab	10.86	106ab	14.28	79ab	11.69	80ab	11.19	348
CC	89ab	10.58	106ab	14.09	79ab	11.45	80ab	10.99	348
PYLD	90a	10.13	108a	13.87	80a	11.36	81a	10.71	348
VIS	87b	8.81	104b	13.74	77b	10.49	78b	10.14	297
					Lodging (score)				
RAND	1.6a	0.5	2.2a	0.86	1.5a	0.62	1.2a	0.4	323
XTH	1.5ab	0.47	2ab	0.8	1.4a	0.62	1.2a	0.46	349
XNDRE	1.5ab	0.43	1.9b	0.79	1.4a	0.58	1.1a	0.34	348
XNDVI	1.5ab	0.48	2ab	0.82	1.4a	0.63	1.2a	0.41	348
CC	1.5ab	0.49	2ab	0.83	1.4a	0.65	1.2a	0.41	348
PYLD	1.5ab	0.5	2ab	0.8	1.5a	0.68	1.1a	0.41	348
VIS	1.4b	0.4	1.9b	0.76	1.4a	0.57	1.1a	0.34	297

[a] RAND = random selections, XTH= selections based on thermal mean, XNDRE = selections based on red edge NDVI mean, XNDVI = selections based on NDVI mean, CC = selections based on canopy size, PYLD = selections based on progeny row seed yield, VIS = breeder selections based on visual phenotype.

[b] Means within a column and trait followed by a common letter are not significantly different at the .05 level of probability based on LSD test.

[c]Means followed by * are significantly different from RAND selections at the .1 level of probability based on LSD test, respectively.

Table 2.6. Pearson's correlation matrix between phenotypic values for soybean entries across location (Loc.) 1, 2, and 3 for 2018 KPE trials and their respective mean value. (n=1288)

	Yield Mean	Yield Loc. 1	Yield Loc. 2	Yield Loc. 3	Mat. Means	Mat. Loc. 1	Mat. Loc. 2	Mat. Loc. 3
Yield Mean								
Yield Loc. 1	0.65*							
Yield Loc. 2	.57*	0.01						
Yield Loc. 3	0.58*	-0.12*	0.36*					
Mat. Means	0.042	-0.43*	0.28*	0.47*				
Mat. Loc. 1	0.09*	-0.32*	0.30*	0.41*	0.91*			
Mat. Loc. 2	0.03	-0.45*	0.27*	0.48*	0.94*	0.78*		
Mat. Loc. 3	0.01	-0.46*	0.23*	0.45*	0.92*	0.75*	0.87*	
Lod. Means	-0.09*	-0.34*	0.15*	0.15*	0.36*	0.33*	0.34*	0.35*
Lod. Loc. 1	-0.07*	-0.28*	0.10 *	0.08*	0.18*	0.19*	0.15*	0.18*
Lod. Loc. 2	-0.07*	-0.31*	0.16*	0.16*	0.43*	0.37*	0.42*	0.42*
Lod. Loc. 3	-0.01	-0.21*	0.10*	0.17*	0.25*	0.21*	0.25*	0.26*
Ht. Means	0.04	-0.24*	0.17*	0.23*	0.35*	0.35*	0.31*	0.32*
Ht. Loc. 1	0.15*	-0.12*	0.20*	0.19*	0.24*	0.32*	0.20*	0.21*
Ht. Loc. 2	0	-0.23*	0.16*	0.16*	0.28*	0.28*	0.26*	0.25*
Ht. Loc. 3	0.02	-0.28*	0.15*	0.27*	0.39*	0.37*	0.36*	0.39*
PYLD	0.23*	0.15*	0.15*	0.18*	0.18*	0.18*	0.17*	0.12*
CC	0.10*	0.06*	0.06*	0.08*	0.01	0.00	0.02	0.02
XNDRE	0.10*	0.14*	0.00	0.03	-0.08*	-0.07*	-0.08*	-0.06*
XNDVI	0.07*	0.07*	0.02	0.05	-0.02	-0.03	-0.01	0.00

XTH	0.04	-0.03	-0.04	0.9*	0.06*	0.05	0.07*	0.06*

	Lod. Means	**Lod. Loc. 1**	**Lod. Loc. 2**	**Lod. Loc. 3**	**Ht. Means**	**Ht. Loc. 1**	**Ht. Loc. 2**	**Ht. Loc 3.**
Lod. Loc. 1	0.81*							
Lod. Loc. 2	0.78*	0.39*						
Lod. Loc. 3	0.63*	0.28*	0.41*					
Ht. Means	0.49*	0.37*	0.44*	0.33*				
Ht. Loc. 1	0.34*	0.32*	0.30*	0.21*	0.79*			
Ht. Loc. 2	0.45*	0.34*	0.41*	0.29*	0.85*	0.56*		
Ht. Loc. 3	0.50*	0.36*	0.43*	0.38*	0.88*	0.60*	0.67*	
PYLD	-0.01	-0.05	0.05	-0.04	0.06*	0.07*	0.03	0.04
CC	-0.02	-0.04	-0.01	0.01	-0.05	-0.05	-0.05	0.00
XNDRE	-0.06*	-0.06*	-0.04	-0.03	-0.15*	-0.12*	-0.12*	-0.11*
XNDVI	0.01	-0.01	0.02	0.03	-0.07*	-0.08*	-0.05	-0.04
XTH	0.09*	0.07*	0.09*	0.06*	0.05	0.04	0.04	0.07*

	PYLD	**CC**	**XNDRE**	**XNDVI**
CC	0.4*			
XNDRE	0.30*	0.75*		
XNDVI	0.31*	0.72*	0.94*	
XTH	-0.02	0.16*	0.25*	0.25*

Pearson correlations between soybean entry means among selection categories for 2018 KPE trials

[a] XTH= selections based on thermal mean, XNDRE = selections based on red edge NDVI mean, XNDVI = selections based on NDVI mean, CC = selections based on canopy size, PYLD = selections based on progeny row seed yield

[b] Lod. = Lodging 1 value for vertical stem, and 5 for completely horizontal stem. Ht. = height of plant in cm. Mat. = maturity in days after August 31st.

[c] (*) indicates significance at a p-value alpha level set at 0.05.

Table 2.7. Comparison of mean and standard deviation (SD) for seed yield, maturity, plant height, and lodging among selection criteria for soybean entries in Kansas Preliminary Early trials in 2019.

Selection criterion[a]	Mean across three locations		Location 4		Location 5		Location 6		No. of entries W/I criterion
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	n
					Seed yield (kg/ha)				
RAND	3341bc	441	3410b	649	3518a	621	3106b	563	210
XNDRE	3448ab*	421	3587a	574	3497a	622	3272a	566	310
XTH	3325c	463	3373b	653	3495a	590	3116b	609	272
CC	3401abc	442	3471ab	582	3551a	636	3187ab	563	210
XNDVI	3500a	387	3613a	562	3606a	572	3299a	538	210
					Maturity (days after August 31)				
RAND	32a	4.35	30a	5.05	34a	5.32	32a	4.53	210
XNDRE	32a	3.23	29a	3.44	33a	4.29	32a	4.06	310
XTH	31a	3.5	29a	3.72	33a	4.51	32a	4.34	272
CC	31a	3.46	29a	3.69	33a	4.23	31a	4.41	210
XNDVI	32a	2.95	29a	3.28	34a	3.82	33a	3.76	210
					Plant height (cm)				
RAND	78a	7.95	84a	10.02	71a	9.45	80a	8.64	210
XNDRE	75b	5.44	80c	6.99	68b	7.11	77b	7.28	310
XTH	78a	6.97	83ab	9.6	71a	8.1	79a	9.11	272
CC	76b	5.71	81bc	7	70ab	7.46	78ab	9.18	210
XNDVI	75b	5.94	79c	7.84	68b	7.2	77b	9.23	210
					Lodging (score)				
RAND	1.5a	0.44	1.7a	0.73	1.1a	0.23	1.6a	0.74	210
XNDRE	1.3b	0.35	1.4b	0.6	1b	0.08	1.4b	0.6	310
XTH	1.5a	0.45	1.7a	0.73	1.1a	0.31	1.7a	0.74	272
CC	1.5a	0.45	1.7a	0.74	1.1a	0.23	1.6a	0.7	210
XNDVI	1.3b	0.37	1.5b	0.65	1b	0.1	1.4b	0.63	210

[a] RAND = random selections, XTH= selections based on thermal mean, XNDRE = selections based on red edge NDVI mean, XNDVI = selections based on NDVI mean, CC = selections based on canopy size, PYLD = selections based on progeny row seed yield, VIS = breeder selections based on visual phenotype.

[b] Means within a column and trait followed by a common letter are not significantly different at the .05 level of probability based on LSD test.

[c] Means followed by * are significantly different from RAND selections at the .1 level of probability based on LSD test, respectively.

Table 2.8. Pearson's correlation matrix between phenotypic values for soybean entries across location (Loc.) 4, 6, and 5 for 2019 KPE trials and their respective mean value. (n=870)

	Yield Means	**Yield Loc. 4**	**Yield Loc. 6**	**Yield Loc. 5**	**Mat. Means**	**Mat. Loc. 4**	**Mat. Loc. 6**	**Mat. Loc. 5**
Yield Loc. 4	0.77*							
Yield Loc. 6	0.76*	0.44*						
Yield Loc. 5	0.67*	0.21*	0.25*					
Mat. Means	0.22*	0.01	0.14*	0.38*				
Mat. Loc. 4	0.15*	-0.01	0.11*	0.27*	0.85*			
Mat. Loc. 6	0.28*	0.12*	0.23*	0.31*	0.86*	0.60*		
Mat. Loc. 5	0.14*	-0.08*	0.02	0.40*	0.86*	0.60*	0.60*	
Lod. Means	-0.15*	-0.20*	-0.21*	0.09*	0.06	0.04	0.01	0.11*
Lod. Loc. 4	-0.10*	-0.14*	-0.18*	0.09*	0.06	0.05	0.02	0.10*
Lod. Loc. 6	-0.09*	-0.14*	-0.14*	0.09*	0.04	0.02	0.01	0.08*
Lod. Loc. 5	-0.24*	-0.22*	-0.24*	-0.06	0.01	-0.02	-0.01	0.06
Ht. means	-0.01	-0.17*	-0.07*	0.22*	0.31*	0.22*	0.22*	0.34*
Ht. Loc. 4	-0.04	-0.12*	-0.08*	0.10*	0.25*	0.18*	0.19*	0.26*
Ht. Loc. 6	0.03	-0.11*	0.04	0.14*	0.24*	0.18*	0.19*	0.23*
Ht. Loc. 5	-0.01	-0.18*	-0.13*	0.28*	0.26*	0.17*	0.17*	0.32*
CC	0.12*	0.09*	0.04	0.12*	-0.13*	-0.10*	-0.14*	-0.11*
XNDRE	0.31*	0.31*	0.27*	0.11*	-0.02	-0.01	0.01	-0.05
XNDVI	0.28*	0.25*	0.23*	0.13*	0.03	0.02	0.05	0.02
XTH	0.02	0.03	0.02	-0.01	0.07*	0.07*	0.08*	0.03

	Lod. Means	Lod. Loc. 4	Lod. Loc. 6	Lod. Loc. 5	Ht. means	Ht. Loc. 4	Ht. Loc. 6	Ht. Loc. 5
Lod. Loc. 4	0.83*							
Lod. Loc. 6	0.84*	0.44*						
Lod. Loc. 5	0.44*	0.21*	0.23*					
Ht. means	0.37*	0.33*	0.27*	0.23*				
Ht. Loc. 4	0.28*	0.26*	0.19*	0.20*	0.81*			
Ht. Loc. 6	0.25*	0.21*	0.20*	0.15*	0.79*	0.52*		
Ht. Loc. 5	0.37*	0.33*	0.27*	0.21*	0.80*	0.49*	0.46*	
CC	0.07*	0.07*	0.07*	-0.02	-0.01	-0.02	-0.02	0.02
XNDRE	-0.34*	-0.23*	-0.28*	-0.27*	-0.31*	-0.25*	-0.23*	-0.26*
XNDVI	-0.21*	-0.16*	-0.17*	-0.15*	-0.21*	-0.17*	-0.18*	-0.17*
XTH	-0.10*	-0.09*	-0.09*	-0.03	-0.05	-0.04	-0.03	-0.05

	CC	XNDRE	XNDVI	TH
XNDRE	0.40*			
XNDVI	0.43*	0.71*		
XTH	-0.36*	-0.10*	-0.35*	

Pearson correlations between soybean entry means among selection categories for 2019 KPE trials

[a] XTH= selections based on thermal mean, XNDRE = selections based on red edge NDVI mean, XNDVI = selections based on NDVI mean, CC = selections based on canopy size

[b] Lod. = Lodging 1 value for vertical stem, and 5 for completely horizontal stem. Ht. = height of plant in cm. Mat. = maturity in days after August 31st.

[c] (*) indicates significance at a p-value alpha level set at 0.05.

Table 2.9. Top 30% comparison of grand mean and standard deviation (SD) for seed yield, maturity, plant height, and lodging among selection criteria for soybean entries in Kansas Preliminary Early trials in 2018.

Selection criterion[a]	Mean across three locations		Location 1		Location 2		Location 3		No. of entries W/I criterion
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	n
					Seed yield (kg/ha)				
RAND	3167a	137	4256a	418	2449a	336	2760a	336	70
XTH	3162a	121	4131a	444	2499a	285	2823a	285	80
XNDRE	3180a	144	4181a	484	2486a	345	2847a	342	101
XNDVI	3192a	154	4113a	494	2516a	399	2879a	340	103
CC	3192a	155	4109a	481	2491a	367	2882a	356	91
PYLD	3177a	145	4154a	427	2519a	360	2798a	356	111
VIS	3195a	157	4212a	431	2489a	378	2847a	334	95
					Maturity (days after August 31)				
RAND	30a	6.5	36a	9.7	31a	7.2	23a	5.9	70
XTH	31a	8.4	35a	10.1	33a	9.4	24a	7.4	80
XNDRE	31a	9.4	37a	12	33a	10.3	25a	8	101
XNDVI	32a	9.6	37a	12	35a	10.5	26a	8.2	103
CC	32a	9.6	36a	11.8	34a	10.3	25a	8.5	91
PYLD	31a	8.2	37a	11.4	34a	9	24a	6.6	111
VIS	32a	9.5	36a	12.3	34a	10.5	25a	7.4	95
					Plant height (cm)				
RAND	92a	8	109a	11	81a	10	84a	9	70
XTH	89ab	7	108a	10	79ab	7	80ab	9	80
XNDRE	89ab	9	107a	12	79ab	10	81ab	9	101
XNDVI	90ab	10	108a	13	80ab	11	81ab	10	103
CC	90ab	10	107a	12	80ab	11	81ab	9	91
PYLD	91a	8	111a	10	81a	9	82ab	9	111
VIS	87b	7	106a	11	76b	8	78b	8	95
					Lodging (score)				
RAND	1.6a	0.42	2.1a	0.81	1.4a	0.53	1.1a	0.3	70
XTH	1.5a	0.41	1.9ab	0.77	1.4a	0.56	1.2a	0.42	80
XNDRE	1.5a	0.43	2.0ab	0.78	1.4a	0.64	1.1a	0.31	101
XNDVI	1.6a	0.44	2.0ab	0.74	1.5a	0.68	1.2a	0.4	103
CC	1.5a	0.47	2.0ab	0.83	1.5a	0.69	1.2a	0.4	91
PYLD	1.5a	0.48	1.9ab	0.76	1.5a	0.69	1.1a	0.41	111
VIS	1.4a	0.35	1.8b	0.67	1.3a	0.57	1.1a	0.26	95

[a] RAND = random selections, XTH= selections based on thermal mean, XNDRE = selections based on red edge NDVI mean, XNDVI = selections based on NDVI mean, CC = selections based on canopy size, PYLD = selections based on progeny row seed yield, VIS = breeder selections based on visual phenotype.

[b] Means within a column and trait followed by a common letter are not significantly different at the .05 level of probability based on LSD test.

Table 2.10. Top 30% comparison of grand mean and standard deviation (SD) for seed yield, maturity, plant height, and lodging among selection criteria for soybean entries in Kansas Preliminary Early trials in 2019.

Selection criterion[a]	Mean across three locations		Location 4		Location 5		Location 6		No. of entries W/I criterion
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	n
					Seed yield (kg/ha)				
RAND	3849a	193	3971a	518	4026a	454	3551a	436	60
XNDRE	3879a	211	3988a	472	3952a	500	3699a	412	113
XTH	3856a	203	3932a	487	3950a	480	3676a	411	76
CC	3857a	215	3869a	478	4081a	502	3622a	371	69
XNDVI	3864a	222	3982a	446	3978a	469	3633a	434	85
					Maturity (days after August 31)				
RAND	33a	3.4	30a	3.8	35a	4.3	34a	4.1	60
XNDRE	32a	2.7	30a	2.6	34a	4.1	33a	3.2	113
XTH	33a	2.7	30a	2.7	34a	4	33a	3.2	76
CC	32a	2.6	30a	2.2	35a	3.8	33a	3.3	69
XNDVI	33a	2.8	30a	3	35a	3.7	33a	3.3	85
					Plant height (cm)				
RAND	79a	7	84a	10	72a	8	80a	7	60
XNDRE	77ab	5	82ab	7	70a	7	79a	7	113
XTH	78ab	6	83ab	9	71a	7	80a	7	76
CC	78ab	5	82ab	7	71a	7	80a	7	69
XNDVI	76b	5	80b	8	69a	7	79a	8	85
					Lodging (score)				
RAND	1.4a	0.39	1.6a	0.67	1a	0	1.6a	0.73	60
XNDRE	1.3a	0.37	1.5a	0.63	1a	0	1.4a	0.65	113
XTH	1.3a	0.35	1.5a	0.61	1a	0.2	1.5a	0.64	76
CC	1.4a	0.43	1.6a	0.65	1a	0.1	1.6a	0.77	69
XNDVI	1.3a	0.37	1.5a	0.63	1a	0	1.5a	0.67	85

[a] RAND = random selections, XTH= selections based on thermal mean, XNDRE = selections based on red edge NDVI mean, XNDVI = selections based on NDVI mean, CC = selections based on canopy size, PYLD = selections based on progeny row seed yield, VIS = breeder selections based on visual phenotype.

[b] Means within a column and trait followed by a common letter are not significantly different at the .05 level of probability based on LSD test.

Table 2.11. Comparison of mean and standard deviation (SD) for seed yield, maturity, plant height, and lodging among selection criteria for soybean entries in Kansas Preliminary Late trials in 2018.

Selection criterion[a]	Mean across three locations		Location 7		Location 8		Location 9		No. of entries W/I criterion
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	n
				Seed yield (kg/ha)					
RAND	3425a	354	3082a	652	3674a	584	3766a	584	68
XTH	3507a	404	3238a	553	3740a	553	3797a	552	72
XNDRE	3464a	495	3268a	639	3678a	639	3758a	639	70
XNDVI	3440a	505	3267a	671	3745a	671	3695a	671	70
CC	3378a	560	3247a	652	3670a	652	3703a	652	71
PYLD	3491a	451	3245a	674	3806a	674	3915a	674	70
VIS	3518a	464	3370a	571	3790a	571	3800a	571	102
				Maturity (days after August 31)					
RAND	49a	3.5	50a	6.8	51a	3.4	46a	4	68
XTH	48a	3.6	47a	6.4	50a	3.2	45a	4.7	72
XNDRE	47a	4.4	48a	7.7	50a	4	44a	4.5	70
XNDVI	48a	4	48a	7.2	50a	2.9	44a	4.8	70
CC	48a	4.3	48a	7.8	50a	3.06	45a	4.9	71
PYLD	48a	3.1	48a	6.6	50a	2.8	45a	3.2	70
VIS	47a	3.5	47a	6.5	50a	2.8	45a	4.4	102
				Plant height (cm)					
RAND	102ab	13.62	111ab	17.39	97a	12.85	97a	15.66	68
XTH	100ab	12.37	110ab	14.4	98a	13.86	93ab	13.31	72
XNDRE	99ab	13.63	110ab	16.69	96a	13.92	92ab	14.58	70
XNDVI	100ab	15.25	110ab	16.32	97a	17.01	93ab	15.89	70
CC	105a	17.12	117a	19.98	100a	17.47	99a	18.58	71
PYLD	98b	14.42	107b	16.44	96a	15.33	91ab	15.3	70
VIS	97b	13	107b	15.02	96a	13.32	90b	14.13	102
				Lodging (score)					
RAND	1.5a	0.47	2.2a	0.88	1.2a	0.48	1.2a	0.43	68
XTH	1.5a	0.44	2.1a	0.85	1.2a	0.44	1.2a	0.41	72
XNDRE	1.4ab	0.34	2.1a	0.76	1.1a	0.26	1.1a	0.3	70
XNDVI	1.4ab	0.4	2a	0.8	1.2a	0.41	1.1a	0.35	70
CC	1.5a	0.39	2.2a	0.78	1.1a	0.38	1.1a	0.34	71
PYLD	1.5a	0.46	2.1a	0.89	1.2a	0.46	1.2a	0.42	70
VIS	1.3b	0.31	1.9a	0.69	1.1a*	0.22	1.1a	0.35	102

[a] RAND = random selections, XTH= selections based on thermal mean, XNDRE = selections based on red edge NDVI mean, XNDVI = selections based on NDVI mean, CC = selections based on canopy size, PYLD = selections based on progeny row seed yield, VIS = breeder selections based on visual phenotype.

[b] Means within a column and trait followed by a common letter are not significantly different at the .05 level of probability based on LSD test.

[c] Means followed by * are significantly different from RAND selections at the .1 level of probability based on LSD test, respectively.

Table 2.12. Comparison of mean and standard deviation (SD) for seed yield, maturity, plant height, and lodging among selection criteria for soybean entries in Kansas Preliminary Late trials in 2019.

Selection criterion[a]	Mean across two locations		Location 10		Location 11		No. of entries W/I criterion
	Mean	SD	Mean	SD	Mean	SD	n
			Seed yield (kg/ha)				
RAND	3317a	447	3162a	550	3455a	590	153
XNDRE	3452a	450	3321a	539	3577a	579	153
XTH	3413a	442	3312a	573	3522a	565	153
CC	3398a	446	3241a	539	3552a	580	153
XNDVI	3415a	460	3297a	523	3547a	579	153
			Maturity (days after August 31)				
RAND	57a	3.26	62a	2.51	53a	4.61	153
XNDRE	57a	3.53	62a	2.93	53a	4.7	153
XTH	57a	3.49	62a	3.06	53a	4.62	153
CC	58a	3.37	62a	2.9	53a	4.51	153
XNDVI	58a	3.77	62a	3.21	54a	4.95	153
			Plant height (cm)				
RAND	94a	11.2	89a	10.8	98a	15.7	153
XNDRE	91a	9.3	86a	9.1	95a	13.3	153
XTH	94a	10.4	89a	10.1	98a	14.4	153
CC	93a	10.1	89a	10.2	96a	14.2	153
XNDVI	92a	9.5	87a	9.8	96a	13.6	153
			Lodging (score)				
RAND	2.5a	2.5	2.7a	2.7	2.3a	0.92	153
XNDRE	2.2b	2.2	2.3b	2.3	2.0b	0.88	153
XTH	2.4ab	2.4	2.6ab	2.6	2.1ab	0.90	153
CC	2.5a	2.5	2.7a	2.7	2.3ab	0.95	153
XNDVI	2.2b	2.2	2.4ab*	2.4	2.1ab	0.92	153

[a] RAND = random selections, XTH= selections based on thermal mean, XNDRE = selections based on red edge NDVI mean, XNDVI = selections based on NDVI mean, CC = selections based on canopy size, PYLD = selections based on progeny row seed yield, VIS = breeder selections based on visual phenotype.

[b] Means within a column and trait followed by a common letter are not significantly different at the .05 level of probability based on LSD test.

[c] Means followed by * are significantly different from RAND selections at the .1 level of probability based on LSD test, respectively.

Table 2.13. Top 30% comparison of grand mean and standard deviation (SD) for seed yield, maturity, plant height, and lodging among selection criteria for soybean entries in Kansas Preliminary Late trials in 2018.

Selection criterion[a]	Mean across three locations		Location 7		Location 8		Location 9		No. of entries W/I criterion
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	n
					Seed yield (kg/ha)				
RAND	3873a	230	3622a	730	4018a	417	4001a	463	14
XTH	3923a	190	3620a	608	3999a	351	4143a	441	22
XNDRE	3950a	171	3782a	338	3973a	372	4087a	467	21
XNDVI	3944a	203	3713a	505	4075a	305	4001a	500	20
CC	3984a	172	3775a	419	4139a	269	4030a	492	18
PYLD	3928a	188	3545a	566	4014a	312	4245a	378	24
VIS	3918a	181	3678a	423	4046a	420	4044a	413	39
					Maturity (days after August 31)				
RAND	51a	2.4	55a	4.4	51a	3.9	47a	2.5	14
XTH	48b	2.4	48b	4.9	50a	2.6	45ab	3.6	22
XNDRE	47b	3.1	47b	5	50a	3.9	43b	3.1	21
XNDVI	47b	3.5	49b	6.1	49a	2.8	44ab	4	20
CC	48b	3.5	50ab	6	49a	3.2	45ab	3.5	18
PYLD	48b	2.5	48b	5.5	50a	2.6	45ab	3.3	24
VIS	47b	2.6	47b	4.3	50a	3	44ab	3.6	39
					Plant height (cm)				
RAND	105a	18	113a	19	103a	18	100a	20	14
XTH	98a	13	108a	13	97a	15	90a	13	22
XNDRE	97a	13	107a	14	96a	16	90a	13	21
XNDVI	100a	17	110a	16	99a	20	92a	16	20
CC	105a	19	114a	19	104a	20	97a	21	18
PYLD	98a	13	106a	15	98a	14	90a	13	24
VIS	96a	12	105a	13	95a	13	87a	12	39
					Lodging (score)				
RAND	1.9a	0.62	2.7a	0.88	1.6a	0.65	1.5a	0.65	14
XTH	1.5ab	0.41	2.2a	0.8	1.2ab	0.53	1.1a	0.29	22
XNDRE	1.4b	0.35	2.0a	0.67	1.1ab	0.36	1.1a	0.3	21
XNDVI	1.4b	0.42	2.0a	0.77	1.2ab	0.41	1.2a	0.37	20
CC	1.5ab	0.42	2.2a	0.73	1.2ab	0.38	1.2a	0.43	18
PYLD	1.5ab	0.56	2.2a	0.93	1.3ab	0.55	1.3a	0.53	24
VIS	1.4b	0.29	2.0a	0.6	1.1b	0.31	1.1a	0.34	39

[a] RAND = random selections, XTH= selections based on thermal mean, XNDRE = selections based on red edge NDVI mean, XNDVI = selections based on NDVI mean, CC = selections based on canopy size, PYLD = selections based on progeny row seed yield, VIS = breeder selections based on visual phenotype.

[b] Means within a column and trait followed by a common letter are not significantly different at the .05 level of probability based on LSD test.

Table 2.14. Top 30% comparison of grand mean and standard deviation (SD) for seed yield, maturity, plant height, and lodging among selection criteria for soybean entries in Kansas Preliminary Late trials in 2019.

Selection criterion[a]	Mean across two locations		Location 10		Location 11		No. of entries W/I criterion
	Mean	SD	Mean	SD	Mean	SD	n
			Seed yield (kg/ha)				
RAND	3871a	253	3736a	429	3988a	506	40
XNDRE	3880a	231	3749a	383	3996a	439	122
XTH	3858a	235	3762a	377	3936a	423	114
CC	3881a	238	3704a	380	4035a	436	50
XNDVI	3877a	239	3738a	377	4001a	441	110
			Maturity (days after August 31)				
RAND	57a	3.3	62a	2.5	53a	4.7	40
XNDRE	58a	3.4	62a	2.6	53a	4.9	122
XTH	58a	3.5	62a	2.9	54a	4.8	114
CC	58a	3.6	62a	2.6	53a	4.8	50
XNDVI	58a	3.5	63a	2.8	54a	4.9	110
			Plant height (cm)				
RAND	100a	14	95a	13	104a	19	40
XNDRE	91b	9	87b	9	95b	12	122
XTH	95ab	11	91ab	11	99ab	14	114
CC	95ab	12	91ab	12	99ab	15	50
XNDVI	91b	10	88b	11	94b	12	110
			Lodging (score)				
RAND	2.1a	0.66	2.3a	0.75	2.0a	0.81	40
XNDRE	1.9a	0.63	2.1a	0.79	1.8a	0.8	122
XTH	2.1a	0.65	2.4a	0.77	1.9a	0.75	114
CC	2.2a	0.7	2.4a	0.88	2.0a	0.81	50
XNDVI	2.0a	0.63	2.2a	0.79	1.8a	0.76	110

[a] RAND = random selections, XTH= selections based on thermal mean, XNDRE = selections based on red edge NDVI mean, XNDVI = selections based on NDVI mean, CC = selections based on canopy size, PYLD = selections based on progeny row seed yield, VIS = breeder selections based on visual phenotype.

[b] Means within a column and trait followed by a common letter are not significantly different at the .05 level of probability based on LSD test.

Table2.15. Pearson's correlation matrix between phenotypic values for soybean entries across location (Loc.) 10 and 11 for 2019 KPL trials and their respective mean value. (n=465)

	Yield Means	**Yield Loc. 11**	**Yield Loc. 10**	**Mat. Means**	**Mat. Loc. 11**	**Mat. Loc. 10**
Yield Loc. 11	0.78*					
Yield Loc. 10	0.78*	0.15*				
Mat. means	0.03	0.13*	-0.09			
Mat. Loc. 11	0.02	0.10*	-0.06	0.95*		
Mat. Loc. 10	0.03	0.15*	-0.10*	0.87*	0.68*	
Ht. Means	0.09	0.02	0.13*	0.08	0.09	0.04
Ht. Loc. 11	0.02	0.01	0.05	0.05	0.05	0.03
Ht. Loc. 10	0.16*	0.04	0.19*	0.08	0.10	0.04
Lod. means	-0.36*	-0.19*	-0.39*	0.24*	0.24*	0.21*
Lod. Loc. 11	-0.32*	-0.24*	-0.29*	0.25*	0.26*	0.19*
Lod. Loc. 10	-0.29*	-0.08	-0.37*	0.17*	0.15*	0.17*
XTH	-0.11*	-0.10*	-0.09	0.13*	0.11*	0.15*
XNDRE	0.21*	0.12*	0.21*	-0.29*	-0.28*	-0.24*
XNDVI	0.15*	0.10*	0.15*	-0.15*	-0.15*	-0.12*
CC	0.13*	0.07	0.13*	-0.22*	-0.21*	-0.20*
	Ht. Means	**Ht. Loc. 11**	**Ht Loc. 10**	**Lod. Means**	**Lod. Loc. 11**	**Lod. Loc. 10**
Ht. Loc. 11	0.88*					
Ht. Loc. 10	0.74*	0.33*				
Lod. means	0.04	0.08	-0.03			

Lod. Loc. 11	0.06	0.05	0.05	0.85*		
Lod. Loc. 10	0.01	0.08	-0.10*	0.86*	0.47*	
XTH	-0.02	0.00	-0.03	0.08	0.08	0.06
XNDRE	-0.16*	-0.14*	-0.12*	-0.35*	-0.28*	-0.33*
XNDVI	-0.144*	-0.11*	-0.13*	-0.28*	-0.22*	-0.26*
CC	-0.03	-0.05	0.00	-0.09*	-0.07	-0.09
	XTH	**XNDRE**	**XNDVI**	**CC**		
XNDRE	-0.24*					
XNDVI	-0.33*	0.73*				
CC	-0.30*	0.45*	0.41*			

Pearson correlations between soybean entry means among selection categories for 2019 KPL trials

[a] XTH= selections based on thermal mean, XNDRE = selections based on red edge NDVI mean, XNDVI = selections based on NDVI mean, CC = selections based on canopy size

[b] Lod. = Lodging 1 value for vertical stem, and 5 for completely horizontal stem. Ht. = height of plant in cm. Mat. = maturity in days after August 31st.

[c] (*) indicates significance at a p-value alpha level set at 0.05.

Table2.16. Pearson's correlation matrix between phenotypic values for soybean entries across location (Loc.) 7, 8, and 9 for 2018 KPL trials and their respective mean value. (n=243)

	Yield Means	**Yield Loc. 7**	**Yield Loc. 8**	**Yield Loc. 9**	**Mat. Means**	**Mat. Loc. 7**	**Mat. Loc. 8**	**Mat. Loc. 9**
Yield Loc. 7	0.74*							
Yield Loc. 8	0.61*	0.20*						
Yield Loc. 9	0.59*	0.13	0.32*					
Maturity Means	-0.11	-0.05	-0.06	0.21*				
Mat. Loc. 7	-0.04	-0.01	-0.05	0.12	0.87*			
Mat. Loc. 8	-0.13*	0.02	-0.19*	0.18*	0.56*	0.23*		
Mat. Loc. 9	-0.15*	-0.12	0.04	0.20*	0.78*	0.45*	0.36*	
Lod. Means	0.05	-0.08	0.05	0.29*	0.29*	0.38*	-0.03	0.16*
Lod. Loc. 7	0.02	-0.15	-0.02	0.26*	0.29*	0.42*	-0.05	0.12
Lod. Loc. 8	0.09	0.02	0.18*	0.19*	0.12	0.10	0.01	0.15*
Lod. Loc. 9	0.09	0.05	0.05	0.16*	0.12	0.18	-0.05	0.06
Height Means	-0.02	-0.02	-0.01	0.18*	0.33*	0.37*	-0.05	0.28*
Ht. Loc. 7	-0.09	-0.02	-0.07	0.13	0.33*	0.37*	0.02	0.24*
Ht. Loc. 8	0.12	0.04	0.19*	0.20*	0.18*	0.24*	-0.16*	0.19*
Ht. Loc. 9	-0.07	-0.06	-0.11	0.17*	0.37*	0.39*	-0.01	0.33*
CC	-0.04	0.00	0.00	-0.14*	-0.04	0.09	-0.21*	-0.10
XNDRE	0.03	0.03	-0.02	-0.12	-0.13	-0.02	-0.11	-0.23
XNDVI	-0.01	0.09	0.02	-0.17*	-0.09	0.02	-0.15	-0.16
XTH	-0.13*	-0.06	-0.06	0.00	0.15*	0.10	0.11	0.17*

PYLD	0.19*	0.02	0.15*	0.22*	-0.10	0.00	-0.11	-0.16*
	Lod. Means	**Lod. Loc. 7**	**Lod. Loc. 8**	**Lod. Loc. 9**	**Ht. means**	**Ht. Loc. 7**	**Ht. Loc. 8**	**Ht. Loc. 9**
Lod. Loc. 7	0.86*							
Lod. Loc. 8	0.64*	0.27*						
Lod. Loc. 9	0.68*	0.36*	0.40*					
Ht. Means	0.32*	0.34*	0.05	0.22*				
Ht. Loc. 7	0.20*	0.28*	-0.11	0.11	0.91*			
Ht. Loc. 8	0.35*	0.29*	0.22*	0.26*	0.85*	0.63*		
Ht. Loc. 9	0.33*	0.34*	0.06	0.23*	0.92*	0.79*	0.67*	
CC	0.03	0.06	-0.03	0.01	0.16*	0.15*	0.14*	0.14*
XNDRE	-0.03	0.00	-0.05	-0.05	-0.06	-0.04	-0.07	-0.07
XNDVI	-0.06	-0.05	-0.06	0.00	-0.02	-0.02	-0.02	-0.02
XTH	-0.02	0.02	-0.05	-0.06	0.07	0.08	0.02	0.09
PYLD	0.04	0.05	0.01	0.03	-0.12	-0.16*	-0.03	-0.13*
	CC	**XNDRE**	**XNDVI**	**XTH**	**PYLD**			
XNDRE	0.71*							
XNDVI	0.81*	0.82*						
XTH	-0.43*	-0.52*	-0.54*					
PYLD	0.13*	0.14*	0.15*	-0.10				

Pearson correlations between soybean entry means among selection categories for 2018 KPE trials

[a] XTH= selections based on thermal mean, XNDRE = selections based on red edge NDVI mean, XNDVI = selections based on NDVI mean, CC = selections based on canopy size, PYLD = selections based on progeny row seed yield

[b] Lod. = Lodging 1 value for vertical stem, and 5 for completely horizontal stem. Ht. = height of plant in cm. Mat. = maturity in days after August 31st.

[c] (*) indicates significance at a p-value alpha level set at 0.05.

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Chapter 3 - Soybean Yield Trials High-Throughput Phenotyping

Abstract

The ability to depict yield associated rankings and predict genotype performance in soybean (*Glycine max* (L.) Merr.) breeding trials across various locations has been relatively unrepeatable. The objective of this study was to evaluate spectral measurements or combinations (vegetation indices) thereof that could predict entry yield ranking by location and evaluate physiological attributes to variables showing a significant relationship to yield. The experiment consisted of four different locations and location 4 consisting of two different trials, resulting in a total of five trials. Each trial ranged from 10 to 52 entries, set up in a randomized complete block design, planted in 4-row plots 3.7m long, spaced .76m apart. Seed yield and spectral measurements were measured from the center two rows of each plot. Multi-Spectral, modified broadband camera, and thermal cameras were used to make spectral measurements. MicaSense measurements evaluated were blue, green, red, red-edge, near-infrared (NIR), blue normalize difference vegetation index (BNDVI), green normalize difference vegetation index (GNDVI), red normalize difference vegetation index (NDVI), normalized difference red-edge (NDRE), and pigment index (PI). Sony measurements evaluated were blue, green, NIR, BNDVI, GNDVI, and PI. Flir camera measurement analyzed was thermal (TH). MicaSense BNDVI, GNDVI, and NDVI showed a significant relationship to yield across multiple trials, however, these results showed to be variable, alternating between positive and negative correlations. Sony cameras BNDVI, and GNDVI measurement had shown a significant relationship to yield across multiple sights as well but altered between positive and negative correlations. No physical plant

characteristics were consistently associated with any significant yielding spectral measurements across all trials.

Introduction

At the forefront of the agricultural cropping systems, producers are attempting to continually achieve higher yielding goals. This is often sought using many different techniques; however, producers rely on improved seed development to help maintain yield advancement. The general progression for yield improvement encourages breeders to focus on developing cultivars that are higher yielding. The improvement of cultivars has largely been in part of a response to selection from large populations (Acquaah, 2007). Genomic selection has been able to rapidly improve selection within populations and offering the ability to depict high yielding attributes within a population (Thomson, 2014). The ability to analyze DNA continues to show improvement, however, these genetic predictions are highly more efficient when their association with plant phenotypes can be depicted (Yu et al., 2016). The association to differentiate phenotypes has not improved at the same rate to genome sequencing, suggesting that improvements in this area are important for further advancement.

The ability to collect phenotypic data with sufficient resolution and accuracy to plant characteristics has been a challenge in plant-based science research (Bai et al., 2016). The typical collection of these plant attributes is labor-intensive and expensive creating a bottleneck of linking data to selected variables. These visual observations are subject to potential bias interpretations (McKenzie and Lambert, 1961). With the advancements in technology, the potential arises to connect genomic data with high throughput phenotypic data, to better advance genomic linkage (Furbank and Tester, 2011). This helps with reducing the cost and time constraints in collecting and characterizing plant phenotypes.

Photosynthesis has shown a strong relationship with crop yield (Dhanapal et al., 2016). Developing a method, that allows for manipulation of photosynthesis's relationship to yield has been suggested as a way for yield advancement (Dhanapal et al., 2016). These results have been verified by using extraction-based methods, but these can be mildly destructive. As an alternative, spectral reflectance has been developed for modeling plant phenotypes, specifically chlorophyll content concerning light adsorption. Research has shown that spectral indices can be used to predict both disease pressure and yield potential within a trial, accounting for up to 41 to 93% of the variation in yield (Menke, 2018). However, field base research has suggested yield estimation using canopy reflectance and the temperature has shown to be variable and inconsistent (Babar et al., 2006). This technology has been useful establishing a relationship between yield and traits such as biomass and canopy reflectance, but many alternatives in methodology and instrumentation exist. The objective of this study was to evaluate the ability of three different spectral imagery platforms (multi-spectral, modified broadband camera, and thermal) to characterize the differences in seed yield, plant height, and maturity, lodging and wilting among commercial soybean varieties, in fortuity of the lesser need of mechanical harvest still allowing for yield ranking. This is in an effort to evaluate cultivars within later trials in a breeding program when comparing established elite lines.

Materials and Methods

Field Trials

The experiment was conducted on soybean cultivars planted at two locations in 2018 and 2 locations 2019 as part of the Kansas Soybean Variety Performance Tests. In 2018 Location 1 was located southeast of Onaga, KS at a latitude of 39°24'51.29"N and longitude of 96° 9'17.78"W. Soil type present at that location was Chase silty clay loam (*mesic Aquertic Argiudolls*). Location 2 in 2018 was located south of Salina at a latitude of 38°40'5.15"N and a longitude of 97°36'14.02"W. The soil type consisted of a Detroit silty clay loam (*mesic Pachic Argiustolls*). In 2019, Location 3 was located north of Manhattan at the research farm at a latitude of 39°12'57.88"N and a longitude of 96°35'31.70"W. The soil type consisted of a Kahola silt loam (*mesic Cumulic Hapludolls*). Location 4 in 2019 was located south of Salina at a latitude of 38°42'34.43"N and a longitude of 97°37'4.92"W. The soil type was a Longford silt loam (*mesic Udic Argiustolls*).

Each experiment was set up using a randomized complete block design with four replications. Cultivars were planted in 4 row plots, 3.7 m long, spaced .76 m apart. Due to herbicide injury, non-dicamba tolerant entries were removed from the evaluations in Locations 1, 2, and 4. In 2018, Location 1 was planted on 5/10/18 with 16 entries in the trial. Location 2 was planted on 5/24/18 with 35 entries. In 2019, Location 3 was planted on 6/10/19 with 52 entries. Location 4 had two trials planted on 6/7/19 consisting of an 38 entries in maturity groups III to mid-IV in the early test (SP10E, T1), and 10 entries in maturity groups late IV to mid V in a late test (SP10L,T2).

Data Collection

The spectral imagery was collected using a Matrice 100 DJI drone (DJI, Shenzhen, China). Gimbal attachments were added to be able to insert cameras of interest for the collection. The drone had a maximum load capacity of 1 kilogram allowing for a flight time of 25 minutes. A built-in autopilot was used for flight data collection. The missions were created in Mission Planner and imported into "Litchi" for automated flight application. Thermal imagery (TH) was collected on a Flir Vue pro R 13mm (Flir Systems Inc., Wilsonville, OR, USA). For spectral imagery, a RedEdge camera by MicaSense (MicaSense Inc., Seattle WA) was used. This camera captured reflectance from five different wavelength bands. The center wavelength of each band consisted of 475 nm (blue), 560 nm (green), 668 nm (red), 840 nm (near-infrared), and 717 nm (red edge). A Sony α5100 (Sony corporation of America, New York, NY) camera was used to collect imagery with 3 bands consisting of blue, green, and near-infrared, modified with broader bands than the Mica Sense camera that were close to 100 nm. Cameras allowed for geotagged data and ground control pointes were used and allowed alignment of imagery. Within the thermal imagery FLIR camera measurements are associated with at-sensor radiometric temperatures showing low radiometric values, being associated with low temperatures and high radiometric values associated with higher temperatures (Sagan et al., 2019). Often to account absolute temperatures in degrees Celsius or Fahrenheit a conversion is necessary to account for atmospheric and emissivity corrections; however, we were only interested in relative cultivar thermal association and plot mean pixel ranking so radiometric value was sufficient. MicaSense camera had come standard with calibrated reflectance panel to compensate for light conditions at time of image capture to give representation of light reaching ground at time of capture. The flights were flown within a two-hour window of solar noon on cloud-free days to allow for limited interference. The drone flight speed 3 m/s at an elevation of 50 m for all flights with an

80% overlap between field passes. The photos were stitched together using Agisoft PhotoScan Professional. The resulting imagery was placed into ArcGIS Pro for raster calculations and extractions. The spectral imagery was extracted from the center two rows of each plot. At Location 1, a flight was conducted on 7/09/18. At Location 2, flights were conducted on 7/08/18, 8/02/18, and 9/22/18. At Location 3, a flight was conducted on 8/29/19. At Location 4, flights were conducted on 9/03/19 and 9/13/19.

Phenotypic traits

Ground notes taken for each plot consisted of maturity, height, lodging and seed yield. The maturity was calculated based on the days after August 31st when 95% of the pods reach a fully matured color. Height was measured as the average length from the ground to the tip of the main stem in cm. Lodging was scored on a scale of 1-5 based on the number of plants leaning. Upright plants that had 0º of lean were given a 1, 2=20º lean, 3=45º lean, 4= 60 º lean, and 5 was given to prostrate plants. The center two rows were harvested with a plot combine to determine seed yield, reported as kg/ha.

Drought conditions enabled the collection of wilting scores at Location 1, 2, and 4 both trial 1 and 2. Visual wilting scores were collected using a scale from 0 – 100. Plots with a 0 represented no wilting, 20 represented slight wilting and rolling of the leaves at the top of the canopy, 40 represented severe rolling of the leaves at the top of the canopy and moderate wilting of the leaves through the rest of the canopy, 60 represented severe wilting throughout the canopy, 80 represented dead leaves throughout the canopy and severely wilted petioles, and 100 represented plant death.

The spectral and thermal phenotyping was completed by calculating the mean pixel value in the plot after establishing plot polygons. Both spectral and thermal values were used. The phenotypic values consisted of the thermal (TH) reading, and blue (BLUE), green (GREEN), red (RED), red edge (RED EDGE), and near-infrared (NIR) reflectance values. Within the thermal imagery, lower pixel values were associated with low temperatures and high pixel values were associated with higher temperatures. Reflectance values for blue, green, and near infrared were collected using both the MicaSense camera (denoted by "M") and the Sony camera (denoted by "S"). Indices calculated for each plot from the reflectance values included: BNDVI, GNDVI, RNDVI, NDRE, and PI. NDRE was calculated as near-infrared (NIR) minus red-edge wavelengths, divided by NIR plus red-edge wavelengths. NDVI was calculated as NIR minus red wavelengths, divided by NIR plus red wavelengths. BNDVI was calculated as NIR minus blue wavelengths, divided by NIR plus blue wavelengths. GNDVI was calculated as NIR minus green wavelengths, divided by NIR plus green wavelengths. PI was calculated as BNDVI minus GNDVI. All of the indices were calculated using data from the MicaSense camera. NDRE and NDVI could not be calculated with the reflectance data from the Sony camera.

$$NDRE = \frac{NIR - RedEdge}{NIR + RedEdge}$$

$$NDVI = \frac{NIR - Red}{NIR + Red}$$

$$BNDVI = \frac{NIR - Blue}{NIR + Blue}$$

$$GNDVI = \frac{NIR - Green}{NIR + Green}$$

$$PI = BNDVI - GNDVI$$

Data was analyzed using SAS. A mean pixel value was calculated for each plot reading. The plot readings were averaged across reps to produce an entry mean, for each location and sampling time. Consecutive numbers were added to each variable name to indicate different sampling times. PROC CORR was used to calculate Pearson's correlations coefficient between phenotypic measurements. A forward regression analysis was ran using PROC REG with an alpha < 0.10 to estimate which spectral measurements provided the best phenotypic predictions.

Results

Among the five trials Location 3 had both the highest and lowest entry yield and highest grand mean seed yield. (Table 3.1). Location 1 had the lowest grand mean in seed yield. Least significant difference (LSD) values ranged from a high at Location 2 at 317 to a low at Location 1 at 223, resulting in significant difference at all trials amongst the entries. Location 3 had the latest grand mean maturity and Location 1 had the earliest with LSD ranging from 1.08 at location 1 to 4.31 at Location 4 T2. Location 3 had the highest grand mean lodging score and Location 2 had the lowest. Location 4 T2 had the lowest LSD at .23 and Location 1 had the highest with .4, showing a significant difference amongst all trials for lodging entry means.

In 2018 at Location 1 yield had a variation from 2064 to 2625 kg/ha, maturity had a range of 23 to 39, lodging ranged from 1 to 1.8, and height ranged from 70 to 99 cm (Table 3.1). The yield was not significantly correlated to any physical attributes (Table 3.2). Wilt 1 and 2 were the only physical attributes correlated (r=.93*) amongst each other. Multispectral camera variables that were correlated significantly to yield were Blue (r=-.67*), Red (r=-.71*), NDVI (r=0.51*), BNDVI (r=0.55*), and GNDVI (r=0.55*). Modified broadband camera had correlations to yield with Blue (r=-0.48*), BNDVI (r=0.56*), and GNDVI (r=0.54*). PI M was the only reflectance measurement that was correlation to plant height, and it showed a positive relationship (r=0.50*). Maturity increases as RED M values increased (r=0.54*), while maturity decreased as BNDVI M (r=0.53*) and NDVI M (-0.55*) increased. No spectral reading was correlated with lodging scores at this location, where lodging scores ranged from X to Y. Wilting scores taken on two separate dates were positively correlated with reflectance data from both cameras. As reflectance values increased, wilting scores increased, with correlations ranging from 0.51 to 0.70. The correlations between wilting and the NIR reading from both cameras only differed slightly.

Trends had developed between Location 1 and 3, as replicated results from measurements relate to yield (Table 3.2, 3.6) had been presented. At these locations there was a significant relationship to GNDVI M (.44 to .55), BNDVI M(.29 , .55), and NDVI M(.46 , .51), to yield. Single band measurement of Red M (-.30, -.71) were also significantly correlated to yield. Red 2 M was significantly correlated to yield at Location 2 as well. In a forward regression model of these two locations Red M had shown to account for 50% (Table 3.3) of the variation in yield at Location 1, however this had not been shown to be replicated at any other locations.

Maturity was significantly associated with several variables; however Red M was replicated the most with showing a significant correlation at four trials alternating between negative and positive correlation (-.65 to .66) showing a significant but inconsistent measurement (Table 3.8, 3.10). The positive and negative relationship came from two different maturity groups at the same location (Location 4 T1, and T2). Location 1 and 3, which had shown to have the most repeatable measurements amongst yielding attributes, and both showed a positive correlation (.31 to .54) to maturity (Table 3.2, 3.6). Red M did not account for any variation within a forward regression model as it relates to maturity (Table 3.3, 3.5, 3.7, 3.9).

Height had shown to have repeatable significant relationship to both TH (-.34 to .65), and GNDVI M (-.34 to .70) at three trials. However, the correlations alternated between negative positive suggesting inconsistency. In a forward regression model GNDVI 2 M was able to account for 42% of the variation (data not shown) in addition to Red 2 M creating a multi regression model accounting for 73% of the variation at Location 4 T2 (Table 3.11). TH was able to account for 13% of the variation in height at Location 2 (Table 3.5). At location 3 TH was able to account for 9% of the variation in height (data not shown) but in a multi regression model with PI1 M was able to account for 27% of the variation (Table 3.7).

Lodging had shown to have a significantly repeatable relationship at Location 2, 3, and 4T1 to NDRE M (-.32 to .52) (Table 3.4, 3.6, and 3.8). The alternating positive to negative correlations suggesting unrepeatability showing the variable move in different directions by locaion. In a forward regression model at Location 3, NDRE was able to account for 6% of the variation (data not shown), and in a multi regression model with TH1, and RedEdge 1M was able to account for 28% of the variation (Table 3.7).

Discussion

At location 2 only Red 2 M was significantly correlated to yield. This could be related to this location having the highest LSD value and the least variation in entry mean yield, suggesting less significant difference amongst entry means were found at this location for yield. Three of the variables had shown a significant relationship to yield at more than one location including; NDVI-M, BNDVI-M, and GNDVI-M. The individual band RED from the multispectral camera had also shown to have a significant relationship to yield in addition to accounting for 50% of the variation in yield at location 1. At individual trials, BNDVI - M had shown to have a significant positive correlation to yield at two of the trials. This relationship was associated with higher yields in Locations 1, and 3. This value showed to have a decrease in maturity as the value increased at Location 1. Within each trial, GNDVI-M showed a significant positive relationship to yield at Locations 1 and 3. GNDVI-M had shown to increase when maturity decreased at location three and increase when lodging decreased at Location 4 T2 ($p< 0.10$). When NDVI-M measurement had shown to increase as yield increased at the Location 1 and 3.

At Location 1 when NDVI-M measurement increased it was found that maturity had decreased as a response.

At Location 3 the multispectral camera consistently showed a significant relationship to yield, whereas modified broadband measurements hand no significant relationship. Amongst the highest correlations to yield such as GNDVI M (r=.44*) and NDRE M (r=.51*) there is secondary significant correlations to height and lodging, which are also significantly correlated to yield. Modified broadband camera had several measurements significantly correlated to maturity but no other physical attributes. Maturity was not significantly associated with yield at this trial, as such it is possible that the multispectral camera measurements are correlated to physical attributes most associated with yield at the given environment.

At Location 4 T1 many of the variables showed a significant relationship to height and maturity, but only RED EDGE 2 M and NIR S showed a significant correlation to yield. This could be in part that this trial had the second largest LSD for yield and the second smallest LSD for height and maturity, requiring more yield difference to show a significance and less variation in height in maturity to show a significance, allowing for more significant relationships between measurements to height and maturity.

The multispectral camera had the greatest success showing consistent significant measurements amongst GNDVI, BNDVI, and NDVI at two location. These measurements were consistent with previous research (Christenson et al., 2015, 2016), (Gitelson, 2012), and (Ma et al., 2001) showing that NDVI measurements in the red, blue, and green wavelengths had shown the highest predictability to yield. Similar to research conducted by (Gitelson, 2012), the greatest response to yield came from GNDVI–M, suggesting that further research could be explored using this measurement specifically in an attempt to improve the repeatability of this index potentially using different cameras. This study also found that these measurements had shown inconsistencies across environments, similar to previous research (Clark, 2016). Similarly, the single band measurement Red had shown a significant relationship to yield as well. Their significant relationship to height, lodging maturity, and wilting appeared to be inconsistent across environments. The values that were most associated with height, lodging, and maturity gave confounding results, altering between positive and negative correlations. It is worth noting that the most consistent measurements to yield showed a significant relationship to yield at location 1 and 3, which are both the lowest and highest yielding trials. This showing that trial grand mean performance was not necessarily an indicator of measurement success.

Conclusion

This research focused on the predictability of yield, within yield trials to determine if spectral measurements could predict entry performance ranking by location. We evaluated the effects on crop physiology when yield showed a significant relationship, to determine if measurements were associated with these physical attributes as opposed to yield. We found that with an alpha level set at 0.05; multispectral camera measurements of BNDVI, NDVI, and GNDVI showed a significant relationship to yield at Location 1 and 3. These were the only measurements showing a consistent relationship across more than 1 environment. The individual band Red M was able to show significant correlation to yield at Location 1, 2, and 3. Within the forward selection regression analysis, we found that many of the variables were able to account for variation within the models, but these models were not repeatable across the trials.

The overall goal of spectral measurements is to simplify a collection of information relating back to yield, offering the ability to accurately select lines to progress in a breeding system. A collection of a set of data needs to be easily identifiable as accurate and repeatable.

Within this study the three measurements we found to be replicable were not consistent across all environments. The predictability in determining these measurements as successful by location seems to be unidentifiable as a standalone measurement. The two environments showing this replication in selection were not similar, in that Location 3 had the highest grand mean and Location 1 having the lowest. Additionally, Location 1 had the second lowest number of entries and Location 3 had the highest number of entries suggesting that degree of freedom was not a limitation amongst 4 of the 5 trials. In a stand-alone selection process, it is difficult to determine if any of these spectral measurements will accurately identify top performing lines consistently. Establishing parameters in which seemingly replicable measurements are accurate across these varying environments is imperative to its future use or at the very least the ability to identify measurements accuracy without the need of performing labor-intensive comparisons. Further technological advancements are needed to create technology or measurements that can adequately compare elite cultivar performances or create indicator to identify when measurements are inaccurately representing the population.

Table 3.1 Means, ranges, and LSD values from agronomic traits for 5 trials

Trial	Mean	LSD (0.10)	Range	
	Seed Yield (kg/ha)			
Location 1	2360	223	2064	2625
Location 2	3531	317	2892	4237
Location 3	4213	245	1853	4960
Location 4 T1	4102	247	3100	4556
Location 4 T2	3818	218	3026	4596
	Maturity (days after Aug. 31st)			
Location 1	29	3.91	23	39
Location 2	33	1.56	25	46
Location 3	45	1.08	39.08	52.44
Location 4 T1	32	1.4	28	41
Location 4 T2	42	4.31	39	44
	Height (cm)			
Location 1	87	5	76	99
Location 2	81	6	66	117
Location 3	96	7	79	113
Location 4 T1	83	6	74	106
Location 4 T2	90	8	86	100
	Lodging			
Location 1	1.2	0.4	1	1.8
Location 2	1	0.32	1	1.8
Location 3	1.4	0.38	1	4.2
Location 4 T1	1.1	0.25	1	2
Location 4 T2	1.1	0.23	1	2

Table 3.2. Phenotypic correlations between wavelength and physiological measurements at Location 1 (n=17).

	Yield	Height	Maturity	Lodging	Wilt 1	Wilt 2
Maturity	-0.44	-.05		-.05	.23	.23
Lodging	-0.45	.41	-.05		.11	.09
Height	-0.16		-.05	.41	.23	.23
Wilt 1	-0.19	.23	.23	.11		.93*
Wilt 2	-0.20	.23	.23	.09	.93*	
BLUE M	-0.67*	0.09	0.37	0.23	0.17	0.26
GREEN M	-0.46	0.30	0.15	0.10	0.36	0.48
RED M	-0.71*	-0.11	0.54*	0.23	-0.17	-0.09
RED EDGE M	-0.13	0.19	-0.08	-0.13	0.51*	0.60*
NIR M	0.20	-0.02	-0.38	-0.26	0.65*	0.66*
BNDVI M	0.55*	-0.08	-0.53*	-0.28	0.34	0.26
GNDVI M	0.55*	-0.26	-0.47	-0.27	0.22	0.10
NDRE M	0.42	-0.25	-0.39	-0.15	0.15	0.02
NDVI M	0.51*	0.02	-0.55*	-0.24	0.46	0.38
PI M	-0.28	0.50*	0.10	0.13	0.17	0.28
BLUE S	-0.48*	0.12	0.25	0.04	0.31	0.39
GREEN S	-0.28	0.20	0.02	-0.11	0.54*	0.63*
NIR S	0.14	-0.01	-0.33	-0.32	0.67*	0.70*
BNDVI S	0.56*	-0.06	-0.46	-0.20	0.05	-0.01
GNDVI S	0.54*	-0.16	-0.36	-0.06	-0.21	-0.31

PI S	0.48	0.02	-0.46	-0.26	0.23	0.21
TH	-0.48	-0.45	0.20	0.10	-0.32	-0.33

(*) indicates significance at an alpha level of .05., (M) indicates MicaSense camera measurement, (S) indicates Sony camera measurement

*, indicates significance at an alpha level (<.05).

Table 3.3. Regression model predictors for yield, height, maturity, lodging, Wilt 1, and Wilt 2 for Location 1.

Independent variable(s)	Dependent variable	R - Square	Pr>F
Red1 M	Yield	0.50	0.0016
PI1 M	Height	0.25	0.0413
NDVI1 M	Maturity	0.30	0.023
NA	Lodging		
NIR1 S	Wilt 1	0.45	0.0031
NIR1 S	Wilt 2	0.45	0.0031

Table 3.4. Phenotypic correlations between wavelength and physiological measurements at Location 2. n=35

	Yield	Height	Maturity	Lodging	Wilt 1	Wilt 2	Wilt 3	Wilt 5
Maturity	0.18	.68*		0.13	-0.18	0.04	-0.25	-0.13
Lodging	0.19	-.02	0.13		-0.1	-0.02	-0.07	0.04
Height	0.24		.68*	-0.02	-0.21	0.07	-0.19	0.05
Wilt 1	0.29	-.21	-0.18	-0.1		.86*	.81*	.47*
Wilt 2	0.20	.07	0.04	-0.02	.86*		.72*	.44*
Wilt 3	0.13	-.19	-0.25	-0.07	.81*	.72*		.43*
Wilt 5	0.34*	.05	-0.13	0.04	.47*	.44*	.43*	
B 1 M	.26	.15	0.23	-0.04	-0.04	-0.02	-0.11	-0.14
B 2 M	.03	.01	0.13	0.17	-0.33	-0.25	-0.25	-0.21
B 3 M	-.06	.19	-0.04	-0.44*	-0.21	-0.22	-0.29	-0.18
G 1 M	.15	-.09	0.21	0.02	-0.15	-0.20	-.23	-.25
G 2 M	.05	-.20	0.02	0.33	-0.19	-0.15	-0.10	-0.14
G 3 M	.13	-.35*	-0.24	-0.11	0.12	-0.08	0.06	-0.07
R 1 M	.28	.23	0.22	-0.07	0.10	0.13	0.00	-0.04
R 2 M	.37*	.05	0.17	0.17	0.22	0.30	-0.01	0.23
R 3 M	-.06	.22	-0.02	-0.57*	-0.16	-0.18	-0.26	0.15
RE 1 M	.02	-.27	0.08	0.19	-0.25	-0.34*	-0.33	-0.25
RE 2 M	.16	.08	0.17	0.17	-0.22	-0.14	-0.29	-0.13
RE 3 M	.10	-.41*	-0.2	0.16	0.14	-0.02	0.15	-0.01
NIR 1 M	-.06	-.34	-0.17	0.24	-0.15	-0.25	-0.09	0.00

NIR 2 M	-.13	.05	-0.09	0.08	-0.31	-0.32	-0.13	-0.29
NIR 3 M	.05	.12	0.23	0.30	-0.06	0.01	-0.01	0.03
BNDVI 1 M	-0.29	-0.18	-0.22	0.02	-0.13	-0.19	-0.04	0.01
BNDVI 2 M	-0.05	-0.24	-0.22	0.04	0.11	0.00	0.14	0.10
BNDVI 3 M	0.05	-0.30	-0.05	0.45*	0.17	0.12	0.25	0.11
GNDVI 1 M	-0.30	-0.18	-0.28	0.01	-0.10	-0.16	0.03	0.07
GNDVI 2 M	-0.11	-0.07	-0.22	-0.21	0.12	-0.01	0.12	0.13
GNDVI 3 M	-0.04	-0.18	0.04	0.54*	0.07	0.11	0.19	0.09
PI 1 M	0.12	-0.21	0.17	0.22	-0.04	0.00	-0.15	-0.20
PI 2 M	0.00	-0.07	0.19	0.07	-0.14	-0.11	-0.20	-0.16
PI 3 M	0.18	-0.26	-0.16	-0.10	0.20	0.03	0.14	0.05
NDRE 1 M	-0.10	-0.22	-0.30	0.14	0.04	0.00	0.16	0.21
NDRE 2 M	-0.12	-0.04	-0.15	-0.32	0.10	-0.03	0.12	0.06
NDRE 3 M	-0.06	-0.12	0.07	0.52*	0.05	0.12	0.18	0.08
NDVI 1 M	-0.29	-0.21	-0.20	0.04	-0.19	-0.25	-0.08	-0.04
NDVI 2 M	-0.32	-0.03	-0.17	-0.10	-0.27	-0.32	-0.04	-0.26
NDVI 3 M	0.06	-0.29	-0.04	0.53*	0.16	0.12	0.25	0.12
B 1 S	0.30	0.11	0.21	0.01	0.06	0.09	-0.08	-0.10
B 2 S	0.08	0.04	0.15	0.15	-0.32	-0.24	-0.24	-0.22
B 3 S	-0.17	0.09	0.04	-0.31*	-0.16	-0.09	-0.26	-0.25
G 1 S	0.22	0.001	0.20	0.15	-0.21	-0.23	-0.33	-0.28
G 2 S	0.09	-0.21	-0.02	0.32	-0.21	-0.21	-0.13	-0.14

G 3 S	0.08	-0.43*	-0.30	0.00	0.07	-0.15	0.01	-0.10
NIR 1 S	-0.03	-0.34*	-0.11	0.25	-0.20	-0.31	-0.23	-0.14
NIR 2 S	-0.07	-0.01	-0.02	0.07	-0.35*	-0.36*	-0.25	-0.27
NIR 3 S	0.04	-0.41*	-0.15	0.36*	0.13	0.02	0.18	0.03
BNDVI 1 S	-0.28	-0.08	-0.17	0.06	-0.19	-0.23	-0.06	-0.01
BNDVI 2 S	-0.31	-0.03	-0.15	-0.12	-0.15	-0.28	-0.01	-0.15
BNDVI 3 S	0.06	-0.28	-0.03	0.42*	0.19	0.15	0.26	0.12
GNDVI 1 S	-0.31	-0.05	-0.24	-0.01	-0.07	-0.09	0.08	0.12
GNDVI 2 S	-0.18	-0.08	-0.22	-0.25	0.20	0.08	0.15	0.17
GNDVI 3 S	0.00	-0.10	0.10	0.49*	0.13	0.20	0.26	0.16
PI 1 S	-0.24	-0.10	-0.12	0.10	-0.25	-0.30	-0.14	-0.08
PI 2 S	-0.24	-0.05	-0.10	-0.03	-0.20	-0.32	-0.08	-0.20
PI 3 S	0.08	-0.33*	-0.07	0.37*	0.19	0.12	0.24	0.10
TH 1	0.04	0.36*	0.27	-0.29	0.02	0.08	0.00	-0.10
TH 2	0.25	0.11	0.17	0.06	0.33*	0.49*	0.08	0.15
TH 3	-0.10	0.28	-0.03	-0.55*	-0.20	-0.18	-0.27	-0.12

(*) indicates significance at an alpha level of .05., (M) indicates MicaSense camera measurement, (S) indicates Sony camera measurement

Table 3.5. Regression model predictors for yield, height, maturity, lodging, Wilt 1, Wilt 2, Wilt 3, and Wilt 5 for Location 2.

Independent variable(s)	Dependent variable	R - Square	Pr > F
NDVI 2 M	Yield	0.1	0.0629
TH 1	Height	0.13	0.0339
NDRE 1 M	Maturity	0.09	0.0792
TH 3, BNDVI 3 S, PI 1 S	Lodging	0.42	0.0947
TH 2	Wilt 1	0.11	0.0497
TH 2	Wilt 2	0.24	0.0027

Table 3.6. Phenotypic correlations between wavelength and physiological measurements at Location 3.

	Yield	Height	Maturity	Lodging
Maturity	-0.17	.56*		-.05
Lodging	-0.48*	.23	-.05	
Height	-0.31*		.56*	.23
BLUE M	-0.10	0.07	0.31*	-0.16
GREEN M	-0.22	0.32*	0.69*	0.01
RED M	-0.30*	0.11	0.31*	0.07
RED EDGE M	-0.18	0.27*	0.72*	0.02
NIR M	0.29*	0.13	0.65*	-0.31*
BNDVI M	0.29*	0.00	0.05	0.04
GNDVI M	0.44*	-0.34*	-0.48*	-0.20
NDRE M	0.51*	-0.26	-0.44*	-0.32*
NDVI M	0.46*	-0.05	0.06	-0.23
PI M	-0.38*	0.40	0.59*	0.23
BLUE S	-0.07	0.15	0.26	-0.12
GREEN S	0.04	0.20	0.63*	-0.13
NIR S	0.22	0.13	0.61*	-0.22
BNDVI S	0.16	-0.09	-0.02	0.01
GNDVI S	0.05	-0.21	-0.56*	-0.03
PI S	0.16	0.06	0.43*	0.04
TH	-0.20	-0.31*	-0.17	0.37*

(*) indicates significance at an alpha level of .05., (M) indicates MicaSense camera measurement, (S) indicates Sony camera measurement

Table 3.7. Regression model predictors for yield, height, maturity, lodging, for Location 3.

Independent variable(s)	Dependent variable	R - Square	Pr>F
NDRE 1 M, NIR 1 S, Green 1 S	Yield	0.49	0.0124
PI1-M, TH 1	Height	0.27	0.0079
RedEdge 1 M, NDVI 1 M, Green 1 M	Maturity	0.59	0.0084
TH 1, NDRE 1 M, RedEdge 1 M	Lodging	0.28	0.0233

Table 3.8. Phenotypic correlations between wavelength and physiological measurements at Location 4, Trial 1. N=38

	Yield	Height	Maturity	Lodging	Wilt1
Maturity	-0.36*	.73*		.60*	.26
Lodging	-0.22	.69*	.60*		.26
Height	-0.28	.49*	.73*	.69*	.49*
Wilt 1	-0.02	.26	.26	.26	
BLUE 1 M	0.04	-0.71*	-0.68*	-0.47*	-0.24
BLUE 2 M	0.12	-0.76*	-0.78*	-0.46*	-0.30
GREEN 1 M	0.14	-0.65*	-0.53*	-0.33*	-0.19
GREEN 2 M	0.28	-0.76*	-0.74*	-0.41*	-0.33*
RED 1 M	-0.02	-0.70*	-0.65*	-0.43*	-0.31
RED 2 M	0.04	-0.73*	-0.70*	-0.43*	-0.36*
RED EDGE 1 M	0.20	-0.49*	-0.25	-0.20	-0.15
RED EDGE 2 M	0.32*	-0.78*	-0.73*	-0.42*	-0.40
NIR 1 M	-0.01	0.48*	0.61*	0.31	0.31
NIR 2 M	0.02	0.48*	0.61*	0.33*	0.27
NDRE 1 M	-0.11	0.65*	0.57*	0.33*	0.31
NDRE 2 M	-0.22	0.76*	0.76*	0.44*	0.40*
NDVI 1 M	0.04	0.66*	0.64*	0.39*	0.33*
NDVI 2 M	0.00	0.69*	0.68*	0.42*	0.36*
PI 1 M	0.13	-0.46*	-0.38*	-0.17	-0.18
PI 2 M	0.28	-0.69*	-0.68*	-0.37*	-0.33*

BNDVI 1 M	0.00	0.69*	0.75*	0.48*	0.34*
BNDVI 2 M	-0.06	0.71*	0.72*	0.44*	0.21
GNDVI 1 M	-0.07	0.70*	0.70*	0.43*	0.30
GNDVI 2 M	-0.19	0.70*	0.76*	0.45*	0.32*
BLUE 1 S	-0.07	0.69*	-0.68*	-0.44*	-0.29
BLUE 2 S	0.16	0.72*	-0.80*	-0.45*	-0.24
GREEN 1 S	-0.06	0.64*	-0.47*	-0.33*	-0.18
GREEN 2 S	0.31	0.74*	-0.69*	-0.37*	-0.23
NIR 1 S	-0.04	-0.67*	0.24	0.05	0.15
NIR 2 S	0.33*	-0.71*	-0.09	-0.05	0.01
BNDVI 1 S	0.03	-0.57*	-0.35*	-0.25	-0.31
BNDVI 2 S	-0.11	-0.66*	-0.71*	-0.33*	-0.18
GNDVI 1 S	-0.26	0.02	0.73*	0.47*	0.33*
GNDVI 2 S	-0.26	-0.16	0.81*	0.47*	0.25
PI 1 S	0.025	-0.45*	0.76*	0.42*	0.24
PI 2 S	0.06	-0.56*	0.76*	0.42*	0.24
TH 1	-0.24	0.65*	0.61*	0.34*	0.27
TH 2	0.13	0.58*	0.74*	0.42*	0.34*

(*) indicates significance at an alpha level of .05., (M) indicates MicaSense camera measurement, (S) indicates Sony camera measurement

Table 3.9. Regression model predictors for yield, height, maturity, lodging, and Wilt for Location 4, Trial 1.

Independent variable(s)	Dependent variable	R - Square	Pr>F
NIR 2 S, NIR 1 S	Yield	0.25	0.0133
RedEdge 2 M, PI 2 S, BNDVI 2 S	Height	0.75	0.0028
BNDVI 2 S, TH 1	Maturity	0.73	0.0051
PI 1 S	Lodging	0.23	0.0026
NDRE 2 M, GNDVI 1 S, Blue 2 S, Green 2 S	Wilt	0.5	0.027

Table 3.10. Phenotypic correlations between wavelength and physiological measurements at Location 4, Trial 2. n=10

	Yield	Height	Maturity	Lodging	Wilt 1	Wilt 2	Wilt 3
Maturity	-0.79*	.27		.62	-.48	-.96*	-.91*
Lodging	-0.69*	-.36	.62		.05	-.65*	-.83*
Height	-0.22		.27	-36	-.38	-.20	-.01
Wilt 1	0.06	-.38	-.48	.05		.55	.39
Wilt 2	0.72*	-.20	-.96*	-.65*	.55		.93*
Wilt 3	0.70*	-.01	-.91*	-.83*	.39	.93	
BLUE 1 M	-0.23*	0.05*	0.02	0.28	0.46	0.12	-0.08
BLUE 2 M	0.28	-0.11	-0.58	-0.29	0.53	0.69*	0.58
GREEN 1 M	-0.41	-0.33	0.07	0.66	0.52	-0.09	-0.28
GREEN 2 M	0.33	-0.55	-0.73*	-0.07	0.56	0.67*	0.56
RED 1 M	-0.57	-0.19	0.66*	0.86*	-0.15	-0.68*	-0.83*
RED 2 M	-0.18	-0.52	0.21	0.62	-0.06	-0.26	-0.44
RED EDGE 1 M	-0.10	-0.27	-0.36	0.19	0.60	0.32	0.23
RED EDGE 2 M	0.34	-0.36	-0.78*	-0.27	0.51	0.73*	0.70*
NIR 1 M	0.18	-0.06	-0.58	-0.15	0.70*	0.61	0.51
NIR 2 M	0.25	0.02	-0.58	-0.27	0.51	0.64*	0.55
BNDVI 1 M	0.49	-0.16	-0.63*	-0.49	0.14	0.48	0.61
BNDVI 2 M	-0.26	0.22	0.49	0.28	-0.47	-0.64*	-0.53
GNDVI 1 M	0.60	0.27	-0.63	-0.83*	0.14	0.68*	0.77*
GNDVI 2 M	-0.18	0.65*	0.39	-0.15	-0.26	-0.28	-0.21

NDRE 1 M	0.38	0.27	-0.27	-0.47	0.11	0.37	0.35
NDRE 2 M	-0.24	0.47	0.54	0.13	-0.26	-0.44	-0.46
NDVI 1 M	0.53	0.07	-0.79*	-0.69*	0.47	0.81*	0.86*
NDVI 2 M	0.34	0.38	-0.59	-0.67	0.42	0.68*	0.75*
PI 1 M	-0.42	-0.35	0.38	0.65*	-0.09	-0.50	-0.54
PI 2 M	0.10	-0.54	-0.23	0.21	0.11	0.09	0.05
BLUE 1 S	-0.36	0.21	0.30	0.34	0.24	-0.13	-0.30
BLUE 2 S	0.34	0.04	-0.63	-0.47	0.55	0.75*	0.70*
GREEN 1 S	-0.30	-0.17	0.23	0.61	0.22	-0.27	-0.44
GREEN 2 S	0.45	-0.13	-0.82*	-0.45	0.48	0.78*	0.77*
NIR 1 S	0.17	-0.127	-0.38	0.01	0.52	0.35	0.25
NIR 2 S	0.47	0.07	-0.79*	-0.61	0.34	0.75*	0.82*
BNDVI 1 S	-0.20	0.00	0.40	0.29	-0.48	-0.56	-0.47
BNDVI 2 S	-0.20	0.00	0.40	0.29	-0.48	-0.56	-0.47
GNDVI 1 S	0.44	0.23	-0.49	-0.82*	-0.04	0.52	0.72*
GNDVI 2 S	0.44	0.23	-0.49	-0.82*	-0.04	0.52	0.72*
PI 1 S	0.15	-0.40	-0.13	0.19	-0.05	-0.08	-0.07
PI 2 S	-0.02	-0.17	0.02	0.19	-0.20	-0.19	-0.16
TH 1	-0.46	-0.26	0.64*	0.58	-0.54	-0.70*	-0.69*
TH 2	-0.48	0.00	0.40	0.21	-0.07	-0.35	-0.27

(*) indicates significance at an alpha level of .05., (M) indicates MicaSense camera measurement, (S) indicates Sony camera measurement

Table 3.11. Regression model predictors for yield, height, maturity, lodging, Wilt 1, Wilt 2, and Wilt3 for Location 4, Trial 2.

Independent variable(s)	Dependent variable	R - Square	Pr>F
GNDVI 1 M, PI 2 M, NDRE 2 M, PI 1 M	Yield	0.94	0.02
GNDVI 2 M, Red 2 M	Height	0.73	0.0246
Green2 S	Maturity	0.67	0.004
Red 1 M, PI 1 M	Lodging	0.89	0.0173
NIR1 M, TH2	Wilt 1	0.7	0.0678
NDVI1 M, BNDVI2 M	Wilt 2	0.8	0.0576
NDVI1 M, Green1 S, NIR1 S, Green2 M	Wilt 3	0.98	0.0493

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Chapter 4 - Index of Issues and Future Outlook

Within the process of collecting data for both experiments, there were complications that arose. It is important to recognize these issues for documentation of future experiments. It is possible that new findings of technical difficulties could correct errors founded in this complex process for future research and aid in the improvements in increasing the accuracy of and development of spectral reading as applied to a breeding program.

Thermal

Complications had been founded in using thermal imagery. This appears to be a complication of both technical limitations and the practice of collection itself. Imagery was often irregular in random areas of stitching. It appeared that there could have been an influence on the surrounding environment within a given image. Blotchy patterns would develop in random locations, and patterns of temperature change would be simply related to the time the drone platform had made passes through the experiment. Temperature differences among the plots appeared to become hotter or cooler based on time of drone suspension.

It is possible that the imagery itself is less representative of an area in order to collect valid data depending on the angle of collection within individual pictures. For example, there may be a need to produce more overlap between image passes at lower altitude to collect information on a smaller and more direct area of interest. There could be an outer area of influence negatively affecting data as the pixels or area of image collection become less perpendicular to the location of the camera collection.

Hypothetically, there could also be an issue with the time of day of collection. Typically, with spectral measurements, collections were made near solar noon to minimize changes in sun position throughout data capture. With thermal imagery, it may be important to re-evaluate this mindset. It is possible that a different time may be necessary to differentiate treatment differences in temperature during the day. Reflecting on this thought, it is believed that there could be potential to see larger variation in thermal response during the peak in daytime temperature rather than sun position.

Sony Camera

The Sony camera used in these experiments had a higher resolution and broader wavebands than the comparative MicaSense camera. However, in the second experiment (Chapter 3) we found that the MicaSense camera proved to be superior in detecting differences among the genotypes. This was surprising in that we had suspected that the Sony camera would develop more data as a result to being able to account for measurements that are more sensitive. We expected that this camera would be able to account for carotenoid differences between the cultivars reflected in the pigment index measurement (PI). This speculation was assumed by its predicted association of carotenoids to stress induced plants.

With this camera, adding more data to the image through wider bands and greater resolution created technical issues of processing ability. These issues was probably related to the computer processing power. As a comparison, stitching within smaller yield trials (Chapter 3) as compared to larger progeny row trials (Chapter 2) we found that processing would take a roughly a week for each yield trial. However, using this same process within the larger trial progeny rows it took multiple weeks to process and often resulted in a computer crash before processing was complete. Therefore, the limitation on processing power as trial size increased may have provided the MicaSense camera with an advantage with comparing the different imagery platform.

Individual Bands

We found in the second experiment (Chapter 3) that reflectance data of individual bands were as good, or superior to spectral indices in characterizing performance among the cultivars. It is interesting that wave measurements in a less complex form of data collection could prove to be superior. It is possible that if an individual band could prove to be superior, the cost of collecting data could be drastically reduced. This is assuming that this same measurement is consistent across different platforms.

In Chapter 3, we found that the red wave band proved superior to NDVI and with that offers the potential to utilize cheaper red green blue (RGB) cameras to collect data. If this could prove to be replicated with cheaper cameras this could offer the ability to select at a more affordable level, and still allow for sensitive measurements.

Weather

Weather and the surrounding environment had great implications on data collection. Ideally, when collecting data the desirable environment would include cloud free days, relatively low or no wind, and warm temperatures. These conditions can be elusive during the growing season and can place limitations on the number of collection dates that can be completed. Often one or more of the requirements for favorable conditions does not occur. This can result in delays in data collection, or collection of data under conditions that may compromise the quality of the data. As a result, repeating flights was common to address issues related to shadow affects that would occur from clouds or wind gusts that produced poor quality data.

Conclusions

At the conclusion of these experiments, we can see the potential for great improvements in selection efficiency to be made within a breeding program. There is an opportunity to explore additional measurements not only in vegetation indices but also within individual wave bands through different variations of cameras that can reflect cultivar performance. It is possible that we are at a time where technology is improving at a more rapid pace than of what is testable. However, with continued evaluation of these ever-evolving technologies there is potential to greatly increase future production and improvements within a breeding program and agriculture.